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SLEEP DISORDERS IN MULTIPLE SYSTEM ATROPHY: A LONGITUDINAL STUDY

Presentata da: Dott.ssa Giulia Giannini

Coordinatore Dottorato **Supervisore**

Prof. Pietro Cortelli **Prof. Pietro Cortelli**

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ABSTRACT

Introduction: A higher frequency of sleep and breathing disorders in Multiple System Atrophy (MSA) populations is documented in literature. The analysis of disease progression and prognosis in patients with sleep and breathing disorders could shed light on specific neuropathology and pathophysiology of MSA.

Objective: To characterize sleep disorders and their longitudinal modifications during disease course in MSA patients, and to determine their prognostic value.

Methods: This is a retrospective and prospective cohort study including 182 MSA patients (58.8% males). Type of onset was defined by the first reported motor or autonomic symptom/sign related to MSA. The occurrence of symptoms/signs and milestones of disease progression and their latency were collected. REM sleep behaviour disorder (RBD) and stridor were video-polysomnography (VPSG)-confirmed. VPSG recordings were analysed in a standardized fashion during the disease course. Survival data were based on time to death from the first symptom of disease.

Results: Isolated RBD represented the first MSA symptom in 30% of patients, preceding disease onset according to international criteria with a median of 3(1–5) years. Patients developing early stridor or presenting with RBD at disease onset showed a more rapid and severe disease progression. These features had independent negative prognostic value for survival. Sleep architecture was characterized by peculiar features which could represent negative markers in MSA prognosis. Patients with stridor treated with tracheostomy showed a reduced risk of death.

Conclusions: This is one of the first studies focusing on longitudinal progression of sleep in MSA. Sleep disorders are key features of disease, playing a role in presentation, prognosis and progression. In our MSA cohort, RBD represented the most frequent mode of disease presentation. Moreover, some specific clinical and instrumental sleep features could represent a hallmark of MSA and could be involved in prognosis and, in particular, in sudden death and death during sleep.

INTRODUCTION

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder characterized by a heterogeneous combination of autonomic failure, cerebellar ataxia, parkinsonian features poorly responsive to levodopa and pyramidal signs (Gilman et al. 2008).

The diagnostic criteria define 3 degrees of certainty for diagnosis (possible, probable, and definite) and 2 phenotypes: parkinsonian (MSA-P) or cerebellar (MSA-C), according to the predominant feature at the time of evaluation (Gilman et al. 2008).

Mean survival in MSA ranges from 6.2 to 10 years (Wenning et al. 2013; Low et al. 2015; Coon et al. 2015; Fanciulli et al. 2015), with few patients surviving more than 15 years (Calandra-Buonaura et al. 2013). Causes of death in MSA commonly include bronchopneumonia, urosepsis or sudden death that often occurs at night and has been attributed to either bilateral vocal-cord paralysis or disruption of the brain-stem cardiorespiratory drive (Fanciulli et al. 2015).

Sleep disorders are frequently seen in MSA populations, some of these overlap across the other parkinsonisms while others are either unique to or far more prevalent in this disease. Sleep disorders include sleep onset and maintenance insomnia, fragmented sleep mainly caused by motor symptoms and urinary dysfunction, poor sleep quality, excessive daytime sleepiness, rapid eye movement (REM) sleep behaviour disorder (RBD), periodic limb movements (PLMs) and sleep-disordered breathing (Abbott et al. 2014; Jecmenica-Lukic et al. 2012; Moreno-Lopez et al. 2011).

Video-polysomnographic features in MSA

Only few studies investigated video-polysomnographic features in MSA showing abnormal sleep architecture. One study on 19 patients reported decreased sleep efficiency and an abnormal sleep structure with increased non-rapid eye movement (NREM) sleep stages 1-2, decreased stages 3-4 and a normal amount of rapid eye movement (REM) sleep (Vetrugno et al. 2004). Another study on 30 MSA cases described decreased sleep efficiency and total sleep time, prolonged sleep latency to sleep onset and REM sleep, and sleep architecture alteration with longer duration of NREM 1 stage and shorter REM sleep (Guo et al. 2013).

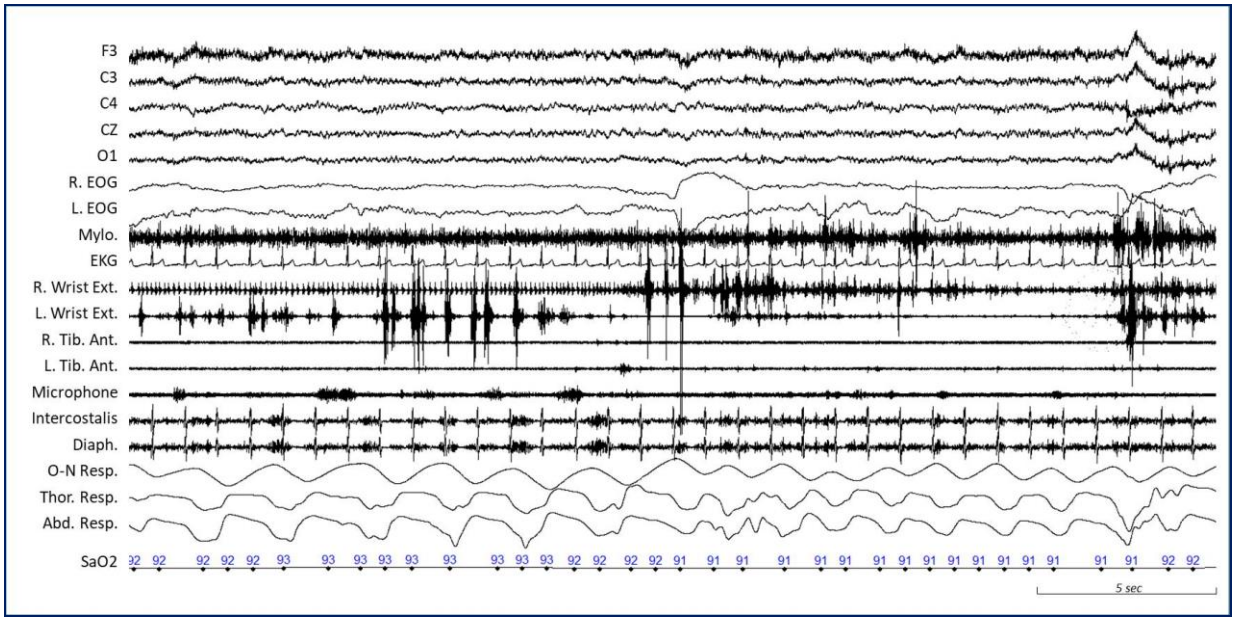
Comparing 45 patients with MSA, 45 patients with Parkinson's disease (PD) and 45 healthy controls, a longer REM sleep-onset time and lower sleep efficiency were reported both in MSA and PD groups, while lower REM sleep percentage was only reported in MSA group. Regarding sleep

fragmentation, the rates of arousals were similar across the groups, but the PLMs index was higher in the MSA group than in the other two (Rekik et al. 2018).

REM sleep behaviour disorder

RBD is a parasomnia characterized by loss of the normal muscle atonia during REM sleep associated with complex, sometimes violent, and dangerous motor behaviours during which patients act out the content of their dreams (American Academy of Sleep Medicine 2014) (Figure 1).

Figure 1: Video-polysomnographic recording showing REM sleep epoch during RBD



Legend: Abd. Resp.=abdominal respirogram; EKG= electrocardiogram; EOG= electro-oculogram; Diaph= diaphragm; L= left; Mylo= Mylohyoideus; O-N Resp.= oro-nasal respirogram; R= right; SaO2= oxygen saturation; Thor. Resp.= thoracic respirogram; Tib. Ant.= tibialis anterior; Wrist Ext.= wrist extensor.

Prevalence of RBD in MSA

Previous prospective and retrospective studies, most of which on a small sample, investigated the prevalence of clinically suspected RBD (Table 1). Of these, 15 articles included prevalence of RBD confirmed by video-polysomnography (VPSG). A meta-analysis performed in 2015 reported a summary prevalence of clinically suspected RBD in MSA ranging from 25 to 100%, with a summary prevalence of 73 % (95% confidence interval [CI]= 62–84 %) in a pooled sample of 324

patients. The prevalence of VPSG-confirmed RBD in MSA ranged from 68.8 to 100%, with a summary prevalence of 88% (95% CI= 79-94%) in the pooled sample (Palma et al. 2015).

Other studies, not included in the meta-analysis, reported prevalence of clinically and VPSG-confirmed RBD in small samples. One study on 30 MSA patients (23 with MSA-C and 7 with MSA-P) reported a clinically suspected RBD prevalence of 46.7% (52.2% MSA-C, 28.6% MSA-P) and a same prevalence of VPSG-confirmed RBD (Guo et al. 2013). Another study on 38 MSA patients (11 with MSA-C and 27 with MSA-P), 36 of which with valid VPSG recordings, showed similar prevalence of VPSG-confirmed RBD in MSA-P (76.0%) and MSA-C (72.7%) (Muntean et al. 2013). A recent study investigated VPSG-confirmed RBD in 55 MSA patients reporting a prevalence of 32.7%, without significant difference between MSA-C and MSA-P groups (50.0% MSA-C, 25.6% MSA-P) (Wang et al. 2020).

The largest retrospective cohort study on 635 MSA patients reported a clinically suspected RBD prevalence of 44.4% (48.6% MSA-C, 41.9% MSA-P). Presence of dream-enactment behaviour was recorded based on documentation in the clinical history (Coon et al. 2015).

Table 1: Studies focused on prevalence of RBD in MSA patients

First author, year	Study sample	Patients underwent VPSG	Clinically suspected RBD	VPSG-confirmed RBD
Plazzi, 1997	39	39	69.2%	89.7%
Tachibana, 1997	21	21	---	90.5%
Iranzo, 2000	20	20	90.0%	90.0%
Silber, 2000	28	28	60.7%	71.4%
Wetter, 2000	10	10	---	70%
Boeve, 2001	13	10	84.6%	90.0%
Ghorayeb, 2002	57	---	47.5%	---
Vetrugno, 2004	19	19	78.9%	100.0%
Schmeichel, 2008	11	5	72.7%	100.0%
De Cock, 2011	49	19	87.8% (91.7% MSA-C, 84.0% MSA-P)	100.0%
Nomura, 2011	16	16	25.0%	68.8%
Muntean, 2013	36	36	---	72.7% MSA-C, 76.0% MSA-P
Guo, 2013	30	30	46.7% (52.2% MSA-C, 28.6% MSA-P)	46.7%
Stanzani-Maserati, 2014	10	10	---	100%
Palma, 2015	42	42 (2 had no REM during the study)	76.2%	81.0% (77.8% MSA-C, 86.7% MSA-P)
Coon, 2015	685	---	44.4% (48.6% MSA-C, 41.9% MSA-P)	---
Wang, 2020	55	55	---	32.7% (50.0% MSA-C, 25.6% MSA-P)

Legend: MSA-C= multiple system atrophy with predominant cerebellar phenotype; MSA-P= multiple system atrophy with predominant parkinsonism; RBD= REM sleep behaviour disorder; VPSG= video-polysomnography.

Phenoconversion to MSA in patients with isolated RBD

Despite almost all patients eventually diagnosed with MSA experience RBD at some point in their disease progression, studies investigating the rate of phenoconversion to synucleinopathies in cohorts of patients with isolated RBD (iRBD) showed that a low percentage of those who converted actually developed MSA. iRBD is a rare condition and population-based studies showed a prevalence of about 1% over the age of 60. Few studies investigated the prevalence of RBD, using polysomnography, on population-based cohorts. In particular, in one study on 348 individuals from South Korea aged 60 years and over, REM tone was quantified with PSG and participants with abnormal tone were contacted by phone and asked about dream-enactment behaviour, revealing an RBD prevalence estimate of 1.15% (Kang et al. 2013). Another study evaluated the prevalence of RBD in a sample of 539 individuals aged 60 years or older, from a Spanish community, using a validated single question for the screening of RBD followed, in those who screened positive, by clinical assessment and VPSG, and showed an estimated prevalence of 0.74% (95% CI = 0.29–1.89) (Pujol et al. 2017). Analysing data from 1997 participants belonging to the population-based HypnoLaus study (mean age= 59 ± 11.1 years, 53.6% women), who completed the Munich Parasomnia Screening questionnaire and had a complete polysomnography at home, authors estimated a prevalence of RBD of 1.06% (95% CI = 0.61–1.50) (Haba-Rubio et al. 2018).

In the last years several international groups performed follow-up studies on iRBD cohorts focusing on the rate of phenoconversion to an overt neurodegenerative disease and on the predictive role of clinical and instrumental markers for phenoconversion.

In 1996 Schenck et al. first reported the phenoconversion of a series of 29 males older than 50 years of age and in 2013 they provided a 16-year update from their previous report (Schenck et al. 1996; Schenck et al. 2013). Other two important research groups closely followed-up iRBD cohorts throughout the disease course. Since 2014, Postuma and collaborators recruited up to 154 patients with iRBD in Montreal (Canada), publishing numerous articles on the longitudinal evolution of this cohort (Postuma et al. 2009; Postuma et al. 2011; Postuma et al. 2012; Postuma, Gagnon, et al. 2015; Fereshtehnejad et al. 2019). Similarly, the group led by Iranzo, from Barcelona (Spain), first followed iRBD patients diagnosed from 1991 to 2003 and, subsequently, followed-up disease-free patients since 2005 to-date in a systematic manner, publishing several articles on increasing number of patients including up to 203 participants in his cohort (Iranzo et al. 2006; Iranzo et al. 2010; Iranzo, Serradell et al. 2013; Iranzo, Tolosa et al. 2013; Iranzo et al. 2014; Fernandez-Arcos et al.

2016). Moreover, other international groups focused on this topic and recently the International RBD Study Group, combined prospective follow-up data making up the largest iRBD cohort counting 1280 participants recruited from 24 centres (Postuma et al. 2015; Postuma et al. 2019).

Taken together, these observational studies have suggested that most iRBD patients who eventually develop a defined neurodegenerative disease, were almost always diagnosed with a synucleinopathy (Fantini et al. 2011; Boot et al. 2012; Wing et al. 2012; Schenck et al. 2013; Arnulf et al. 2015; Mahlknecht et al. 2015; Fernández-Arcos et al. 2016; Youn et al. 2016; Li et al. 2017; Zhou et al. 2017; Fereshtehnejad et al. 2019; Postuma et al. 2015; Postuma et al. 2019) (Table 2). These studies, generally performed in single centres and on small sample, reported a frequency of phenoconversion ranging from 12.5% to 80.8%. This variability in results could be related to the heterogeneity of methods and follow-up duration (Fantini et al. 2011; Boot et al. 2012; Wing et al. 2012; Schenck et al. 2013; Arnulf et al. 2015; Mahlknecht et al. 2015; Fernández-Arcos et al. 2016; Youn et al. 2016; Li et al. 2017; Zhou et al. 2017; Fereshtehnejad et al. 2019). More recently, a largest study analysed prospective follow-up data on 1280 iRBD patients from 24 centres of the International RBD Study Group showing that 352 (28%) converted to an overt neurodegenerative syndrome (median time to phenoconversion = 8.0 years), 16 (4.5%) of which converted into MSA (Postuma et al. 2019).

Considering only the sample who phenoconverted from iRBD, the rate of those who developed MSA ranged from 0% (Fantini et al. 2011; Boot et al. 2012; Wing et al. 2012; Mahlknecht et al. 2015) to 16.7% (Li et al. 2017), with the remaining patients developing PD, DLB or dementia (Table 2).

One retrospective study focused on a subgroup of patients who experienced iRBD for at least 15 years before evolving into PD, PD dementia (PDD), DLB, or MSA. In 27 patients (88.9% males) with clinical history of RBD, 14 of whom with VPSG-confirmed RBD, initial parkinsonian symptomatology was observed in 13 patients, initial cognitive impairment was reported by 13 patients and primary autonomic symptomatology was the first symptom in one patient. At the last evaluation the following diagnoses were performed: MSA in 1 (3.7%) patient, PD in 4 (14.8%) patients, PDD in 6 (22.2%) patients, PD-MCI in 3 (11.1%) patients, MCI in 2 (7.4%) patients and DLB in 11 (40.8%) patients. The median latency from RBD to neurodegenerative syndrome symptoms onset was 25 years (range 15–50 years) (Claassen et al. 2010).

