Sleep cardiac dysautonomia and EEG oscillations in Amyotrophic Lateral Sclerosis

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ABSTRACT

Study Objectives: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease due to loss of motor neurons. However, the Autonomic Nervous System (ANS) can also be involved. The aim of this research was to assess the sleep macro- and micro-structure, the cardiac ANS during sleep and the relationships between sleep, autonomic features and clinical parameters in a cohort of ALS patients.

Methods: Forthy-two consecutive ALS patients underwent clinical evaluation and full-night video-polysomnography (video-PSG). Only 31 patients met inclusion criteria (absence of comorbidities, intake of cardioactive drugs, or recording artifacts) and were selected for assessment of sleep parameters, including cyclic alternating pattern (CAP) and heart rate variability (HRV). Subjective sleep quality and daytime vigilance were also assessed using specific questionnaires.

Results: Although sleep was subjectively perceived as satisfactory, compared to age- and sex-matched healthy controls, ALS patients showed significant sleep alteration: decreased total sleep time and sleep efficiency, increased nocturnal awakenings, inverted stage 1 (N1) / stage 3 (N3) ratio, reduced REM sleep and decreased CAP rate, the latter supported by lower amounts of A phases with an inverted A1/A3 ratio. Moreover, a significant reduction in HRV parameters was observed during all sleep stages, indicative of impaired autonomic oscillations.

Conclusion: Our results indicate that sleep is significantly disrupted in ALS patients despite its subjective perception. Moreover, EEG activity and autonomic functions are less reactive, as shown by a decreased CAP rate and a reduction in HRV features, reflecting an unbalanced autonomic modulation.

Keywords: Amyotrophic lateral sclerosis (ALS); heart rate variability (HRV); sleep and neurodegenerative disorders; autonomic nervous system (ANS)

Statement of significance: amyotrophic lateral sclerosis is a neurodegenerative disease characterized by prominent motor involvement, with alteration also in other systems like autonomic nervous system and sleep. In our polysomnographic study of a cohort of ALS patients we confirmed that sleep structure in these patients is altered and for the first time we found an alteration of CAP with decreased CAP rate due to reduced amount of A1 phases; moreover, we demonstrated a subclinical autonomic dysfunction present since the early stages of the disease, as shown by reduced heart rate variability (HRV) in all sleep stages. HRV assessment during sleep is a not-invasive early tool to investigate autonomic function and decreased HRV is associated with increased risk in mortality for cardiac sudden arrest, and low HRV is a strong predictor of increased mortality for coronary events, which occurs more frequently at night.

It is fundamental performing a PSG study since the earliest stages of the disease.

Introduction

Amyotrophic lateral sclerosis (ALS), is an adult-onset inexorably progressive, devastating and fatal neurodegenerative disease, characterized by progressive loss of spinal, bulbar and cortical motor neurons with consequent painless paralysis of striatal skeletal and bulbar muscles, with dysphagia, dysarthria and respiratory impairment, up to respiratory failure. So far, no reversible treatment for ALS is available, and death occurs typically within 2-3 years after disease onset.[1] Although ALS has traditionally been considered a pure motor neuron disease, with the concomitant presence of symptoms and signs involving other systems suggesting alternative diagnoses, the evidence of involvement of other domains (e.g. sensitive, cognitive, autonomic nervous system) has been documented, configuring ALS as a multisystem disorder.[2–4]

Autonomic dysfunctions with impairment of cardiovascular control in ALS have been observed especially in the advanced stages of the disease and during sleep.[3,5,6] In addition, degeneration of intermediolateral column neurons[6] and involvement of the hypothalamus[7] have been described. Dysautonomia, in particular reduced heart rate variability (HRV) and increased sympathetic tone have been identified as mortality risk factors in patients with cardiac disease.[5,8] Sudden death and cardiovascular dysfunction due to dysautonomia are probably more frequent during sleep in patients affected by ALS.[9,10]

Sleep fragmentation and sleep disorders may be early manifestations in ALS patients, although underdiagnosed. Several factors may disturb sleep in these patients, namely sleep disordered breathing, nycturia, sleep fragmentation, restless legs syndrome, nocturnal cramps, pain, depression, difficulty in changing position, problems in swallowing, and troublesome cough.[11–13] Most sleep studies in ALS have predominantly focused on the chronic nocturnal respiratory insufficiency and hypoventilation[14] that commonly anticipate the onset of awake respiratory failure, because of the physiologic vulnerability of respiration during sleep.

Sleep is an ideal condition to study the Autonomic Nervous System (ANS), as the automatic control of vegetative functions occurs without complete consciousness and reduced external influences.

Nevertheless, few overnight polysomnographic studies have been performed in patients affected by ALS[13,15], and a complete evaluation of the ANS during sleep and of sleep EEG microstructure through the study of the Cyclic Alternating Pattern (CAP)[16,17] is still lacking.

The present study aims to better characterize the clinical features of ALS beyond the motor features, through extensive evaluation of nocturnal sleep, including sleep quality, EEG oscillations and autonomic function during both sleep and quiet wakefulness.

Methods

Among the patients affected by ALS referred to the motor neuron disease outpatients service at the University of Cagliari, thirty-one were consecutively enrolled in the study at the Sleep Center, University of Cagliari. Diagnosis of ALS was made according to El-Escorial revised criteria.[18] Exclusion criteria are reported in Table 1. Twenty-six sex- and age-matched healthy controls were selected according to the criteria detailed in Table 1.

Each subject underwent a clinical and instrumental examination.

The study was approved by local ethical committee (protocol no. 2013/3205).

Clinical evaluation.