Table 2: Studies investigating phenoconversion in cohorts of patients with iRBD

First author, year	Study sample	PSG	Overall Follow-up of the study	Conversion risk	Phenoconversion	Prevalence of MSA in converters
Fantini, 2011	24 (75.0% males)	Yes	26.3 months (SD 5.0)	3 (12.5%) patients converted at follow-up	n =2 PD	0.0 %
Boot, 2012	44 (77.3% males)	No	46 months (IQR 31-47)	15 (34.1%) patients evolved in neurodegenerative disease. Median time between onset of dream-enactment behaviour and diagnosis of MCI / PD = 20.7 years	n = 1 PD n = 14 MCI	0.0 %
Wing, 2012	91 (82.4% males)	Yes	5.6 years (SD 3.3)	19 (20.9%) patients evolved in neurodegenerative disease. The estimated 5-year and 9-year risks of any neurodegenerative disorder for the overall study cohort were 8.5% and 38.1%.	n = 8 PD n = 8 AD n = 1 DLB n = 2 vascular dementia	0.0 %
Schenck, 2013	26 (100% males)	Yes	16 years	21 (80.8%) of patients who were initially diagnosed with iRBD eventually developed parkinsonism/dementia Mean time to conversion= 14.2±6.2 years (range: 5-29 years)	n = 13 PD n = 3 DLB n = 2 MSA n = 1 dementia (unspecified); n = 2, clinically diagnosed AD with autopsy-confirmed combined AD + Lewy body disease pathology.	9.5%
Arnulf, 2015	69 (81.2% males)	Yes	3 years (range: 1–15)	16 (23.2%) converted into neurodegenerative disorders. Median time from RBD onset to parkinsonism/dementia = 16 years.	n = 6 parkinsonism n = 6 dementia n = 2 dementia plus parkinsonism n= 2 MSA	12.5%

Mahlknecht, 2015	34 (85.3% males)	Yes	4.9 years (SD 0.3)	9 (26.5%) patients with iRBD developed Lewy body disease. Mean interval from iRBD diagnosis.to conversion = 5.5 ± 4.7 years	n = 6 PD n = 3 DLB	0.0 %
Fernández-Arcos, 2016	203 (79.8% males)	Yes	5.0 years (range: 0.1-17)	69 (34.0%) received a diagnosis of defined neurodegenerative syndrome after a median follow-up of 5 years.	n =22 PD n = 32 DLB n =2 MSA n =13 MCI	2.9%
Youn, 2016	84 (69.1% males)	Yes	4.1 years (SD 2.1, range 1.0-10.3)	18 (21.4%) patients developed neurodegenerative disorders. The estimated risk of developing neurodegenerative diseases was 9% at 3, 18% at 6 and 35% at 6 years from the diagnosis of iRBD, respectively.	n = 9 PD n = 4 DLB n = 1 MSA n = 3 AD n = 1 spinocerebellar ataxia	5.6%
Li, 2017	43 patients (79.1% males)	Yes	5 years	18 (41.9%) developed neurodegenerative synucleinopathy diseases. Median interval from the estimated onset of iRBD symptoms to conversion= 10.5 years	n = 9 PD n = 2 DLB n = 3 MSA n = 4 PD/MCI	16.7%
Zhou, 2017	179 patients (79.1% males	Yes	5.8 ± 4.3 years	50 (27.9%) patients developed neurodegenerative diseases. Median time of conversion to neurodegenerative diseases = 9 years from RBD onset, 3.1 years from RBD diagnosis.	n = 27 PD n = 7 DLB n = 2 MSA n = 14 AD	4.0%
Fereshtehnejad, 2019	154 patients	Yes	8.2 years (SD 9.0)	55 (36%) converted to an overt neurodegenerative syndrome. Mean interval between baseline evaluation and phenoconversion = 4.6 ± 2.5 years.	n= 25 PD n= 4 MSA n=26 dementia	7.3%

Postuma (IRBDSG*), 2015	279 (79.6% males)	Yes	3.8 years (SD 1.4)	93 (33.3%) developed a neurodegenerative disease. Risk of neurodegenerative disease was 15% after 2 years, 25% after 3, 36% after 4, and 41% after 5 years. Mean interval between baseline and disease diagnosis = 2.5 ±1.7 years.	n =39 PD n =7 MSA n =47 dementia (n=28 probable DLB)	7.5%
Postuma (IRBDSG*), 2019	1280 patients (82.5% males)	Yes	3.6 years (max 19)	352 (27.5%) converted to an overt neurodegenerative syndrome. Phenoconversion rate of 6.25% per year (10.6% after 2 years, 17.9% after 3 years, 31.3% after 5 years, 51.4% after 8 years, 60.2% after 10 years, and 73.5% after 12 years). Mean interval between baseline evaluation and phenoconversion was 4.6±3.5 years.	n= 199 parkinsonism (16 probable MSA) n= 153 dementia first.	4.6%

Legend: AD= Alzheimer's Disease; DLB= dementia with Lewy bodies; IRBDSG *= International REM Sleep Behaviour Disorder Study Group; n= sample; PD= Parkinson's disease; MCI= Mild Cognitive Impairment; MSA= Multiple System Atrophy; SD= Standard Deviation.

Phenoconversion to MSA in patients with Isolated Autonomic Failure

Another pre-motor cohort is represented by patients with Isolated Autonomic Failure (IAF).

As there is an overlap of autonomic dysfunction in patients with MSA and those with pure autonomic failure (PAF), it may be challenging to differentiate these two disorders in early stages, when only isolated autonomic failure is present.

The presence of RBD in the context of autonomic failure was suggested to be confined to those evolving to MSA and to be absent in those with PAF in a previous case series, making RBD a clinical marker of MSA (Plazzi et al. 1998). However, subsequent case series have reported RBD in patients with PAF (Weyer et al. 2006; Kashihara et al. 2008; Miglis et al. 2017), with symptoms occurring after the onset of autonomic failure (Miglis et al. 2017).

Disagreement in results may be a consequence of the differences in design, sample size, population characteristics, follow-up duration, and diagnostic criteria used in the studies.

Few studies on cohorts of patients with IAF have investigated the role of RBD in predictive of phenoconversion.

The largest follow-up studies reported to date on the natural history of a cohort of patients with a 5-year history of IAF, who fulfil current criteria for PAF (n= 50, mean disease duration 13.8 ± 7.1), documented a global percentage of 32% of phenoconversion to a manifest synucleinopathy (converters n= 16, 10 with MSA). This study found that the latency of RBD onset, not its presence, allows discrimination of the converters group. In fact VPSG-confirmed RBD, which was more frequent in the converters group without reaching statistical significance, occurred significantly earlier in this group during the disease course (2 [−2; 5] vs. 10 [4; 13] years, p= 0.0281). These data entail a higher risk of phenoconversion to other synucleinopathies in patients with IAF with early-onset RBD (hazard ratio [H-Ratio] 8.05, p = 0.016) (Giannini et al. 2018).

A multicentre prospective study on the natural history of 100 patients with IAF, 74 of those followed up longitudinally, reported a cumulative incidence of phenoconversion to central synucleinopathy of 34% (converters n= 25, 6 with MSA), during a limited 4-year follow-up period (≈14%/year). This study did not report RBD in the small group of patients retaining the PAF phenotype at the last follow-up (n= 12) and showed that the presence of RBD was strongly associated with phenoconversion to other synucleinopathies (Kaufmann et al. 2017). In a monocentric retrospective cohort study on 318 patients (79 patients followed up for at least 3 years) with IAF, in which the estimated conversion rate ranged between 12% and 48% (38 of 318 and 38

of 79, respectively), 22 patients developed MSA. Conversely compared to previous findings, RBD was not found to be associated with a higher risk of phenoconversion and was seen in about half of patients with stable PAF, but its presence or absence was not consistently documented (Singer et al. 2017).

However, the latter two studies included all patients with IAF, irrespective of the disease duration, with subtle nonspecific neurologic deficits at the time of enrolment and followed patients for a short period (4-year follow-up). Our previous study (Giannini et al. 2018) included only patients with IAF with a disease duration >5 years without subtle nonspecific neurologic deficits at the time of enrolment who were followed up for a longer period, allowing better estimation of the phenoconversion rate in patients currently defined as having PAF.

RBD as initial manifestation of MSA

Finally, some studies showed that, in patients with synucleinopathies, RBD frequently predates motor and cognitive deficits preceding overt manifestations by decades. Despite its higher prevalence on MSA, prevalence of RBD as initial manifestation of disease has been poorly investigated. Few studies primarily investigated RBD as mode of MSA onset. However, these data were reported also in other studies focused on different aims. To date, 11 studies reported RBD preceding the disease onset, only 7 diagnosed with VPSG, showing a prevalence ranging from 15.6% to 60.0% (Plazzi et al. 1997; Iranzo et al. 2000; Iranzo et al. 2005; Vetrugno et al. 2004; Nomura et al. 2011; Guo et al. 2013; Stanzani-Maserati et al. 2014; De Cock et al. 2011; Palma et al. 2015; Coon et al. 2015; McKay et al. 2018).

The first study evaluating clinical and VPSG data reported that 27 out of 39 patients with MSA (69.2%) had a history of RBD, 12 (30.8%) of which referred this sleep disorder before the disease onset (Plazzi et al. 1997). Another study on 20 MSA patients revealed a clinically-suspected and VPSG-confirmed RBD in 18 subjects and a prevalence of iRBD in 30% (n= 6/20) of the total sample (Iranzo et al. 2000). A study of the same group comparing RBD features in 26 patients with MSA and 45 patients with PD with VPSG-confirmed RBD found that RBD preceded the onset of motor symptoms in 14 out of 26 patients with MSA (53.8%) (Iranzo et al. 2005). A video-polysomnographic study on 19 MSA patients recorded RBD in all patients, in 3 (15.6%) of them isolated RBD preceded autonomic and/or motor onset (Vetrugno et al. 2004). One study compared clinical and polysomnographic characteristics of RBD between 16 MSA patients and 49 PD

patients, and found VPSG-confirmed RBD in 11 patients, 7 (43.8%) of which reported the development of RBD symptoms before disease onset (Nomura et al. 2011). The study on 30 MSA patients reported a VPSG-confirmed RBD prevalence of 46.7% (n= 14) and a prevalence of RBD onset before disease onset of 10% (n= 3) (Guo et al. 2013). In a study on 10 MSA patients investigated with VPSG, RBD was reported in all patients and preceded the disease onset in 6 (60.0%) out of 10 patients (Stanzani-Maserati et al. 2014).

In the other four studies prevalence of isolated RBD was calculated on the basis of clinically-suspected RBD because RBD diagnosis was based on history taking, VPSG was performed in a subgroup of iRBD sample or the rate of VPSG-confirmed RBD in patients presenting with RBD was not specified in the results section. One study based on bed-partner interview revealed a clinical-suspected RBD prevalence of 87.8% and iRBD prevalence of 30% (De Cock et al. 2011). In this study 22 out 49 patients performed VPSG but the rate of VPSG-confirmed RBD in patients developing this sleep disorder before MSA onset is not specified. One cross-sectional multicentre study based on sleep questionnaires observed that 29 out of 64 patients with MSA (45.3%) presented RBD symptoms before the onset of motor deficits, but the amount of VPSG-confirmed RBD in this subgroup of patients is not specified in the article (Palma et al. 2015). In a large retrospective study on 685 patients with MSA, history of sleep symptoms suggesting RBD was reported in 304 participants and in 34% of cases sleep symptoms preceded motor and autonomic ones (Coon et al. 2015). Finally, in a recent retrospective study on 30 patients with MSA, 5 (16.7%) participants reported a history suggesting RBD as first symptom of disease, which was confirmed by VPSG in 2 patients (McKay et al. 2018).

Although RBD represents an early clinical marker of neurodegeneration and may be relevant for addressing possible candidates for future neuroprotective therapies, to date VPSG studies investigating RBD in wide cohorts of patients with MSA are lacking.

VPSG features in MSA with RBD

One MSA patient with RBD was longitudinally investigated by VPSG during the disease course. Decreased behaviours/movements with time were confirmed on polysomnography/video recording in this patient, and sleep talking became less frequent and intense. At a more advanced stage, the ratio of REM Sleep Without Atonia (RSWA) to the whole of REM sleep increased in this patient,

while the sleep architecture as well as the percentage of REM sleep were maintained (Tachibana et al. 2004).

A VPSG study on 2 MSA patients reported a reduced frequency of RBD episodes during the disease course and described the appearance of a disrupted sleep pattern with sleep stages no longer identifiable, associated with a nearly continuous motor and verbal abnormal behaviours, and ambiguous and rapid oscillation of state-determining polysomnographic variables. Features of abnormal Stage 1 NREM and REM sleep, together with an instable chin muscle tone, recurred rapidly and irregularly, in a sort of undifferentiated sleep state. These features were consistent with status dissociatus and were interpreted as progression of RBD (Vetrugno et al. 2009).

Sleep-related breathing disorders

Respiratory abnormalities and different sleep-related breathing disorders can occur in MSA (Chokroverty 1994; Abbott et al. 2014; Iranzo et al. 2007; Osaki et al. 2009). Sleep-related breathing disorders in MSA included snoring, obstructive sleep apnoea/hypopnoea syndrome, central sleep apnoea, paradoxical breathing, nocturnal tachypnoea with quite normal waking respiratory rate syndrome, dysrhythmic breathing or irregular patterns and Cheyne-Stokes respiration or a variant of the Cheyne-Stokes pattern (Chokroverty 1994; Vetrugno et al. 2007). Dysrhythmic breathing, described for the first time from Chokroverty, is defined as non-rhythmic respiration with irregular rate, rhythm, and amplitude which may occur exclusively during sleep or may worsen during sleep, causing repeated nocturnal hypoxemias and awakenings (Chokroverty 1994).

Stridor

Stridor in MSA is a strained, high-pitched, harsh respiratory sound, mainly inspiratory, caused by laryngeal dysfunction leading to narrowing of the rima glottidis. It may occur only during sleep or it may be present both during sleep and wakefulness (Cortelli et al. 2019; Ozawa et al. 2016).

One study described this distinctive noise occurring in inspiration in MSA patients finding a fundamental acoustic frequency of 260–330 Hz, different from that of ordinary soft palate snoring (Kakitsuba et al. 1997). The analysis of acoustic features of stridor in 22 patients with MSA revealed that stridor can be decomposed into rhythmic and semirhythmic waveforms comprising formats and harmonics, whose presence suggests a vocal cord origin, and these features

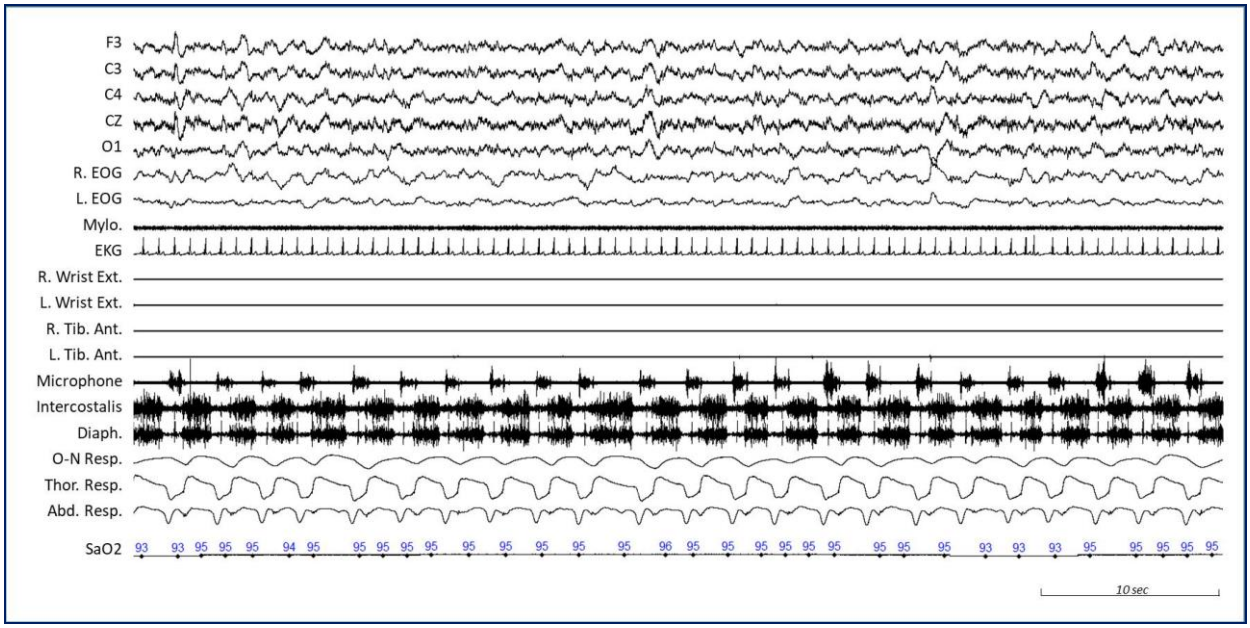
differentiate stridor from snoring (composed by irregular-shaped sound with no formats and harmonics) (Koo et al 2016).

Studies with VPSG, which include audio recording and concurrent evaluation of vocal cord motion by fiberoptic laryngoscopy, showed that the high-pitched sound identified as stridor was associated with impaired vocal cord abduction, paradoxical adduction or both, during inspiration and expiration, leading to narrowing of the rima glottidis. This indicates that inspiratory vibration of the narrowed vocal cord folds causes stridor (Cortelli et al. 2019).

One VPSG study exploring breathing activity and EMG activity of the respiratory muscles, observed that stridor was accompanied by overactivation of intercostalis and diaphragmatic muscles (Vetrugno et al. 2007). Figure 2 showed video-polysomnographic recording showing stridor associated by overactivation of intercostalis and diaphragmatic muscles.

Prevalence of stridor in MSA ranges from 12% to 42%, and might develop at any time point in the disease process (Giannini et al. 2016; Tada et al. 2007; Coon et al. 2015; Silber et al. 2000; Iranzo et al. 2004; Vetrugno et al. 2004; Figueira et al. 2014; Wenning et al. 1994). Only two studies focused on stridor as initial manifestation of MSA showing that 4%-5.2% of patients presented stridor at disease onset (Uzawa et al. 2005; Giannini et al. 2016).

Figure 2: Video-polysomnographic recording showing stridor



Legend: Abd. Resp.=abdominal respirogram; EKG= electrocardiogram; EOG= electro-oculogram; Diaph= diaphragm; L= left; Mylo= Mylohyoideus; O-N Resp.= oro-nasal respirogram; R= right; SaO2= oxygen saturation; Thor. Resp.= thoracic respirogram; Tib. Ant.= tibialis anterior; Wrist Ext.= wrist extensor.

VPSG features in MSA with stridor

Only few studies investigated VPSG features between patients with and without stridor.

Concerning sleep architecture, one study performed on 19 consecutive MSA patients, 8 with stridor and 11 without stridor, did not report differences between patients with and without sleep-related respiratory disturbances (Vetrugno et al. 2004). Conversely, the study performed by Rekik on 17 patients with stridor and 15 without stridor found that the first group had higher NREM 1 percentage and tended to have lower REM sleep percentage (Rekik et al. 2018).

Concerning autonomic features, the study performed by Isono et al. reported increased respiratory frequency during sleep compensating for a reduction of tidal volume in 10 MSA with stridor (Isono et al. 2001). Similarly, in the study performed by Vetrugno et al., patients with stridor had breathing rate comparable to patients without stridor during wake (19 ± 5 vs. 19 ± 2), but higher rates during both NREM (23 ± 8 vs. 18 ± 3) and REM sleep (23 ± 9 vs. 19 ± 3). The stridor group, already tachypnoic during wakefulness, had a significant increase in breathing rate from wake to NREM and REM sleep (19 ± 5 vs. 23 ± 8 vs. 23 ± 9, $p < 0.05$).

Paradoxical breathing was found in about half of the patients, more frequent in patients with stridor (Vetrugno et al. 2004).

All studies did not report differences in mean SaO2. In particular, in the study of Vetrugno, mean SaO2 during sleep remained the same in patients with and without stridor (Vetrugno et al. 2004). The study of Rekik showed that mean oxyhemoglobin saturation during sleep (92.9 ± 3.8% vs. 93.6 ± 2.8%, p= 0.14), as well as the mean oxyhemoglobin saturation during REM sleep (93.3 ± 5.3% vs. 93.9 ± 3.1%, p= 0.24) was similar in the two groups (Rekik et al. 2018).

There were no significant differences between patients with and without stridor in mean heart rate during wake and sleep stages. However, patients with stridor showed no significant decrease during NREM and higher heart rate during REM sleep compared to wake (71 ± 13 in NREM vs. 73 ± 12 in REM vs. 72 ± 11 in wake), while patients without stridor had significantly lower heart rate between wake and NREM sleep (69 ± 10 in wake vs. 65 ± 10 in NREM vs. 66 ± 10 in REM) (Vetrugno et al. 2004).

Concerning motor features, in patients with stridor, intercostalis muscle and limb motor overactivity was always more prevalent throughout wake and sleep, when compared to patients without stridor. Patients with stridor showed an higher PLMS prevalence while RBD was found in all MSA patients with and without stridor (Vetrugno et al. 2004).

Other sleep-related breathing disorders

Among the sleep-related breathing disorders, nocturnal tachypnea with quite normal waking respiratory rate, snoring, nocturnal paradoxical breathing, obstructive sleep apnoea/hypopnoea syndrome, dysrhythmic breathing with Cheyne-Stokes or irregular patterns and central apnoea were reported (Vetrugno et al. 2007; Bhattacharya et al. 2020).

A retrospective study compared sleep characteristics of 45 MSA patients having undergone a VPSG to those of 45 patients with PD and 45 healthy controls, matched for age and sex. In MSA, 28/45 (62.2%) patients had sleep breathing disorders, including (overlapping samples) stridor (n= 17, 38%), obstructive sleep apnoea (n= 14, 31%), central sleep apnoea (n= 4, 9%), and ataxic breathing (n = 1) (Rekik et al. 2018).

The frequency of obstructive sleep apnoea in large studies of MSA patients ranges from 15% to 69% (Flabeau et al. 2017; Vetrugno et al. 2004; Plazzi et al. 1997). However, mean prevalence of sleep apnoea syndrome ranged from 39- 45% when only studies with unbiased recruitment were analysed (Meissener et al. 2014; Vetrugno et al. 2004; Shimohata et al. 2007; Ghorayeb et al. 2005; Manni et al. 1993; Plazzi et al. 1997; Iranzo et al. 2000; Silber et al. 2000). There are no data focusing on the premotor frequency of this sleep disorder (Jecmenica-Lukic et al. 2012; Vetrugno et al. 2004; Plazzi et al. 1997).

Central sleep apnoea is less common than obstructive sleep apnoea and tends to occur at late stages of the disease, however few MSA cases with central apnoea as presenting feature are described (Cormican et al. 2004; Glass et al. 2006; Iranzo et al. 2007). One case report described a 61-year-old male presented with apparent idiopathic central sleep apnoea who, after 4 years developed features of autonomic, cerebellar and extrapyramidal dysfunction consistent with a diagnosis of MSA (Cormican et al. 2004). One study described 6 patients who presented respiratory insufficiency as the initial and most prominent feature of disease and who were ultimately recognized to have MSA. All patients required tracheostomy: 3 were emergently examined for acute respiratory distress, the other 3 cases showed obstructive sleep apnoea unresponsive to continuous positive airway pressure therapy. Stridor was early detected during disease course in all patients (Glass et al. 2006). This study suggested that the respiratory problems overshadowed the neurologic complaints in all of these cases, and the ultimate diagnosis of MSA was unsuspected or at least not definite at presentation. Early clues to the diagnosis were autonomic symptoms, which

were eventually present in all 6 patients, and dream-enactment behaviour, which was present in 3 cases. Motor symptoms and signs were present early in 5 patients but were mild (Glass et al. 2006). Finally, one study suggested that MSA patients had very poor chemosensitivity to hypoxia when compared to PD patients, whereas chemosensitivity to hypercapnia was normal. Moreover, among patients with idiopathic late-onset cerebellar ataxia the 6 ones who later evolved to MSA-C had an impaired hypoxic ventilatory response that appears to be a good marker enabling earlier diagnosis of MSA in patients presenting with idiopathic late-onset cerebellar ataxia (Tsuda et al. 2002).

Role of sleep disorders on survival

Role of stridor on survival

Some studies investigated the role of sleep disorders on MSA survival, mainly focusing on stridor. The prognostic role of stridor remains controversial (Cortelli et al. 2019) and this could be ascribable to heterogeneity in design, method, study population, MSA diagnostic certainty (clinical vs. autopsy based) and stridor diagnosis across studies (Table 3).

The first study on this topic, reporting survival curves of 30 patients with MSA who underwent polysomnography, showed a shorter survival time from polysomnography to death in patients with stridor (n = 11) compared to those without (median survival 3 vs. 4 years, respectively, $p < 0.05$). However, survival curves obtained from disease onset did not significantly differ between groups and diverged after 7 years (Silber et al. 2000). On the contrary, seven retrospective studies did not find an association between the presence of stridor during the disease course and shortened survival (Giannini et al. 2016; Coon et al. 2015; Yamaguchi et al. 2003; Tada et al. 2007; Krim et al. 2007; Lalich et al. 2014; Starhof et al. 2016; Cortelli et al. 2019).

One study evaluated laryngeal stridor in 104 MSA patients, 83 (stridor to non-stridor ratio 33:50) of these were followed up and 57 died at the time of analysis. The median survival period in the stridor group and the non-stridor group was 8.0 and 9.0 years, respectively, without difference between groups ($p= 0.2302$). The incidence of sudden death was more frequent in the stridor group ($p=0.0054$) (Yamaguchi et al. 2003). However, in this study patients initially classified in the non-stridor group were considered without stridor also during the follow-up because the final visit was performed by telephone.

Table 3: Studies focused on the prognostic role of stridor

First Author, year	Study sample	N. patients with stridor	Stridor diagnosis	Results
Silber, 2000	42 (30 with follow-up)	11	VPSG	Shorter survival time from polysomnography to death in patients with stridor compared to those without (median survival 3 vs. 4 years, respectively, $p < 0.05$); survival curves obtained from disease onset did not significantly differ between groups and diverged after 7 years
Yamaguchi, 2003	83	33	Checked by medical staff during hospitalization (partially determined by fundamental frequency analysis and laryngoscopy)	No difference between stridor and non-stridor group (8.0 vs. 9.0 years, $p = 0.2302$).
Tada, 2007	49	18	N.A.	H-Ratio= 1.21,95% CI= 0.63–2.34, p= 0.57 (H-Ratio= 1.84, 95% CI= 0.55–6.16, $p = 0.32$ for sudden death)
Krim, 2007	86	17	N.A.	Associated with shortened survival in the univariate model (RR=3.36, p= 0.006), not retained in the multivariate model
Lalich, 2014	38	25	Partially with VPSG	Presence of stridor was not associated with adverse prognosis (median survival 24.9 in patients with stridor vs. 61.7 months in patients without stridor, p= 0.6735).
Coon, 2015	685	176	Clinical history	H-Ratio=1.38, 95% CI=1.14–1.68, p=0.001
Giannini, 2016	136	42	VPSG	H-Ratio=1.20 (0.80–1.81), p= 0.386
Starhof, 2016	99	44	N.A.	No difference in median survival between stridor and non-stridor group [7.1 (1.7–12.1) vs. 7.3 (2.8–15) years, p= 0.499]

Legend: H-Ratio= Hazard Ratio; N.A.= Not available; RR= Relative Risk; VPSG= video-polysomnography.

One study on 86 MSA patients, 17 with clinical diagnosis of stridor, found a relative risk to death of 3.36 (p= 0.006) in patients with this symptom in the univariate analysis but this results was not confirmed in the multivariate analysis (Krim et al. 2007).

In one study performed on 38 MSA patients with vocal fold motion impairment, 25 of those presenting stridor, the diagnosis of which was performed by VPSG in a portion of the sample, showed that the presence of stridor was not associated with adverse prognosis (median survival 24.9 months in patients with stridor vs. 61.7 months in patients without stridor, p= 0.6735) (Lalich et al. 2014).

The study conducted on 49 patients with pathologically confirmed MSA showed that the presence of stridor had no significant effect on survival time (H-Ratio= 1.21,95% CI= 0.63–2.34, p= 0.57) and on the risk of sudden death (H-Ratio= 1.84, 95% CI= 0.55–6.16, p= 0.32). In that study, however, the assessment and definition of stridor were not specified in the method section (Tada et al. 2007). Another study on a larger population (n= 685) investigated the role on survival time of numerous variables, including stridor, the diagnosis of which was based on clinical history. This symptom was associated with shortened survival (from symptom onset to death, H-Ratio= 1.38, 95% CI= 1.14–1.68, p= 0.001) but lost its predictive value in the presence of other variables in the multivariate model (Coon et al. 2015).

Another study on 99 patients (n= 44 with stridor), found no differences in median survival between patients with and without stridor [7.1 (1.7–12.1) vs. 7.3 (2.8–15) years, p= 0.499] (Starhof et al. 2016).

Results of our previous study published in 2016 on 136 patients reported that the overall survival did not differ between patients with MSA with stridor (n= 42) and without stridor. In this study the diagnosis of stridor was confirmed with VPSG. This study demonstrated, for the first time, that early stridor onset (≤ 3 years from disease onset) is an independent risk factor for shorter survival (Giannini et al. 2016).

Concerning the characteristics of stridor and survival, only one study analysed stridor classifying it into rhythmic and semirhythmic types with the Multi-Dimensional Voice Program. Of 22 MSA patients with stridor, 15 have subsequently died, Kaplan Meier's survival curve showed that outcome of patients with rhythmic waveform was significantly less favourable than the outcome of patients with semirhythmic waveform (Koo et al. 2016).

Role of stridor treatment on survival

Stridor treatment is based mainly on tracheostomy or continuous positive airway pressure (CPAP) (Cortelli et al. 2019), but guidelines for the use of these treatments in patients with MSA have yet to be established, and few studies with small sample sizes have analysed the role of treatment type as a predictor of survival. Tracheostomy is usually the surgical procedure of choice for stridor and involves the positioning of a fenestrated cannula, maintained closed during the day, in order to allow phonation (Kuhlo et al. 1969). Skin-lined tracheostomy have been proposed for the treatment of severe stridor in MSA because provides a greater opening of the stoma, higher stability over time, less risk of granulation tissue and reversibility, and it does not require a cannula during the night (Campanini et al. 2004).

Our retrospective study on 42 MSA patients with stridor showed that those treated with tracheostomy had longer overall disease duration, longer disease duration after stridor onset, and longer disease duration after treatment compared with those treated with CPAP. Kaplan-Meier curve did not reveal a difference in survival between these treatments even if there was a trend toward longer disease duration in patients who had undergone tracheostomy compared with those treated with CPAP ($p= 0.0850$), with a difference in the incidence rate of death (9 vs. 11 per 100 person-years, respectively) (Giannini et al. 2016). Only another previous descriptive study compared these 2 types of stridor treatment and observed survival in a small sample of MSA patients. In this study, 2 of the 4 patients receiving tracheostomy died 1 year after the sleep evaluation, and the other 2 patients were alive 1.9 and 7 years later, while all 5 patients treated with CPAP died a mean of 2.4 years after the sleep evaluation. Of the patients treated with CPAP, one had poor device compliance and another one presented audible stridor despite device use. In this small study, authors suggested that CPAP had no effect on survival (Silber et al. 2000).

Two studies focused on the role of CPAP in MSA survival. In one study 13 MSA patients with stridor receiving CPAP had similar median survival when compared to 26 MSA patients without stridor (77 vs. 88 months, $p= 0.6914$) (Iranzo et al. 2004). Sudden death was reported in 2 of 13 patients following CPAP initiation (Ghorayeb et al. 2005).

One study, conducted on 49 patients with definite MSA, showed that tracheostomy reduces the risk of death (H-Ratio= 0.21, 95% CI= 0.08–0.56, $p < 0.01$) and of sudden death (H-Ratio= 0.15, 95% CI= 0.02–0.98, $p < 0.05$) in MSA (Tada et al. 2007).

Therefore, to date, findings on stridor treatment remained inconclusive. The Consensus declared that tracheostomy might improve survival in patients with stridor and that CPAP can be useful in the symptomatic control of stridor but it is uncertain if this treatment improves survival (Cortelli et al. 2019).

More recently, one study evaluated the benefit/tolerance of various modes of ventilation. The recommended type of ventilation was decided as a function of the presence and the type of sleep apnoea: patients received fixed CPAP when stridor was isolated, auto-adjusting CPAP when it was combined with obstructive sleep apnoea, and adaptive servo-ventilation when combined with central sleep apnoea. Except for three initial refusals and two yet untreated patients, fixed CPAP (n= 9), auto-adjusting CPAP (n= 8) and adaptive servo-ventilation (n= 2) were well-tolerated (limited leaks and good compliance) and successfully controlled stridor plus sleep apnoea. The ventilation was well tolerated with good compliance (> 4 h of use/night) in ten (83%) patients. The mean compliance with the ventilation was 6.3 ± 1.5 h/night (range: 2 h 53 min - 9 h 07 min). Stridor disappeared under ventilation in 12/12 (100%) of the patients. The median survival times from the disease onset were not different between the 12 patients who were treated for stridor (93 months) and the 28 patients without any stridor at baseline (119 months, p= 0.57). Treated patients had survival times similar to those of patients without any sleep breathing disorder (Rekik et al. 2018).

Role of sleep apnoeas on survival

A recent study assessed the role of sleep apnoea syndrome on survival in 28 MSA patients who underwent polysomnography and were followed up. In this study patients with apnoea-hypopnea index (AHI) ≥ 10/h were considered as having sleep apnoea syndrome (n= 11). In univariate Cox analysis sleep apnoea was associated with mortality but, when adjusting for disease duration and disease scale score, this association was no longer significant (Flabeau et al. 2017). In another study, authors compared survival in patients with sleep breathing disorders but not stridor (obstructive sleep apnoea and central sleep apnoea), showing that there was no difference between patients with treated (n = 19) and untreated (n = 7) sleep breathing disorders, nor between patients with treated sleep breathing disorders (n = 19) and those without any sleep breathing disorders (n = 17) (Rekik et al. 2018).

Summary

In summary, a large corpus of literature documented a higher frequency of sleep and breathing disorders in MSA populations. Some sleep features could represent early markers of neurodegeneration and the analysis of disease progression and prognosis in patients with sleep and breathing disorders could shed light on specific neuropathology and pathophysiology of MSA.

In particular, although RBD represents an early clinical marker of neurodegeneration and may be relevant for addressing possible candidates for future neuroprotective therapies, to date VPSG studies investigating RBD as initial manifestation of disease in wide cohorts of patients with MSA are lacking.

Early stridor onset (within 3 years from the first motor/autonomic MSA symptom) has been recently reported as independent risk factor of survival but its role on disease progression remains to be determined.

Furthermore, few studies and on small samples described the sleep structure, the cardiorespiratory pattern, the breathing and motor control alterations in MSA. To date, the longitudinal evolution of these polysomnographic features during the disease course has not been investigated, although this could provide additional clues on the role of brain-stem cardiorespiratory drive on disease progression, survival and risk of sudden death in MSA.

GOALS OF THE PROJECT

The main goal of the study was to characterize sleep disorders and their longitudinal modifications during the disease course in MSA patients, and to determine their prognostic value.

In order to achieve these goals, we defined the following specific aims:

1. to investigate the prevalence of patients with RBD predating MSA onset;
2. to investigate disease progression and prognosis in patients with RBD predating MSA onset;
3. to investigate disease progression and prognosis in patients with early onset of stridor;
4. to describe the VPSG features and their longitudinal modifications during the disease course and to identify their prognostic value on survival.

METHODS

Study population and methods

This is a retrospective and prospective cohort study including 182 patients with a final diagnosis of MSA, according to international criteria (Gilman et al. 2008).

In this study 56 patients were recruited from prospective studies of the Department of Biomedical and NeuroMotor Sciences and followed up during the disease course (BoProPark Study - RFPS2006-7-336374, CE 09070, and Natural History of Multiple System Atrophy, CE: 17083).

At baseline patients underwent a clinical evaluation and neurological examination.

The following instrumental/laboratory tests were performed at baseline: 1) brain MRI or CT (if MRI was not possible); 2) neuropsychological evaluation (Stanzani-Maserati et al. 2014); 3) VPSG; 4) head-up tilt test (HUTT) and other cardiovascular reflex tests (Baschieri et al. 2015); 5) effect of levodopa (if applicable) assessed by a) a standardized oral levodopa kinetic-dynamic test, b) improvement of part III of the Unified Parkinson's Disease Rating Scale after increasing levodopa up to 1 g/die (Calandra et al. 2016); 6) cardiac 123I-metaiodobenzylguanidin (MIBG)-SPECT; 7) cerebral 123I-ioflupane-SPECT.

Patients were clinically revaluated every 6 months from enrolment to detect occurrence of parkinsonian, cerebellar, autonomic or pyramidal signs/symptoms and milestones of disease progression (O'Sullivan et al. 2008). Diagnosis of possible or probable MSA and predominant phenotype was updated at the last follow-up evaluation, according to international criteria (Gilman et al. 2008).

Patients and/or their relatives were contacted by telephone and questioned regarding the clinical course and the time and the cause of death (if applicable) when the patient missed a clinical evaluation within 12 months.

Further, we retrospectively selected 126 individuals attending the Movement and Autonomic Disorders Clinic of the Department of Biomedical and NeuroMotor Sciences, University of Bologna, between 1991 and October 2017 with a clinical diagnosis of MSA. Only patients evaluated at least once a year during the disease course were included. Three neurologists expert in movement disorders (P.C., G.C.B., P.G.) independently confirmed the diagnosis of MSA from data available at the last follow-up evaluation according to international criteria (Gilman et al. 2008). Their consensus and the absence of non-supporting features for MSA were mandatory for inclusion in the study.

Patients were categorized as probable or possible MSA according to the consensus criteria and classified as MSA-P or MSA-C on the basis of the predominant motor involvement at the time of the last follow-up visit (Gilman et al. 2008).

According to clinical practice, information on family and patient history, new symptom onset, treatment changes, response and adverse effects, and annual neurological evaluations including blood pressure measurement in clinostatic position and within three minutes of standing had been collected at each follow-up visit. The following instrumental/laboratory tests, performed during the disease course if required for differential diagnosis or to elucidate specific conditions suspected by history or examination, were analysed when available: 1) brain CT or MRI 2) neuropsychological evaluation (Stanzani-Maserati et al. 2014); 3) HUTT and other cardiovascular reflex tests (Baschieri et al. 2015); 4) effect of levodopa assessed by a) a standardized oral levodopa kinetic-dynamic test, b) improvement of part III of the Unified Parkinson's Disease Rating Scale after increasing levodopa up to 1 g/die (Calandra et al. 2016); 5) cardiac 123I-metaiodobenzylguanidin (MIBG)-SPECT; 6) cerebral 123I-ioflupane-SPECT.

Clinical data collection

The following clinical data were collected in patients prospectively recruited and analysed from medical records in patients included in the retrospective cohort:

1) age at disease onset defined as the age in years at the time of the first reported motor or autonomic symptom or sign that could be related to MSA (Gilman et al. 2008);

2) age and cause of death;

3) disease duration defined as the interval in years from first symptom onset to death or to the time of the analysis;

4) symptoms at initial presentation;

5) occurrence of parkinsonian, cerebellar, autonomic or pyramidal signs/symptoms;

6) sleep disturbances, collected from patients and checked with close relatives interview (when available), including subjective complaints, sleep quality, occurrence of snoring and others noises during sleep consistent with apnoea and/or stridor, presence of motor and behaviours during sleep consistent with Restless Legs Syndrome and/or RBD;

7) occurrence of cognitive impairment;

8) occurrence of the following milestones of disease progression: frequent falls (at least 3 falls per year or documentation of frequent or several falls), wheelchair dependence, severe dysphagia or percutaneous endoscopic gastrostomy (PEG), unintelligible speech, urinary catheterization (O'Sullivan et al. 2008).

For each symptom, sign and milestone of disease progression, timing and latency of occurrence from disease onset were collected.

Symptoms and signs were categorized as early if presenting within 3 years of disease onset.

RBD and stridor were defined when VPSG-confirmed. RBD was defined according to the International Classification of Sleep Disorders ICSD-III (American Academy of Sleep Medicine 2014). RBD onset was defined as the onset of the symptoms suggestive of RBD reported by the bed partner. In case of event registration during VPSG, the video was shown to the bed partner to ensure that the behaviour was the same reported in anamnesis.

Time of stridor treatment (CPAP or tracheostomy) and latency of occurrence from stridor onset were collected. For patients treated with CPAP tolerability of device and hours per day of utilization were gathered in a standardized fashion.

Survival data were defined on the basis of time to death from the first symptom of disease.

Sleep recording and analysis

VPSG was performed including: electroencephalography (F3-A2; C3-A2; CZ-A2; O1-A2); two electrooculography electrodes; surface electromyography of submental and intercostal muscles, right and left anterior tibialis, right and left extensor communis carpi; airflow measured by thermocouple transducers, thoracic and abdominal effort; electrocardiography and peripheral hemoglobin saturation measured by a sensor placed on a finger. EEG-synchronized continuous audio and video recordings were performed by means of infrared cameras.

VPSG recordings were analysed from 2 sleep technicians (A.C. and F.M.), two neurologists expert in sleep disorders (G.C.B. and F.P.) and one neurologist trained in sleep disorders (G.G.).

The following features were collected and analysed:

- Total bed time, total recording time, total sleep period, sleep efficiency, latency to sleep and to REM sleep onset, total sleep time (TST); percentages of NREM 1, NREM 2, NREM 3 and REM sleep, and Wake After Sleep Onset (WASO) were scored according to the criteria of the American Academy of Sleep Medicine (American Academy of Sleep Medicine 2014)
- The arousal index was the number of arousals per hour of sleep (Atlas Task Force of the American Sleep Disorders Association 1992; American Academy of Sleep Medicine 2014).
- The sleep fragmentation index (SFI) was calculated as the total number of awakenings and sleep stage shifts divided by total sleep time (Haba-Rubio et al. 2004)
- Apnoea was defined as a complete cessation of airflow for more than 10 seconds measured by the thermistor. Hypopnea was defined as at least 30% reduction in nasal pressure signal excursions from baseline and associated with at least 3% desaturation from pre-event baseline or with an arousal. The apnoea–hypopnea index (AHI) was the number of apnoeas plus hypopneas per hour of sleep. Obstructive sleep apnoea syndrome was defined according to the International Classification of Sleep Disorders ICSD-III (American Academy of Sleep Medicine 2014).
- Sleep time with snoring was defined as the number of epochs with at least 50% of snoring and the percentage of time of snoring (sleep time with snoring/total sleep time) was then calculated.
- Presence of stridor was defined according to the Consensus criteria on stridor in MSA (Cortelli et al. 2019).

- PLMs index was calculated according to the International Classification of Sleep Disorders ICSD-III (American Academy of Sleep Medicine. 2014).
- For the measurement of EMG activity in REM sleep, trained technicians and neurologists visually quantified “any” (tonic, phasic, or a combination of both) EMG activity in the mentalis muscle and phasic EMG activity in muscles in 3-second mini epochs (American Academy of Sleep Medicine 2014, n. 54). The percentage of RSWA was the ratio between the number of epochs without atonia and the total number of epochs. We considered a cut-off of RSWA greater than 27% of REM sleep time, following the SINBAR method (Frauscher et al. 2012).
- Mean breathing rate (BR) and heart rate (HR) were calculated during the dark period (from light OFF to light ON). The mean value of each variable was calculated in each epoch of quiet wakefulness and sleep (NREM 1, NREM 2, NREM 3 and REM) stage. The difference between sleep stages and wakefulness values (ΔBR and ΔHR) was also calculated and expressed as % decline of sleep values over wakefulness values (Grimaldi et al. 2012).

Standard protocol approvals, registrations, and patient consents

The study was conducted in agreement with the principles of good clinical practice. The study protocol was approved by the local ethics committee of the local health service of Bologna, Italy (Cod. CE: 09070 and 17093). All patients gave written informed consent for study participation.

Statistical analysis

All clinical and polysomnographic variables were collected using an ad hoc anonymized and standardized form and entered into an ad-hoc database for statistical analysis.

Normality of continuous parameters distribution was checked using the Skewness-Kurtosis test, variables were expressed as mean ± standard deviation (SD) or median along with interquartile ranges (IQR) when appropriate. Continuous variables were compared by using t-test or Wilcoxon rank-sum, as appropriate. Categorical variables were described by their absolute and/or relative frequencies and compared using Chi square test.

Repeated measures ANOVA was performed to investigate significant main effects for all patients across time. Kruskall-Wallis test with Bonferroni post-hoc was utilized for multiple comparisons across time when appropriate.

Kaplan-Meier curves were used to analyse survival probability from disease onset, and the log-rank test was performed to compare survival between patient subgroups. To identify variables associated with survival in MSA and in the stridor subgroup, univariate and multivariable Cox regression analyses were performed. Parameters with a value of $p < 0.1$ on univariate analysis were entered into the multivariable model. The following variables were studied: age at disease onset, sex, predominant clinical phenotype, presence of stridor, and symptoms of disease onset. The same variables and the latency of stridor onset were considered in the stridor subgroup.

A p-value lower than 0.05 (2-sided) was considered significant. Statistical analyses were performed using the statistical software STATA®, version 14.0.

RESULTS

A total of 182 (107 males) patients with MSA were included in the study: 3 patients met the consensus criteria for definite MSA, 139 for probable MSA, and 40 for possible MSA. Ninety-two patients were classified as having MSA-C and the other 90 as having MSA-P. Mean age at disease onset was 57.3 ± 8.3 years, mean disease duration was 7.8 ± 3.9 years. At the time of the study 141 (77.5%) were deceased.

Demographic and clinical features of the study sample are shown in Table 4.

According to current consensus criteria (Gilman et al. 2008), at disease onset 41% of patients complained pure motor symptoms (21% cerebellar, 18% parkinsonism and 2% cerebellar symptoms and parkinsonism), while 32% of patients reported isolated autonomic symptoms (27% urinary involvement, 3% symptomatic OH and 2% both). In 24% of the total sample, both motor and autonomic symptoms were present at disease onset (10% with parkinsonism, 11% with cerebellar symptoms and 3% with cerebellar symptoms and parkinsonism). In 6 patients (4%) the first symptom of disease was undefined (Figure 3A).

Considering sleep as mode of onset, 30% (n= 55) of patients presented isolated RBD and 14% (n= 25) presented RBD associated with motor/autonomic symptoms (5% with autonomic, 3% with cerebellar and 6% with autonomic, cerebellar and parkinsonian symptoms) (Figure 3B).

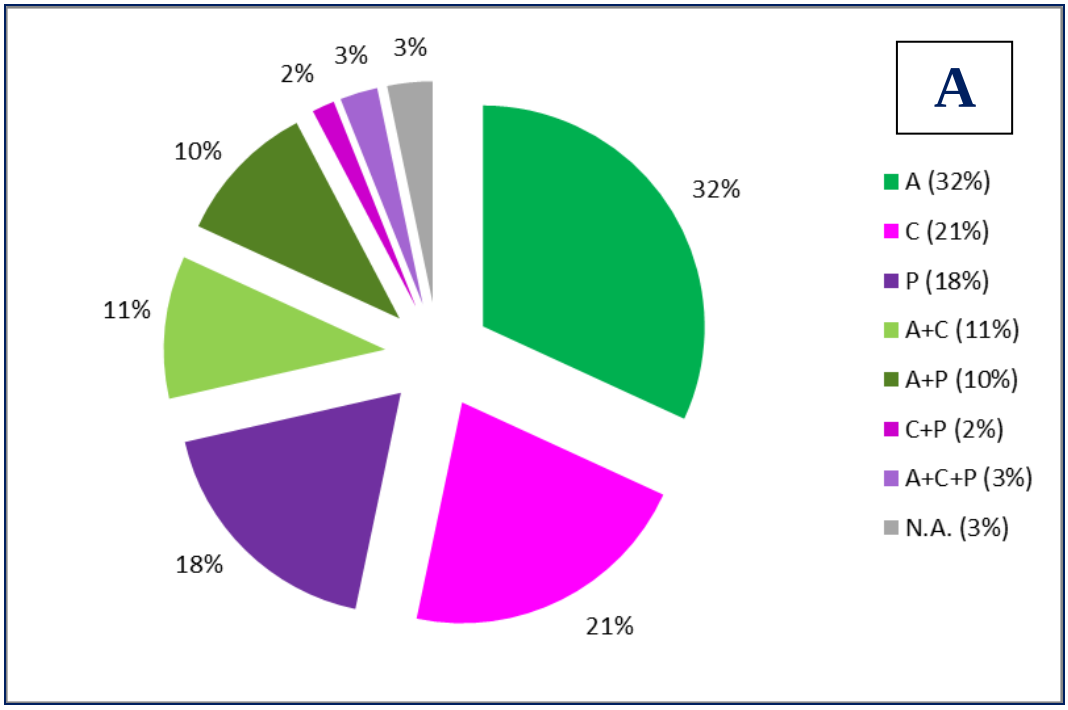
Table 4: Demographic and clinical characteristics of the study sample

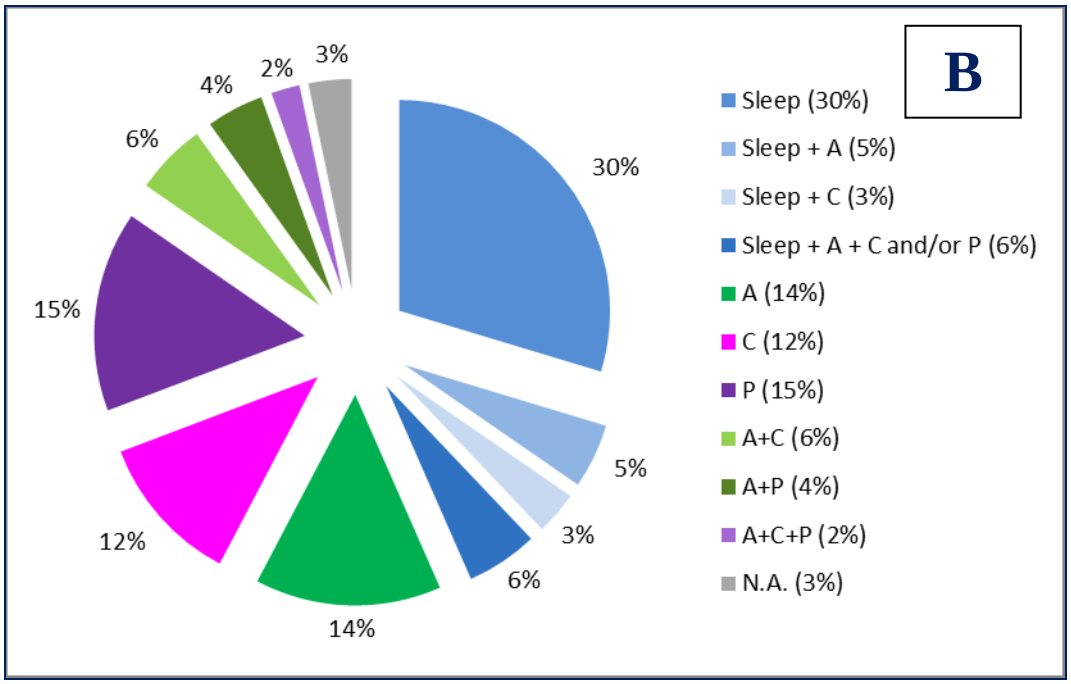
		MSA MOTOR SUBTYPE		
	Total MSA sample	**MSA-C**	**MSA-P**	**p-value[1]**
	182	92	90	
Sex				
Male, *n (%)*	107 (58.8)	56 (60.9)	51 (56.7)	0.565
Female, *n (%)*	75 (41.2)	36 (39.1)	39 (43.3)	
Age at onset, *y*	57.29 ± 8.35	56.9 ± 7.8	57.7 ± 8.9	0.5440
Disease duration, *y*	7.79 ± 3.86	7.6 ± 3.9	8.0 ± 3.8	0.4841
Died				
Yes, *n (%)*	141 (77.5)	71 (77.2)	70 (77.8)	0.922
No, *n (%)*	41 (22.5)	21 (22.8)	20 (22.2)	
Long survival[2], *n (%)*	11 (6.0)	5 (5.4)	6 (6.7)	0.727
Symptom of disease onset				
Autonomic, *n (%)*	101 (57.4)	51 (55.4)	50 (59.5)	0.584
Cerebellar, *n (%)*	66 (37.5)	62 (67.4)	4 (4.8)	< **0.001**
Parkinsonism, *n (%)*	60 (34.1)	7 (7.6)	53 (63.1)	< **0.001**
Milestones of disease progression				
Frequent[3] falls, *n (%)*	105 (57.7)	50 (54.4)	55 (61.1)	0.098
Wheelchair dependence, *n (%)*	107 (58.8)	58 (63.0)	49 (54.4)	0.601
Urinary catheterization, *n (%)*	72 (39.6)	37 (40.2)	35 (38.9)	0.224
Unintelligible speech, *n (%)*	53 (29.1)	29 (31.5)	24 (26.7)	0.759
Severe dysphagia/PEG, *n (%)*	41 (22.5)	20 (21.7)	21 (23.3)	0.567
Stridor, *n (%)*	75 (41.2)	43 (46.7)	32 (35.6)	0.311
Stridor at onset, *n (%)*	10 (5.5)	8 (8.7)	2 (2.2)	0.055
Latency of stridor onset, *y*	3 (1-5)	3 (1-5)	4 (3-5.5)	0.0622
Disease duration after stridor onset, *y*	3.9 ± 2.8	4.6 ± 2.8	3.2 ± 2.7	**0.0482**
VPSG-confirmed RBD, *n (%)*	138 (75.8)	73 (79.4)	65 (72.2)	0.101
Latency of RBD onset, *y*	0 [(-2) – 2]	0 [(-2) – 2]	0 [(-2) – 3]	0.2126

Data are expressed as mean ± standard deviation or median (interquartile range)
Statistically significant p-values are denoted in bold.

Legend: [1]= between C and P; [2]= disease duration ≥ 15 years; [3]= frequent was defined at least 3 falls per year or documentation of frequent or several falls; *n*= sample size; MSA= Multiple System Atrophy; MSA-C= multiple system atrophy with predominant cerebellar phenotype; MSA-P= multiple system atrophy with predominant parkinsonism phenotype; PEG= percutaneous endoscopic gastrostomy; RBD= REM sleep behaviour disorder; VPSG= video-polysomnography; *y*= years.

Figure 3: Mode of disease onset in MSA cohort, according to international criteria (A) and considering sleep (B)





Legend: A=Autonomic onset; C= cerebellar symptoms; N.A.= Not available, P= parkinsonism.

RBD predating and following disease onset

On the total sample, 154 patients underwent VPSG for stridor and/or RBD suspicion at history taking. RBD diagnosis was confirmed in 138 patients (75.8% of the total sample).

Among the latter, RBD predating (RBD-pre) disease onset occurred in 52 patients and RBD following (RBD-post) disease onset in 76 patients (for 10 patients the year of RBD onset was undefined).

The mean age at RBD-pre onset was 55.1 ± 7.9 years and the median duration before the first autonomic or motor symptom/sign of MSA was 3 (1–5) years. Among patients with RBD-pre, 22 presented with isolated autonomic failure (25 urinary symptoms, 6 symptomatic OH), 11 with autonomic failure associated with motor symptoms (6 with cerebellar syndrome, and 5 with parkinsonism), 13 with cerebellar syndrome, 5 with parkinsonism and 1 with combined cerebellar and parkinsonian symptoms.

Features of the MSA population with RBD-pre and RBD-post are compared in Table 5.

Mean age at disease onset was lower in RBD-pre group (55.1 ± 7.9 vs. 58.3 ± 8.7, p= 0.0371). Compared to the RBD-post subgroup, patients with RBD-pre showed a more frequent autonomic onset of MSA (63.5% vs 50%, p= 0.132) and less frequent parkinsonism (21.2% vs. 40.8%, p=0.020). At the last follow-up RBD-pre group more frequently showed a cerebellar phenotype (61.5% vs 48.7%), but this difference did not reach statistical significance.

During the course of disease, RBD-pre revealed more frequently pyramidal signs (90.4% vs. 75.0%, p= 0.029) and with a shorter latency of onset [0 (0-1) vs. 1 (0-3), p= 0.042). Concerning autonomic features, no significant differences were revealed about the occurrence of urinary symptoms and symptomatic OH but RBD-pre patients presented earlier onset of urinary urgency/frequency [0 (0-1) vs. 1 (0-3), p= 0.0421], urinary incontinence [3 (1-6) vs. 4 (2-8), p= 0.028], and symptomatic OH [1 (0-3) vs. 3 (1-6), p= 0.0030]. Moreover, RBD-pre group showed an earlier stridor onset [2 (2 – 4) vs. 4 (3 – 6), p= 0.017].

Concerning milestones of disease progression, RBD-pre showed a shorter latency of urinary catheterization [4 (3-6) vs. 6 (5-9), p= 0.024], severe dysphagia/PEG [6 (4-7) vs. 8 (5-10), p= 0.0384] and wheelchair dependency [5 (4-6) vs. 6 (4-8.5), p= 0.0179] when compared to the RBD-post group. Earlier unintelligible speech was revealed in RBD-pre patients without reaching a statistical significance [5.5 (4-7) vs. 7 (5-9), p= 0.074].

Table 5: Clinical features and latency of signs/symptoms onset in patients with RBD-pre and RBD-post

	RBD-PRE	**RBD-post**	**p-value**
	52	76	
Males, *n (%)*	29 (55.8)	42 (55.3)	0.955
Age at MSA onset, *y*	55.1 ± 7.9	58.3 ± 8. 7	**0.0371**
Died, *n (%)*	36 (69.2)	52 (68.4)	0.923
Disease duration, *y*	7.2 ± 2.9	8.4 ± 4.5	0.1054
Long survival [1], *n (%)*	1 (1.9)	7 (9.2)	**0.0044**
MSA subtype			
MSA-P, *n (%)*	20 (38.5)	39 (51.3)	0.152
MSA-C, *n (%)*	32 (61.5)	37 (48.7)	
Symptoms at MSA onset			
Parkinsonism, *n (%)*	11 (21.2)	31 (40.8)	0.020
Cerebellar, *n (%)*	20 (38.5)	28 (36.8)	0. 853
Autonomic, *n (%)*	33 (63.5)	38 (50.0)	0.132
Symptoms during the course of disease			
Parkinsonism, *n (%)*	41 (78.8)	68 (89.5)	0.097
Latency of parkinsonism, *y*	3 (0-4)	1 (0-4)	0.1007
Cerebellar, *n (%)*	44 (84.6)	69 (90.8)	0.286
Latency of cerebellar symptoms, *y*	1 (0-3)	1.5 (0-4)	0.4185
Pyramidal signs, *n (%)*	47 (90.4)	57 (75.0)	**0.029**
Latency of pyramidal signs, *y*	3 (2-5)	4 (3-6)	**0.0374**
Urinary urgency/frequency, *n (%)*	47 (90.4)	66 (86.8)	0.541
Early urgency/frequency onset, *n (%)*	49 (94.2)	61 (80.3)	**0.0026**
Latency of urinary symptoms, *y*	0 (0-1)	1 (0-3)	**0.0421**
Urinary retention, *n (%)*	29 (58.0)	47 (62.7)	0.601
Early urinary retention onset, *n (%)*	22 (73.3)	27 (58.7)	0.192
Latency of urinary retention, *y*	2 (1-4)	3 (0-5)	0.5360

Urinary incontinence, *n (%)*	32 (62.8)	49 (64.5)	0.843
Early urinary incontinence, *n (%)*	37 (71.2)	40 (52.6)	**0.036**
Latency of urinary incontinence, *y*	3 (1-6)	4 (2-8)	**0.028**
Symptomatic OH, *n (%)*	34 (66.7)	56 (74.7)	0.329
Early Symptomatic OH onset, *n (%)*	23 (65.7)	16 (40.0)	0.090
Latency of symptomatic OH, *y*	1 (0-3)	3 (1-6)	**0.0030**
Stridor, *n (%)*	30 (57.7)	34 (46.6)	0.220
Early stridor onset, *n (%)*	17 (32.7)	13 (17.1)	**0.041**
Latency of stridor, *y*	2 (2 – 4)	4 (3 – 6)	**0.017**
Milestone of disease progression			
Frequent[2] falls, *n (%)*	32 (61.5)	51 (67.1)	0.541
Latency of frequent falls, *y*	4 (2.5-6)	4 (2-6)	0.9586
Urinary catheterization, *n (%)*	24 (46.2)	34 (44.7)	0.958
Latency of urinary catheterization, *y*	4 (3-6)	6 (5-9)	**0.024**
Unintelligible speech, *n (%)*	14 (26.9)	29 (38.2)	0.175
Latency of unintelligible speech, *y*	5.5 (4-7)	7 (5-9)	0.074
Dysphagia/PEG, *n (%)*	11 (21.2)	24 (31.6)	0.184
Latency of dysphagia/PEG, *y*	6 (4-7)	8 (5-10)	**0.0384**
Wheelchair dependency, *n (%)*	30 (57.7)	51 (67.1)	0.204
Latency of wheelchair dependency, *y*	5 (4-6)	6 (4-8.5)	**0.0179**

Data are expressed as n (%), mean ± SD, or median (interquartile range).
Statistically significant p-values are denoted in bold (p value≤0.05).

Legend: [1]= disease duration ≥ 15 years; [2]= frequent was defined at least 3 falls per year or documentation of frequent or several falls; MSA-C= multiple system atrophy with predominant cerebellar phenotype; MSA-P= multiple system atrophy with predominant parkinsonism phenotype; OH= orthostatic hypotension; PEG= percutaneous endoscopic gastrostomy; RBD= REM sleep behaviour disorder; y= years.

Early and late stridor onset

On the total sample, 75 patients were diagnosed with stridor: 35 showed an early stridor onset (≤ 3 years from disease onset) and 40 a late stridor onset (> 3 years from disease onset).

Features of the MSA population with early and late stridor onset are compared in Table 6.

Compared to patients with late stridor onset, patients with early stridor onset showed a higher age at disease onset (58.7 ± 8.1 vs. 53.5 ± 8.0, p= 0.0069) and more frequently presented with autonomic onset (82.9% vs 50.0%, p= 0.004).

During the disease course, patients with early stridor onset showed an earlier onset of pyramidal signs [3 (3-4) vs. 5 (3-6), p= 0.0292], urinary urgency/frequency [0 (0-1) vs. 2 (0-4), p= 0.010], urinary incontinence [2.5 (1-4) vs. 5 (3-8), p= 0.0037]. A shorter latency of symptomatic OH [2 (1-4) vs. 3 (0-5), p= 0.1153] and urinary retention [1 (0-3) vs. 3 (0-5), p= 0.1311] was reported without reaching statistical significance. Moreover, the early stridor group more frequently presented early RBD (85.71% vs. 65.00%, p= 0.005).

Concerning milestones of disease progression, early stridor onset group showed a shorter latency of urinary catheterization [4 (1-4) vs. 6 (4-8), p= 0.0004], unintelligible speech [4.5 (4-5.5) vs. 7 (5-8), p= 0.0212], severe dysphagia/PEG [4 (4-5) vs. 7 (5-9), p= 0.0335] and wheelchair dependency [4 (4-5.5) vs. 7 (5-8), p= 0.0019], when compared to the late stridor onset group.

Table 6: Clinical features and latency of signs/symptoms onset of MSA patients with stridor

		MSA patients with Stridor		
	Total sample with stridor	**Sample with early onset (≤ 3 years)**	**Sample with late onset (> 3 years)**	**p-value**
	75	35	40	
Males, *n (%)*	39 (52.0)	20 (57.1)	19 (47.5)	0.404
Age at MSA onset, *y*	55.9 ± 8.4	58.69 ± 8.1	53.50 ± 8.0	**0.0069**
Died, *n (%)*	55 (73.3)	27 (77.1)	28 (70.0)	0.485
Disease duration, *y*	8.0 ± 3.9	6.2 ± 2.3	9.6 ± 4.2	**0.0001**
Long survival [1], *n (%)*	6 (8.0)	0 (0.0)	6 (15.0)	**0.0017**
MSA subtype				
MSA-P, *n (%)*	32 (42.7)	13 (37.1)	19 (47.5)	0.366
MSA-C, *n (%)*	43 (57.3)	22 (62.9)	21 (52.5)	
Symptoms at MSA onset				
Parkinsonism, *n (%)*	17 (22.7)	6 (17.1)	11 (27.5)	0.259
Cerebellar, *n (%)*	23 (30.7)	10 (28.6)	13 (32.5)	0. 659
Autonomic, *n (%)*	49 (65.3)	29 (82.9)	20 (50.0)	**0.004**
Symptoms during the course of disease				
Parkinsonism, *n (%)*	66 (88.0)	30 (85.7)	36 (90.0)	0.569
Latency of parkinsonism, *y*	3 (0-4)	2.5 (1-3)	3 (0-5)	0.4871
Cerebellar, *n (%)*	66 (88.0)	30 (85.7)	36 (90.0)	0.569
Latency of cerebellar symptoms, *y*	2 (0-4)	2 (0-3)	2 (0-4)	0.4796
Pyramidal signs, *n (%)*	61 (81.3)	29 (82.9)	32 (80.0)	0.928
Latency of pyramidal signs, *y*	4 (3-6)	3 (3-4)	5 (3-6)	**0.0292**
Urinary urgency/frequency, *n (%)*	66 (88.0)	30 (85.7)	36 (90.0)	0.362
Early urgency/frequency onset, *n (%)*	54 (72.0)	30 (85.7)	24 (60.0)	**<0.0001**
Latency of urinary symptoms, *y*	0.5 (0-3)	0 (0-1)	2 (0-4)	**0.010**
Urinary retention, *n (%)*	42 (56.0)	18 (51.4)	24 (60.0)	0.379
Early urinary retention onset, *n (%)*	28 (37.3)	14 (40.0)	14 (35.0)	0.105
Latency of urinary retention, *y*	2 (0-4.5)	1 (0-3)	3 (0-5)	0.1311

Urinary incontinence, *n (%)*	49 (65.3)	22 (62.9)	27 (67.5)	0.800
Early urinary incontinence, *n (%)*	20 (26.7)	12 (34.3)	8 (20.0)	0.096
Latency of urinary incontinence, *y*	4 (1.5-6)	2.5 (1-4)	5 (3-8)	**0.0037**
Symptomatic OH, *n (%)*	61 (81.3)	31 (88.6)	30 (75.0)	0. 101
Early Symptomatic OH onset, *n (%)*	39 (52.0)	23 (65.7)	16 (40.0)	0.090
Latency of symptomatic OH, *y*	3 (1-4)	2 (1-4)	3 (0-5)	0.1153
VPSG-confirmed RBD, *n (%)*	72	34 (97.1)	38 (95.0)	0.294
Early VPSG-confirmed RBD onset, *n (%)*	56 (74.7)	30 (85.7)	26 (65.0)	**0.005**
Latency of RBD, *y*	0 [(-2) -2]	-0.5 [(-3) -1]	-0.5 [(-3) - 3]	0.3681
Milestone of disease progression				
Frequent[2] falls, *n (%)*	48 (64.0)	19 (54.3)	29 (72.5)	0.312
Latency of frequent falls, *y*	4 (2-6)	3 (2-5)	5 (3-6)	0.1077
Urinary catheterization, *n (%)*	36 (48.0)	14 (40.0)	22 (55.0)	0.288
Latency of urinary catheterization, *y*	5 (4-6)	4 (1-4)	6 (4-8)	**0.0004**
Unintelligible speech, *n (%)*	25 (33.3)	8 (22.9)	17 (42.5)	0.178
Latency of unintelligible speech, *y*	6 (4-8)	4.5 (4-5.5)	7 (5-8)	**0.0212**
Dysphagia/PEG, *n (%)*	23 (30.7)	5 (14.3)	18 (45.0)	**0.005**
Latency of dysphagia/PEG, *y*	7 (5-9)	4 (4-5)	7 (5-9)	**0.0335**
Wheelchair dependency, *n (%)*	51 (68.0)	22 (62.9)	29 (72.5)	0.474
Latency of wheelchair dependency, *y*	6 (4-8)	4 (4-5.5)	7 (5-8)	**0.0019**

Data are expressed as n (%), mean ± SD, or median (interquartile range).
Statistically significant p-values are denoted in bold (p value≤0.05).

Legend: [1]= disease duration ≥ 15 years; [2]= frequent was defined at least 3 falls per year or documentation of frequent or several falls; MSA= multiple system atrophy; MSA-C= multiple system atrophy with predominant cerebellar phenotype; MSA-P= multiple system atrophy with predominant parkinsonism phenotype; OH= orthostatic hypotension; PEG= percutaneous endoscopic gastrostomy; RBD= REM sleep behaviour disorder; VPSG= video-polysomnography; y= years.

VPSG features

At the time of analysis, 81 VPSG were systematically analysed in a standardized fashion, on 33 MSA patients. For 16 patients four sequential VPSG were available during the follow-up and were therefore analysed. Sleep architecture resulted in the following percentages: NREM 1 12.2 % (range 1.14-55.8%, n.v. 6-8.7%), NREM 2 42.2% (range 21.4-79.5%, n.v. 49.6–55.8%), NREM 3 23.3% (range 0-51.2%, n.v. 15-21.2%), REM sleep 15.5% (range 0-43.6%, n.v. 17.8-20.8%). Sleep efficiency was reduced and amounted to 63% (range 17-92%, n.v. 83.7-90%) (Boulos et al. 2019).

Patients who underwent VPSG in the first 3 years of disease onset showed a NREM 1 percentage of 14.5 ± 8.1, NREM 2 of 44.0 ± 15.9, NREM 3 of 24.6 ± 13.8 and REM of 16.8 ± 9.9. The sleep latency was 14.9 ± 8.9 minutes, the REM latency of 123.3 ± 86.9 minutes. The WASO was 146.9 ± 58.7 minutes. Overall the sleep efficiency was 60.4 ± 16.6. The SFI within the first 3 years was 24.8 ± 6.7 and the arousal index was 22.9 ± 11.1.

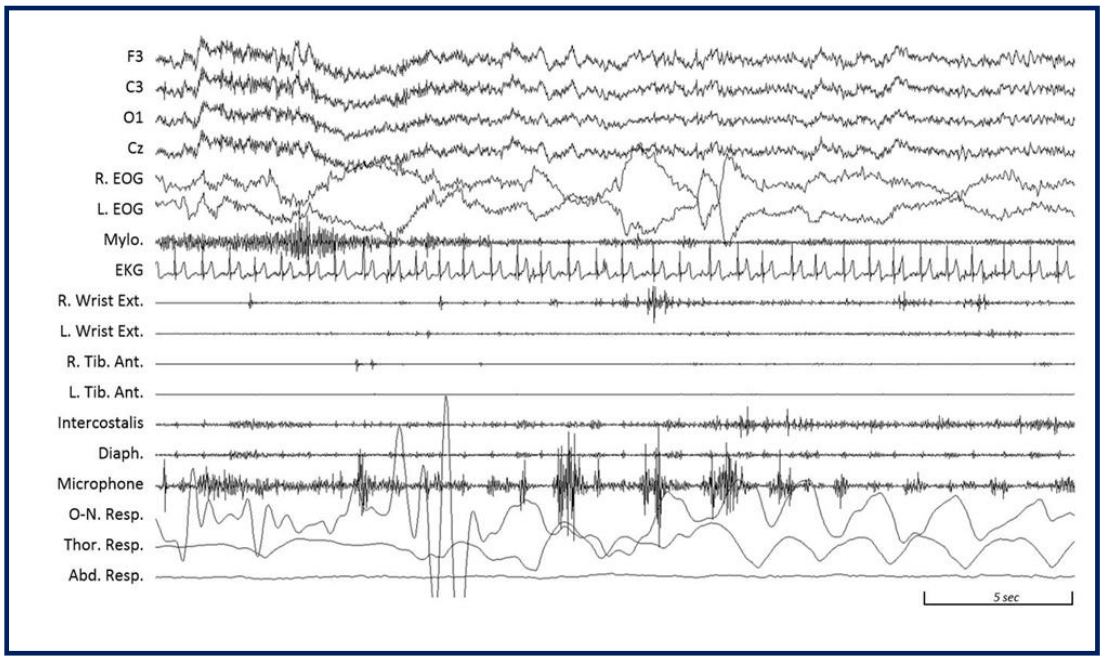
No differences in sleep structure were found despite an increased percentage of NREM 1 and a reduced percentage of REM during the disease course were found. A disrupted sleep pattern occurs in an intermediate-late stage of disease: K-complexes and spindles, hallmark figures of NREM 2, were no longer identifiable, intrusion of REM into NREM 2 was observed and occurrence of spindles during REM sleep was frequently reported. Moreover, a rapid transition among sleep stages was found with an increased SFI during the disease course (from 25.3 ± 8.3 in the first VPSG to 30.2 ± 2.2 in the last one).

Two patients performed the last VPSG in a late stage of disease (disease duration= 11 and 14 years), 1 and 4 months before death, showing sleep stages no longer identifiable with abnormal NREM 1 mixed with RSWA, and slow eye movements intermixed with rapid eye movements, in a sort of undifferentiated sleep state (Figure 4).

Concerning motor patterns in the whole MSA sample, in the first years from disease onset patients presented violent and intense RBD, with a vivid content characterized by threats or attacks by unfamiliar people/animals, and motor features characterized by impressive, abnormal and well-defined movements which are parts of dream-enactment behaviours (violent assaults, fight against an attacker to protect oneself, etc.). During these episodes we observed limb punches and kicks, patients getting out of bed, grabbing and throwing a pillow, shouting/speaking/crying/yelling with intelligible words. During the disease course, in an intermediate-late phase of disease, less intense and violent RBD episodes were registered during which patients talked and moved the mouth,

smiled, gesticulated (raising or moving the arm) showing a minor injury risk. However, in the late stage of disease RBD episodes became more frequent and almost continuous during the REM stage.

Figure 4: Video-polysomnographic recording showing disrupted sleep architecture in the late stage of disease



Legend: Abd. Resp.=abdominal respirogram; EKG= electrocardiogram; EOG= electro-oculogram; Diaph= diaphragm; L= left; Mylo= Mylohyoideus; O-N Resp.= oro-nasal respirogram; R= right; SaO2= oxygen saturation; Thor. Resp.= thoracic respirogram; Tib. Ant.= tibialis anterior; Wrist Ext.= wrist extensor.

Percentages of phasic, tonic and phasic/tonic REM progressively increased (phasic= from 26.8 ± 11.7 to 32.9 ± 13.7, 37.1 ± 11.7 and 43.6 ± 25.9; tonic= from 34.7 ± 15.8, to 44.3 ±23.5, 50.5 ± 24.1 and 62.8 ± 2.3; phasic/tonic= from 50.8 ± 24.3, to 61.2 ± 25.9, 62.1 ± 20.2 and 75.2 ± 12.0) but without reaching statistical significance (Table 7).

Concerning sleep-related breathing disorders we observed that patients developing stridor showed sporadic high-pitched inspiratory sound, mainly in NREM 3 and less frequently in NREM 2 in their previous VPSG. In some cases, although no sound was recorded, overactivation of intercostalis muscles during expiration was observed. Stridor became more frequent and continuous during the disease course, involving also NREM 1 and REM sleep before treatment (CPAP or tracheotomy). In one case stridor was also recorded during wakefulness.

Median AHI progressively increased during the disease course [from 2.6 (1.2-5.7) to 3.4 (0.2-12.5), 7.8 (5.2-9.5) and 12.9 (10.9-15.2)] together with the central apnoea index [from 0.6 (0-1.54) to 0.23 (0-7.15), 2.6 (0-7.4) and 3.7 (0-7.4)] (Table 7).

Concerning autonomic features during sleep, BR progressively increase during the disease course in all sleep stages without reaching a statistical significance. However, statistical significance was found when the first and the last VPSG were compared. In particular the following BR were found: in NREM 1 from 17.8 ± 2.8 to 23.5 ± 12.0, in NREM 2 from 17.7 ± 2.6 to 23.0 ± 9.9, in NREM 3 from 18.0 ± 2.7 to 23.0 ± 9.9, in REM sleep from 18.5 ± 2.7 to 24.0 ± 9.9 (Table 7). Considering ΔBR between sleep stages and wakefulness no differences were found. However, along with the disease duration, ΔBR, which was negative at the first VPSG became positive at the last VPSG, showing therefore an increase in BR from wakefulness to sleep stages. The following percentages of decline of sleep stage values over wakefulness values were observed: in NREM 1 -5.8 % in the first VPSG and +4.7% in the last VPSG, in NREM 2 -8.8% in the first VPSG and +5.6% in the last VPSG, in NREM3 -11.7% in the first VPSG and +5.6% in the last VPSG, in REM +2.2% in the first VPSG and + 7.3% in the last VPSG.

Similarly, the HR (heart rate) progressively increased during the disease course in NREM 1, NREM 2, NREM 3 and REM without reaching overall significance. However, comparing the last VPSG with the first one a difference was found (in NREM 1 from 67.9 ± 8.0 to 86.0 ± 11.3, in NREM 2 from 66.8 ± 7.8 to 85.5 ± 10.6, in NREM 3 from 67.3 ± 7.9 to 85.0 ± 9.9, in REM from 68.8 ± 7.7 to 85.5 ± 10.6) (Table 7). Moreover, in our MSA sample absence of the physiological reduction in HR from wakefulness to sleep stages was observed. Comparing ΔHR between sleep stages and wakefulness no differences were found. In the first VPSG a mild ΔHR decrease was observed during sleep, especially in NREM 3 (-4.2 % in NREM 1, -4.3% in NREM 2, -8.6% in NREM 3 and -2.8% in REM). The longitudinal monitoring documented a progressive lack of this mild ΔHR decrease in NREM sleep (-2.5% in NREM 1, -3.0% in NREM 2, -1.1% in NREM 3) and an increased ΔHR in REM sleep (+ 4.6%).

Table 7: VPSG features

	VPSG 1	VPSG 2	VPSG 3	VPSG 4	p value
	n= 33	n= 16	n= 16	n= 16	
Sleep latency, *min*	16.7 ± 9.9	20.2 ± 11.8	23.5 ± 11.9	20.2 ± 9.1	0.5128
NREM 1, *%*	13.1 ± 8.9	16.8 ± 9.5	11.9 ± 6.9	17.5 ± 8.7	0.6368
NREM 2, *%*	43.4 ± 14.9	49.0 ± 17.9	39.9 ± 16.6	48.1 ± 8.6	0.2544
NREM 3, *%*	23.4 ± 12.2	20.7 ± 11.4	29.2 ± 13.1	22.8 ± 3.7	0.5849
REM, *%*	19.9 ± 10.7	12.7 ± 9.6	18.8 ± 13.0	11.6 ± 2.2*	0.0893
WASO, *min*	135.6 ± 67.8	139.6 ± 92.7	168.7 ± 92.8	122.3 ± 48.7	0.7822
TST, *min*	275.5 ± 76.2	268.9 ± 76.6	258.3 ± 50.4	269.3 ± 80.0	0.959
TRT, *min*	427.1 ± 41.1	427.8 ± 98.0	449.8 ± 102.1	411.5 ± 48.2	0.8560
Sleep efficiency, *%*	64.3 ± 16.7	63.5 ± 17.2	59.5 ± 17.9	64.5 ± 12.0	0.9336
REM Latency, *min*	129.8 ± 93.9	108.8 ± 104.6	80.5 ± 56.3	116.0 ± 32.0	0.6445
SFI, *n*	25.3 ± 8.3	29. 2 ± 11.6	21.0 ± 8.22	30.2 ± 2.2*	0.215
Arousal Index, *n*	21.6 ± 11.2	21.3 ± 11.1	17.9 ± 11.7	21.8 ± 14.1	0.9073
REM phasic, *%*	26.8 ± 11.7	32.9 ± 13.7	37.1 ± 11.7	43.6 ± 25.9	0.4116
REM tonic, *%*	34.7 ± 15.8	44.3 ±23.5	50.5 ± 24.1	62.8 ± 2.3*	0.2211
REM phasic/tonic, *%*	50.8 ± 24.3	61.2 ± 25.9	62.1 ± 20.2	75.2 ± 12.0	0.1323
AHI, *n*	2.6 (1.2-5.7)	3.4 (0.2-12.5)	7.8 (5.2-9.5)	12.9 (10.9-15.2)	0.1342
Central Apnoea index, *n*	0.6 (0-1.54)	0.23 (0-7.15)	2.6 (0-7.4)	3.7 (0-7.4)	0.8005
BR Awake, *n*	17.5 ± 2.9	18.1 ± 2.8	20.2 ± 7.4	23.5 ± 14.8*	0.1338
BR NREM 1, *n*	17.8 ± 2.8	17.9 ± 2.9	19.8 ± 6.6	23.5 ± 12.0*	0.1461
BR NREM 2, *n*	17.7 ± 2.6	17.9 ± 3.0	19.2 ± 5.7	23.0 ± 9.9*	0.1696
BR NREM 3, *n*	18.0 ± 2.7	17.6 ± 3.2	19.0 ± 5.2	23.0 ± 9.9*	0.2024
BR REM, *n*	18.5 ± 2.7	18.5 ± 3.2	20.3 ± 6.3	24.0 ± 9.9*	0.1466
HR Awake, *n*	70.4 ± 10.5	69.8 ± 11.3	69.9 ± 8.3	88.5 ± 14.8*	0.1348
HR NREM 1, *n*	67.9 ± 8.0	67.0 ± 11.7	67.8 ± 8.0	86.0 ± 11.3*	0.0613
HR NREM 2, *n*	66.8 ± 7.8	67.0 ± 11.3	67.2 ± 8.9	85.5 ± 10.6*	0.2024
HR NREM 3, *n*	67.3 ± 7.9	67.5 ± 12.2	68.5 ± 9.6	85.0 ± 9.9*	0.0893
HR REM, *n*	68.8 ± 7.7	69.7 ± 10.7	68.5 ± 10.7	85.5 ± 10.6*	0.0973

Data are expressed as n (%), mean ± SD, or median (interquartile range).
Statistically significant p-values are denoted in bold (p value≤0.05).

Legend: *= significant between the first and last VPSG; BR= breathing rate, HR= Heart Rate; NREM= non-rapid eye movement; NREM 1= non-rapid eye movement sleep stage 1; NREM2= non-rapid eye movement sleep stage 2; NREM 3= non-rapid eye movement sleep stage 3; REM= Rapid eye movement sleep stage; SFI= sleep fragmentation index; TRT= total recording time; TST= total sleep time; WASO= Wake After Sleep Onset.

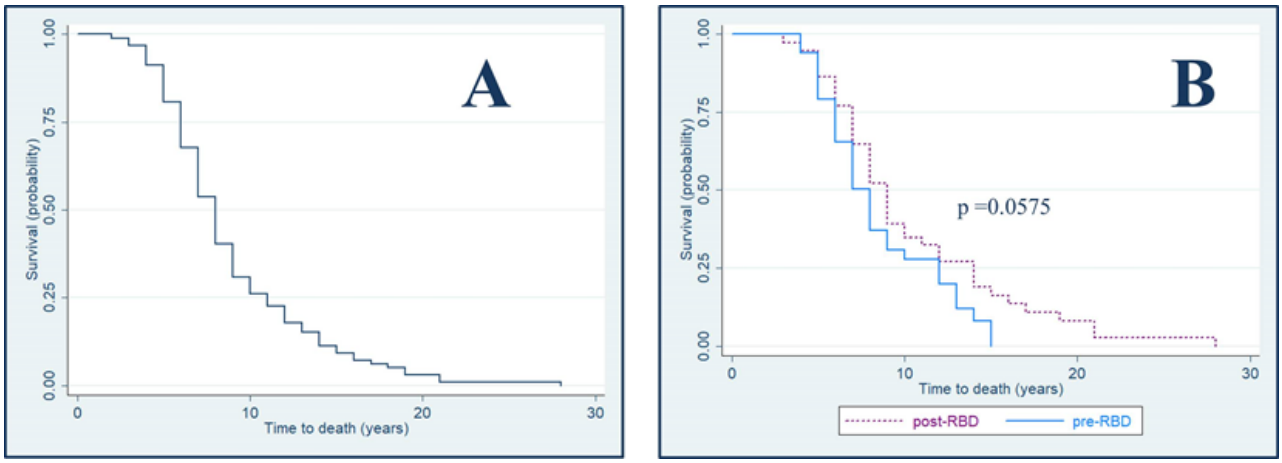
Survival analysis

Kaplan-Meier estimates of death in the overall population are shown in Figure 5A. In this analysis, the median duration of illness was 7.79 years.

The risk of death estimated by Kaplan- Meier analysis (Figure 5B) was higher in patients with RBD-pre but without reaching statistical significance (p= 0.0575, log-rank test).

The incidence rate of death was 10 per 100 person years in the RBD-pre group and 7 per 100 person-years in the RBD-post group.

Figure 5: Kaplan-Meier survival curves for probability of death from disease onset



Legend: A= in all patients; B= in patients with REM sleep behaviour disorder predating disease onset and following disease onset.

Univariate Cox regression analyses identified the following as factors associated with survival: age at disease onset (H-Ratio= 1.05, 95% CI= 1.03-1.07, p <0.001), autonomic onset (H-Ratio= 1.56, 95% CI=1.10-2.22, p= 0.013), early symptomatic OH (H-Ratio= 2.88, 95% CI=1.76-4.73, p<0.001), early urinary urgency/frequency (H-Ratio= 2.62, 95% CI= 1.58-4.33, p < 0.001), early urinary retention (H-Ratio= 2.24, 95% CI=1.32-3.80, p= 0.003), early urinary incontinence (H-Ratio= 2.15, 95% CI= 1.24-3.75, p= 0.007), early RBD (H-Ratio= 3.52, 95% CI= 1.76-7.05, p < 0.001). Presence of stridor was not associated with survival (Table 8).

In the multivariable model early RBD, early symptomatic OH and early urinary incontinence remained an independent predictor of mortality after adjustment for other variables. Early RBD showed an adjusted H-Ratio= 3.12 (95% CI= 1.38-17.11, p= 0.014).

Table 8: Variables associated with survival in patients with MSA in the univariate Cox regression analysis

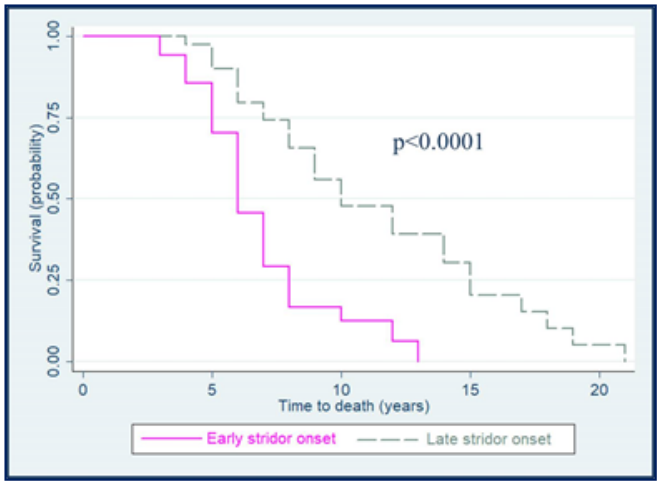
Variable	*n*	Unadjusted H-Ratio (95% CI)	p-value
Age at disease onset	182	1.05 (1.03-1.07)	**<0.001**
Sex			
Female	75	0.83 (0.59-1.18)	0.300
Male	107	1.0 (reference)	
Clinical phenotype			
MSA-P	92	0.90 (0.65-1.26)	0.549
MSA-C	90	1.0 (reference)	
Symptom of disease onset			
Autonomic	101	1.56 (1.10-2.22)	**0.013**
Cerebellar	66	0.95 (0.67-1.36)	0.793
Parkinsonism	60	0.97 (0.68-1.39)	0.875
Symptom of disease onset			
Early symptomatic OH	79	2.88 (1.76-4.73)	**<0.001**
Early urinary urgency/frequency	130	2.62 (1.58-4.33)	**<0.001**
Early urinary retention	57	2.24 (1.32-3.80)	**0.003**
Early urinary incontinence	35	2.15 (1.24-3.75)	**0.007**
Stridor (VPSG)			
Yes	75	0.88 (0.62-1.26)	0.495
No	91	1.0 (reference)	
N.A.	16		
Early RBD (VPSG)	105	3.52 (1.76-7.05)	**<0.001**

Statistically significant p-values are denoted in bold (p value≤0.05).

Legend: CI= confidence interval; H-Ratio= hazard ratio; MSA-C= multiple system atrophy with predominant cerebellar phenotype; MSA-P= multiple system atrophy with predominant parkinsonism phenotype; N.A.= not available; OH= orthostatic hypotension; RBD= REM sleep behaviour disorder; VPSG= video-polysomnography.

The risk of death estimated by Kaplan-Meier analysis was higher in patients developing early stridor ($p<0.0001$, log-rank test) (Figure 6). The incidence rate of death was 12 per 100 person-years in the early stridor onset group and 7 per 100 person-years in the late stridor onset group.

Figure 6: Kaplan-Meier survival curves for probability of death (from disease onset) in patients with early and late stridor onset



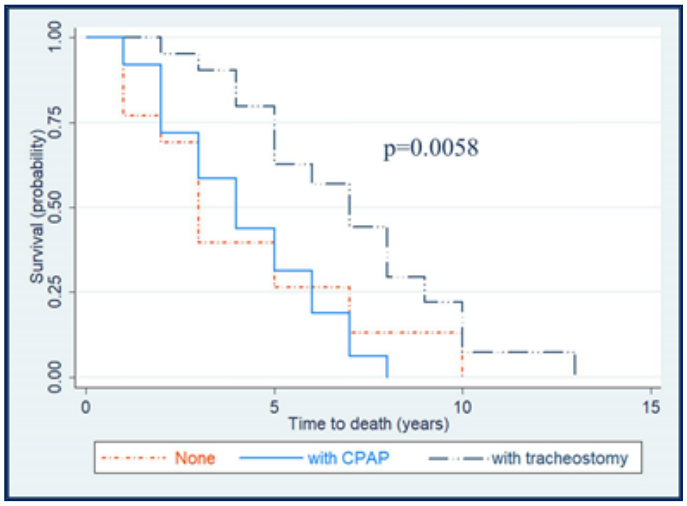
In the stridor subgroup, univariate Cox regression analyses identified the following as factors associated with survival: autonomic onset (H-Ratio= 1.84, 95% CI= 1.01-3.39, p= 0.049), early urinary urgency/frequency (H-Ratio= 2.38, 95% CI= 1.10-5.17, p= 0.028), early stridor onset (H-Ratio= 3.17, 95% CI= 1.74-5.77, p < 0.001), early RBD (H-Ratio= 19.28, 95% CI= 2.51-47.84, p= 0.004). Early stridor onset remained an independent predictor of mortality after adjustment for other variables (H-Ratio= 2.55, 95% CI= 1.25-5.22, p= 0.010).

Concerning stridor treatment, Kaplan-Meier curves from stridor onset showed a difference in survival among patients without treatment (n= 24), those treated with tracheostomy (n= 22), and those treated with CPAP (n= 29) (p= 0.0058, log-rank test) (Figure 7). This statistical significance was attributable to the difference in mortality between patients treated with tracheostomy and those without treatment (p= 0.0230, log-rank test) and between patients treated with tracheostomy and those treated with CPAP (p= 0.0012, log-rank test). Incidence rate of death was 23 per 100 person-years in patients without treatment, 21 per 100 person-years in those treated with CPAP and 12 per 100 person-years in those treated with tracheostomy.

In the univariate Cox regression analyses, patients without treatment showed an increased risk to death when compared to those treated with tracheostomy (H-Ratio= 2.31, 95% CI= 1.02-5.21, p= 0.045) without difference to those treated with CPAP (H-Ratio= 0.98, 95% CI= 0.44-2.17, p= 0.959).

Finally, patients treated with CPAP showed an increased risk of death compared to those treated with tracheostomy (H-Ratio= 2.96, 95% CI= 1.41-6.22, p= 0.004).

Figure 7: Kaplan-Meier survival curves for probability of death (from stridor onset) in patients with stridor without treatment, treated with tracheostomy and treated with CPAP



DISCUSSION

This is one of the first studies focusing on the longitudinal progression of sleep in MSA.

This retrospective and prospective study on a large cohort of MSA patients resulted in the following clinically relevant findings:

1) isolated RBD is the mode of disease presentation in 30% of patients, preceding the disease onset, diagnosed according to the international criteria, with a median of 3 (1–5) years;

2) patients with RBD preceding the disease onset showed a more frequent autonomic onset and less frequent parkinsonism onset, presented more frequently and with a lower latency pyramidal signs, showed an earlier onset of urinary urgency/frequency, urinary incontinence, symptomatic OH and stridor, and presented shorter latencies for several milestones of disease progression;

3) MSA patients developing stridor within 3 years from disease onset presented more frequently with autonomic onset, showed an earlier onset of pyramidal signs, urinary urgency/frequency, urinary incontinence and RBD, and revealed a more rapid disease progression with shorter latency of four milestones of disease progression;

4) sleep architecture was characterized by peculiar features which could represent negative markers in MSA prognosis such as increased NREM 1 and decreased REM stages, disrupted sleep and motor patterns occurring throughout the progression of disease, and progressive sleep-related breathing disorders and breathing/heart rate alterations;

5) early RBD onset and early stridor onset had a negative prognostic value for survival, maintained after adjustment for other confounding variables;

6) concerning stridor treatment, patients treated with tracheostomy showed a longer disease duration after stridor onset with a reduced risk to death.

RBD as mode of disease onset

First, the 30% (n= 55) of patients presented with isolated RBD and developed the first autonomic or motor symptom/sign of MSA after a median of 3 (1–5) years. Moreover, the 14% (n= 25) of patients showed RBD associated with motor/autonomic symptoms at disease onset (5% with autonomic, 3% with cerebellar and 6% with autonomic, cerebellar and parkinsonian symptoms). These results showed that this sleep disorder represents the most frequent first symptom of MSA. Despite the high prevalence of RBD during the disease course, the rate of RBD as initial manifestation of MSA has been poorly investigated. Eleven studies reported RBD preceding the

disease onset, only 7 diagnosed with VPSG, showing a prevalence ranging from 15.6% to 60.0% (Plazzi et al. 1997; Iranzo et al. 2000; Iranzo et al. 2005; Vetrugno et al. 2004; Nomura et al. 2011; Guo et al. 2013; Stanzani-Maserati et al. 2014; De Cock et al. 2011; Palma et al. 2015; Coon et al. 2015; McKay et al. 2018). The first study evaluating clinical and VPSG data reported that 27 out of 39 patients with MSA (69.2%) had a history of RBD, 12 (30.8%) of which referred this sleep disorder before the disease onset (Plazzi et al. 1997). Another study on 20 MSA patients revealed a clinically-suspected and VPSG-confirmed RBD in 18 subjects and a prevalence of iRBD in 30% (n= 6/20) of the total sample (Iranzo et al. 2000). A study of the same group comparing RBD features in 26 patients with MSA and 45 patients with PD with VPSG-confirmed RBD found that RBD preceded the onset of motor symptoms in 14 out of 26 patients with MSA (53.8%) (Iranzo et al. 2005). A video-polysomnographic study on 19 MSA patients recorded RBD in all patients, in 3 (15.6%) of them isolated RBD preceded autonomic and/or motor onset (Vetrugno et al. 2004). One study compared clinical and polysomnographic characteristics of RBD between 16 MSA patients and 49 PD patients, found VPSG-confirmed RBD in 11 patients, 7 (43.8%) of which reported the development of RBD symptoms before disease onset (Nomura et al. 2011). The study on 30 MSA patients reported a VPSG-confirmed RBD prevalence of 46.7% (n= 14) and a prevalence of RBD onset before disease onset of 10% (n= 3/30) (Guo et al. 2013). In a study on 10 MSA patients investigated with VPSG, RBD was reported in all patients and preceded the disease onset in 6 (60.0%) out of 10 patients (Stanzani-Maserati et al. 2014).

In the other four studies prevalence of iRBD was calculated only on the basis of clinically-suspected RBD because RBD diagnosis was based on history taking, VPSG was performed in a subgroup of iRBD sample or the rate of VPSG-confirmed RBD in patients presenting with RBD is not specified in the results section. One study based on bed-partner interview revealed a clinical-suspected RBD prevalence of 87.8% and isolated RBD prevalence of 30% (De Cock et al. 2011). In this study 22 out 49 patients performed VPSG but the rate of VPSG-confirmed RBD in patients developing this sleep disorder before MSA onset is not specified. One cross-sectional multicentre study based on sleep questionnaires observed that 29 out of 64 patients with MSA (45%) presented RBD symptoms before the onset of motor deficits, but the amount of VPSG-confirmed RBD in this subgroup of patients was not specified in the article (Palma et al. 2015). In a large retrospective study on 685 patients with MSA, history of sleep symptoms suggesting RBD was reported in 304 participants and in 34% of cases sleep symptoms preceded motor and autonomic ones (Coon et al.

2015). Finally, in a recent retrospective study on 30 patients with MSA, 5 (16.7%) participants reported a history suggestive of RBD as the first symptom of disease, which was confirmed by VPSG in 2 patients (McKay et al. 2018).

These data suggested that patients with iRBD could represent a pre-motor cohort. However, despite almost all MSA patients experience RBD at some point of disease course, studies investigating the rate of phenoconversion from iRBD to synucleinopathies showed that only a small percentage of converters develop MSA. Few studies investigated predictors of phenoconversion on patients with iRBD. One recent study on the largest cohort of iRBD patients (n=1280) investigated potential predictors of phenoconversion identifying motor symptoms, objective motor examination, olfactory deficit, mild cognitive impairment, erectile dysfunction, an abnormal DAT scan, colour vision abnormalities, constipation, RSWA and age as markers (Postuma et al. 2019). However, this study considered factors predicting the overall conversion rate from iRBD to an overt neurodegenerative disease, but not the ones specific for MSA conversion. A recent study analysed 154 polysomnography-proven patients with iRBD, of whom 55 phenoconverted to defined parkinsonism (n= 25 PD, n= 4 MSA) or dementia (n= 26 DLB). Compared to the other synucleinopathies (DLB/PD), the four patients who eventually developed MSA were significantly younger (54.2 ± 7.8 vs. 70.4 ± 7.5, p= 0.001) and had preserved cognition (MoCA= 26.8 ± 2.5 vs. 21.3 ± 5.5, p= 0.044) and colour vision (FM100= 87.0 ± 60.1 vs. 227.0 ± 122.9, p= 0.005) at the time of phenoconversion. MSA-converters may also have had less impaired olfaction (UPSIT % normal= 77.3 ± 44.4 vs. 57.6 ± 26.6) and more severe urinary symptoms (1.2 ± 0.4 vs. 0.7 ± 0.9) at phenoconversion (with marginal statistical significance levels) (Fereshtehnejad et al. 2019). Therefore, further studies on larger sample and with diagnosis stratification are necessary to identify clinical and instrumental features with higher diagnostic value on phenoconversion.

Comparison between RBD-pre and RBD-post groups

Patients with RBD preceding the disease onset showed a more rapid progression of the disease. Comparing RBD-pre and RBD-post, the first group showed more frequently an autonomic onset (63.5% vs. 50%, p= 0.132) and less frequently parkinsonian symptoms/signs (21.2% vs. 40.8%, p= 0.020). At the last follow-up the RBD-pre group showed more frequently a cerebellar phenotype (61.5% vs. 48.7%), but this difference did not reach statistical significance.

During the course of the disease, RBD-pre revealed more frequently pyramidal signs (90.4% vs. 75.0%, p=0.029) with lower latency of onset [0 (0-1) vs. 1 (0-3), p= 0.042], and earlier onset of urinary urgency/frequency [0 (0-1) vs. 1 (0-3), p= 0.0421], urinary incontinence [3 (1-6) vs. 4 (2-8), p=0.028], symptomatic OH [1 (0-3) vs. 3 (1-6), p= 0.0030] and stridor [2 (2 – 4) vs. 4 (3 – 6), p= 0.0172.5]. Concerning milestones of disease progression, RBD-pre showed a shorter latency of urinary catheterization [4 (3-6) vs. 6 (5-9), p= 0.024], severe dysphagia/PEG [6 (4-7) vs. 8 (5-10), p= 0.0384] and wheelchair dependency [5 (4-6) vs. 6 (4-8.5), p= 0.0179] when compared to the RBD-post group. Earlier unintelligible speech was underlined in RBD-pre patients without reaching a statistical significance [5.5 (4-7) vs. 7 (5-9), p= 0.074].

Comparison between early and late stridor onset groups

In the stridor subgroup, patients developing stridor within the first 3 years of disease, more frequently presented with autonomic onset (82.9% vs. 50.0%, p= 0.004). No difference in frequency of symptoms was found between patients with early and late stridor onset. However, considering the latency from disease onset, patients with early stridor onset showed an earlier onset of pyramidal signs [3 (3-4) vs. 5 (3-6), p= 0.0292], urinary urgency/frequency [0 (0-1) vs. 2 (0-4), p=0.010] and urinary incontinence [2.5 (1-4) vs. 5 (3-8), p= 0.0037]. A shorter latency of symptomatic OH [2 (1-4) vs. 3 (0-5), p= 0.1153] and urinary retention [1 (0-3) vs. 3 (0-5), p= 0.1311] was reported without reaching statistical significance. Moreover, the early stridor group more frequently presented early VPSG-confirmed RBD onset (85.71% vs. 65.00%, p= 0.005). The early stridor onset group developed a more progressive and severe disease, reaching 4/5 milestones of disease progression with shorter latency in respect to the late stridor onset group: urinary catheterization [4 (1-4) vs. 6 (4-8), p= 0.0004], unintelligible speech [4.5 (4-5.5) vs. 7 (5-8), p= 0.0212], dysphagia/PEG [4 (4-5) vs. 7 (5-9), p= 0.0335], wheelchair dependency [4 (4-5.5) vs. 7 (5-8), p= 0.0019].

These findings highlight a more rapid progression and severe disease in patients who early developed stridor and RBD which showed early autonomic involvement, a shorter latency of milestones of disease progression achievement and finally a higher risk of death. Therefore, a careful assessment and a closer follow-up of autonomic dysfunction and stridor are required in these subgroups of MSA patients.

The bidirectional relationship between RBD and stridor onset and the early autonomic onset of these patients could shed light on a common pathogenic pathway residing in key brainstem areas involved in sleep, cardiovascular control and automatic respiration regulation (Benarroch et al. 2018). This correlation could be linked to the highly topographic and functional interconnection of brainstem neuronal networks (parabrachial nucleus, pre-Botzinger complex, rostral ventrolateral medulla, pontine micturition centre, pedunculopontine tegmental nucleus, sublaterodorsal tegmental nucleus, locus ceruleus) whose degeneration in MSA has been widely documented (Benarroch et al. 2003; Benarroch et al. 2018; Benarroch et al. 2019). Therefore, the early occurrence of RBD and stridor during the disease course entails an early involvement of brainstem nuclei, which leads to early autonomic dysfunction, stridor, and other sleep and breathing disorders.

VPSG features and their longitudinal modification

VPSG features modified during the disease course, some of them could represent specific features involved in survival, sudden death and death during night. Sleep architecture in the first years of disease showed an increased percentage of NREM 1 and reduced percentages of REM stage, compared to normal value, with an overall reduced sleep efficiency. Although no statistical significance was found in the general model, a significant reduction of REM stage percentage (from 19.9 ± 10.7 in the first VPSG to 11.6 ± 2.2 in the last VPSG) and a trend of NREM 1 increasing (from 13.1 ± 8.9 in the first VPSG to 17.5 ± 8.7 in the last VPSG) were reported. Moreover, during the disease course an increased SFI was calculated (from 25.3 ± 8.3 in the first VPSG to 30.2 ± 2.2 in the last one), and this datum is an index of unstable sleep with rapid transitions among different stages.

A qualitative analysis of sleep architecture, performed by sleep disorders experts, showed that sleep figures (K-complexes and spindles) were no longer identifiable in the intermediate-late phase of disease. Moreover, intrusion of REM sleep into NREM 2 stages was observed and presence of spindles during REM sleep was frequently reported. Further studies comparing different disease entities should be performed to investigate the diagnostic value of these features and their diagnostic accuracy.

Concerning motor pattern, the episodes of RBD, violent and isolated at the beginning of the disease, became more frequent (subcountinous in some cases) and subtler during the disease course. In the same way, the percentages of phasic, tonic and phasic/tonic REM progressively increased from the

first to the last VPSG evaluation, demonstrating increased and persistent motor activation. Our data confirm the results reported in a previous studies on small MSA samples. A decrease of behaviours/movements over time was confirmed by polysomnography/video recording in one patient, the ratio of RSWA to the whole REM sleep also increased in this patient, while the sleep architecture as well as the percentage of REM sleep were maintained (Tachibana et al. 2004). A VPSG study on 2 MSA patients reported a reduced frequency of RBD episodes during the disease course and described the appearance of a disrupted sleep pattern with sleep stages no longer identifiable associated with a nearly continuous motor and verbal abnormal behaviours and ambiguous and rapid oscillation of state-determining polysomnographic variables. Features of abnormal NREM 1 and REM sleep, together with instable chin muscle tone, recurred rapidly and irregularly in a sort of undifferentiated sleep state (Vetrugno et al. 2009). However, these findings should be confirmed in a larger sample.

Sleep-related breathing disorders were characterized by an increased frequency of stridor during the night and by a progressive increase of AHI and central apnoea index, without differences in mean oxygen saturation. These data should be confirmed in a larger sample, by means of objective measures of percentages of stridor during sleep and with stratification for treatment (CPAP or tracheotomy) to better evaluate the influence of treatment on these aspects.

Concerning autonomic features during sleep, both BR and HR progressively increased during the disease course in all sleep stages, without reaching a statistical significance. However, comparing the first and the last VPSG, differences in BR and HR in all sleep stages were found. In the first VPSG a mild reduction in ΔBR was found in NREM, especially in NREM 3, while a positive ΔBR was found in REM sleep. In the late phase of the disease, a progressive increase of ΔBR in all sleep stages was documented suggesting an abnormal autonomic regulation. Studies describing changes in spontaneous breathing during sleep in normal humans reported a decrease in minute ventilation during steady slow wave sleep (stable NREM 2 and NREM 3), compared with wakefulness (Krieger 1985). It is unclear whether the decrease in minute ventilation is due to a decrease in tidal volume or in respiratory frequency or in both. In animals the decrease in minute ventilation is due to a decrease in respiratory rate incompletely compensated by an increase in tidal volume. However, the changes in ventilation observed during NREM sleep in humans are different and studies on this topic remain inconclusive. Quantitative measurements of ventilation in REM sleep showed a variable decrease in minute ventilation compared to that in wakefulness, and discrepant results were

found when compared to that in NREM sleep. Discordant results were reported also in tidal volume, inspiratory flow and respiratory rate in REM in humans (Krieger 1985). As data on the physiological BR rate in humans remain inconclusive, further studies taking also into consideration healthy controls are necessary to investigate these features.

Behavioural respiratory drive is minimal during deep NREM sleep and may be activated intermittently during REM sleep, either erratically or by dream contents (Schafer and Schlafke 1998, Benarroch et al. 2019). In fact, physiological breathing during REM sleep is described as an irregular pattern, with short breathing pauses and phases of rapid shallow breathing. This irregularity is caused by sudden changes in both respiratory amplitude and frequency, which are independent of variations in chemoreceptors or vagal afferent activity (Orem et al. 2000). In animals this irregular pattern persists during hypoxia and hypercapnia as well as after vagotomy and chemodenervation, therefore it has been suggested that this breathing pattern is produced by activation of the behavioural respiratory control system during REM (Krieger 1985). Moreover, irregularity of breathing at sleep onset (NREM 1 and NREM 2 before steady sleep) is reported in healthy humans. This respiratory pattern was called periodic breathing because it consisted in oscillations in breathing amplitude, regularly decreasing and increasing. Breathing instability at sleep onset could be the result of the combination of the different set points of ventilation (higher PCO_2 level and lower ventilation) during sleep and oscillations between arousal, NREM 1 and NREM 2 (Krieger 1985).

Therefore, breathing instability at sleep onset (NREM 1 and NREM 2 before steady sleep) and breathing irregularity during REM sleep has been reported in healthy humans (Krieger 1985; Carskadon MA et al. 2000; Benarroch et al. 2019). These features could be more frequent and pronounced in MSA patients both in the intermediate phase of the disease when rapid transitions among unstable sleep stages with increased SFI and REM intrusions into NREM 2 are observed, and even more in the late phase when sleep stages are no longer identifiable and abnormal NREM 1 is mixed with RSWA (status dissociatus). Indeed, breathing instability and irregularity could represent negative prognostic factors, playing a role in sudden death and death during sleep in MSA.

Finally, in our MSA sample the absence of the physiological reduction in HR from wakefulness to sleep stages was reported. In healthy controls, a reduction in HR and blood pressure occurs during NREM sleep, becoming more pronounced as sleep progresses from stage 1 to stage 3. From NREM

to REM sleep a progressive sympathetic predominance is observed associated with HR and blood pressure values comparable with those during wakefulness (Calandra-Buonaura et al. 2016). In our MSA sample no difference was found between NREM sleep and wakefulness and an increased HR was observed in REM sleep, especially in the late phase of disease.

These data, taken together, suggested abnormal cardio-respiratory regulation during sleep with lack of physiological circadian decrease of these features during sleep stages. The cardio-respiratory drive alteration during sleep in MSA, associated with the rapid transition among sleep stages and with blunted hypoxic response during sleep in this disease (Glass et al. 2006), could contribute to increasing the risk of respiratory failure, of impaired response to hypoxemia causing hypoxic ventilatory decline (Tsuda et al. 2002) and of cardiac rhythm alteration in response to respiratory pattern, leading to sudden death and death during night. Loss of neurons in brainstem nuclei involved in cardiovascular and respiratory modulation during the wake-sleep cycle and in key brainstem areas involved in respiratory pattern generators, reported in MSA (Benarroch et al. 2003), may contribute to sleep apnoea, respiratory dysrhythmia, reduced respiratory chemosensitivity, impaired arousal responses to hypoxia, stridor, and death during sleep in these patients (one of the major causes of death in MSA).

Survival analysis

The incidence rate of death was 10 per 100 person-years in the RBD-pre group and 7 per 100 person-years in the RBD-post group, and therefore the risk of death estimated by Kaplan- Meier analysis was higher in the RBD-pre group although this did not reach statistical significance ($p= 0.0575$, log-rank test). The risk of death estimated by Kaplan- Meier analysis was higher in patients developing early stridor ($p < 0.0001$, log-rank test) and the incidence rate of death was 12 per 100 person years in the early stridor onset group and 7 per 100 person-years in the late stridor onset group. In the univariate and multivariate Cox regression model we considered clinical features and early onset of symptoms/signs showing that both early RBD and early stridor were independent predictors of mortality (H-Ratio= 3.12 and 3.17, respectively), also after adjustment for other well-recognized predictors of mortality such as autonomic features (OH and urinary symptoms).

Finally, regarding stridor treatment, Kaplan-Meier curves from stridor onset showed a difference in survival among patients without treatment, those treated with tracheostomy, and those treated with CPAP ($p= 0.0058$). This statistical significance was attributable to the difference in mortality

between patients treated with tracheostomy and the other two groups. Incidence rate of death was 23 per 100 person-years in patients without treatment, 21 per 100 person-years in those treated with CPAP and 12 per 100 person-years in those treated with tracheostomy. Both patients without treatment and treated with CPAP showed an increased risk of death at univariate Cox regression when compared to those treated with tracheostomy. Multivariate analysis taking also into consideration autonomic factors, early stridor onset, central apnoea index and CPAP compliance should be performed to confirm these data.

These results, conducted on a larger sample, confirmed our previous data on 42 MSA patients with stridor showing that patients treated with tracheostomy had longer overall disease duration, longer disease duration after stridor onset, and longer disease duration after treatment compared with those treated with CPAP. In our previous article, Kaplan-Meier curve did not reveal a difference in survival between these treatments even if there was a trend toward longer disease duration in patients who had undergone tracheostomy compared with those treated with CPAP (p= 0.0850), and a difference on incidence rate of death (9 vs. 11 per 100 person-years, respectively) (Giannini et al. 2016). One previous descriptive study compared these 2 types of stridor treatment and observed survival in a small sample of MSA patients. In this study, 2 of the 4 patients receiving tracheostomy died 1 year after the sleep evaluation, and the other 2 patients were alive 1.9 and 7 years later, while all 5 patients treated with CPAP died a mean of 2.4 years after the sleep evaluation. Of the patients treated with CPAP, one had poor device compliance and another one presented audible stridor despite device use. In this small study, authors suggested that CPAP had no effect on survival (Silber et al. 2000). Another study on 49 patients with definite MSA focused only on the role of tracheostomy on survival showing that tracheostomy reduces the risk of death (H-Ratio= 0.21, 95% CI= 0.08–0.56, $p < 0.01$) and of sudden death (H-Ratio= 0.15, 95% CI= 0.02–0.98, $p < 0.05$) in MSA (Tada et al. 2007).

Two studies focused on the role of CPAP in MSA survival. In one study 13 MSA patients with stridor receiving CPAP had similar median survival when compared to 26 MSA patients without stridor (77 vs. 88 months, p= 0.6914) (Iranzo et al. 2004). Sudden death was reported in 2 of 13 patients following CPAP initiation (Ghorayeb et al. 2005). Recently one study reported similar median survival times calculated from the disease onset between the 12 patients who were treated for stridor (93 months) and the 28 patients without any stridor at baseline (119 months, p= 0.57). In this study patients treated with stridor received fixed CPAP, auto-adjusting CPAP or adaptive

servo-ventilation as function of the presence and the type of sleep apnoea: patients received fixed CPAP when stridor was isolated, auto-adjusting CPAP when it was combined with obstructive sleep apnoea, and adaptive servo-ventilation when combined with central sleep apnoea (Rekik et al. 2018).

Strengths and limits of the study

The strengths of our study are that all patients were seen and diagnosed in a single centre, ensuring uniformity of data. Patients of a retrospective cohort were evaluated at least once a year during the disease course, instrumental/laboratory tests were performed during the disease course when specific conditions were suspected by history or examination, and data were systematically collected.

Moreover, cardiovascular autonomic failure and stridor were instrumentally documented. In order to reduce the recall bias of RBD onset, event registration from VPSG was shown to bed partners to ensure it was the same as that reported in patient recall, and diagnosis of RBD was retained only when confirmed by VPSG.

However, different limits should be discussed. VPSG features were systematically analysed from the authors only in a subgroup of the overall sample and our results should be confirmed in our whole MSA sample and eventually after adjustment for disease duration. Moreover, for specific VPSG features, comparison with healthy controls and with patients affected by Parkinson's Disease or/and with Autonomic Failure, is mandatory to investigate if these results are hallmarks of MSA or are more frequently reported in this cohort. A stratification between patients with and without stridor, and patients with and without stridor treatment, could highlight different VPSG features (such as autonomic parameters) associated with these conditions, as suggested in small previous case series. Finally, some features qualitatively observed need to be analysed by means of objective methodology.

CONCLUSION

Sleep disorders are key features of MSA playing a role in presentation, prognosis and progression of disease. Patients who early developed stridor during MSA course or presented with RBD at disease onset showed a more rapid and severe disease progression, mainly due to a rapid involvement of autonomic features (both OH and urinary dysfunctions) and to an early achievement of milestones of disease progression. Although autonomic failure is a negative predictor on prognosis per se, we demonstrated that early stridor and RBD onset are independent risk factors on MSA survival. The VPSG analysis suggested that sleep architecture, motor pattern, sleep-related breathing disorders and autonomic parameters modified during the disease course. Some specific features, such as the rapid transition among sleep stages, the higher sleep fragmentation index, the continuous motor activation, the progression of sleep-related breathing disorders and, in particular, the modifications of autonomic parameters and the absence of their physiological circadian decrease during sleep stages, could underline a disrupted cardiorespiratory brainstem drive. These factors could represent a hallmark of MSA and could be involved in prognosis and in particular in sudden death and death during sleep, causes of death neither frequent in other neurodegenerative diseases nor in isolated autonomic failure. Further studies on a larger sample and with control groups are necessary to confirm our data. Our findings could contribute to the definition of new international criteria, helping to better define MSA prognosis and to recognize patients with a poor prognosis, stratifying them for domain of disease onset, even in view of upcoming trials.

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