Each patient underwent thorough history, neurological and general examination, and questionnaires for screening sleep disorders:[19] Epworth Sleepiness Scale (ESS),[20] Pittsburgh Sleep Questionnaire Index (PSQI),[21] Berlin Questionnaire (BQ),[22] Restless Legs Syndrome Diagnostic Interview (RLSDI), and if positive Restless Legs Syndrome Severity Rating Scale (IRLSRS),[23] REM behavior Disorder Screening Questionnaire (RBDSQ)[24]. ALS severity was measured by the Revised ALS Functional Rating Scale (ALSFRS-revised),[25] and by the ALS Severity Scale (ALSSS)[26]). The meaning of these questionnaires and evaluating scales is detailed in Table 2.

Instrumental evaluation.

Each subject underwent a full-night video-polysomnography (video-PSG) recording (Morpheus – Micromed®), carried out in a standard sound-attenuated sleep laboratory, attended by an expert technician. The following data were included in the PSG study: EEG (3 channels: one frontal, one central, and one occipital, referred to the contralateral earlobe); electrooculogram (electrodes placed 1 cm above the right cantus and 1 cm below the left cantus); electromyogram (EMG) of submental muscle, EMG of bilateral tibialis anterior muscle; one single-lead ECG. During the same night, sleep respiratory pattern was assessed by means of nasal airflow (nasal pressure cannula), thoracic and abdominal respiratory effort, and oxygen saturation (pulse-oximetry).

Sleep stages, respiratory and legs activity were scored manually by a clinical neurophysiologist expert in sleep medicine (PC), following standard criteria.[27]

The CAP scoring was automatically performed with the Embla® RemLogic™ PSG Software (Embla Systems) and then visually checked and manually corrected on the basis of standard criteria [17] by a neurologist expert in sleep medicine (GM). The following CAP variables were measured: CAP time (time of CAP in NREM sleep), CAP rate (the percentage ratio of CAP time to total NREM sleep time) in total NREM sleep, number of A phases, and the prevalence of each phase A subtype (A1, A2, A3). For more details on the scoring rules of CAP see Fig S1.

Quantitative analysis of HRV was performed in Matlab (The Mathworks, Natick, MA) using the ECG signal by a biomedical engineer (SM).

Two separate HRV analyses were performed for each subject: a global analysis of the whole night, starting from sleep onset and ending at sleep offset and an analysis of 5-minute segments of the RR. The 5-minutes segments were flagged as belonging to wake, NREM, light NREM sleep (N1 and N2), deep NREM sleep (N3) and total NREM sleep if at least 80% of the segment was scored as the corresponding sleep stage. Mixed segments were discarded. Then, for each subject, we calculated the median value of each feature over the epochs corresponding to a certain sleep stage. We decided to perform both a stage-to-stage analysis and a full-night analysis for several reasons. In fact, full-night analysis allows to look at sleep as a "continuum" state independent from the empiric division

into well-defined and stiff stages of 30 seconds, thus offering a more comprehensive look at longer-term dynamics (e.g. VLF power), which can otherwise be limited by the duration of each single sleep stage. On the other hand, the stage-to-stage analysis allows to point up all the continuous autonomic changes in correlation to the specific sleep stages.

The included HRV features were recommended by the Task Force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology:[28] 7 time-domain features: mean NN interval (NN), standard deviation of the NN intervals (SDNN), standard deviation of the average NN intervals (SDANN), mean of the 5-minute standard deviations of NN intervals calculated over the entire recording (SDNN index), square root of the mean squared differences of successive NN intervals (RMSSD), proportion of interval differences of successive NN intervals greater than 50 ms (pNN50) and proportion of interval differences of successive NN intervals greater than 20 ms (pNN20); and 5 frequency-domain features: Very low frequency (VLF), low frequency (LF), high frequency (HF), the total spectral power (total power), and the LF/HF ratio (LF/HF).[29–32] Further information about HRV analysis is reported in Method S1.

We elected to calculate both time- and frequency-domain HRV features since each class complements the other in terms of the information it provides on the cardiac autonomic control. In fact, while there are significant known correlations between time- and frequency-domain features, e.g. between pNN50 and HF power, or between SDANN and total power, the full ensemble provides the optimal description of the activity of the ANS.

Statistical analysis

The statistical analyses were performed using Stata software (version 13, StataCorp, College Station, US), Prism 7 for Mac OS X - GraphPad software and Matlab (Mathworks, Natick, MA) with a type I error set at 0.05. Continuous parameters were expressed mean ± standard-deviation or median [interquartile range], according to statistical distribution. The assumption of normality was evaluated using D'Agostino-Pearson Normality test. Description of specific tests used is reported in

the results section and in the tables' legends. More precisely, the comparisons between patients and controls were conducted using Student t-test or Mann-Whitney test if the assumptions of t-test were not met, HRV features extracted for the whole night and for the different sleep states were compared between ALS patients and healthy controls by means of a non-parametric Kruskal-Wallis t-test. The homoscedasticity was analyzed using the Fisher-Snedecor test. As proposed by some statisticians (Feise, 2002; Rothman, 1990)[33,34] a particular focus was also given to the magnitude of differences, in addition to inferential statistical tests expressed using p-values. Then, the assessment of relationships between quantitative parameters (for example between HRV features in time- and frequency- domain of whole night recording and ALSFRS score, or between clinical parameters and sleep scoring parameters) was measured using Spearman's correlation coefficient, applying a Sidak's type I error correction for multiple comparisons. For correlated data (several measures for a same subject), random-effects models were carried out to take into account between and within patient variability. The normality of residuals was checked for each random-effects model. When appropriate, a logarithmic transformation was proposed to achieve the normality of dependent variables.

Results

Thirty-one patients (mean age 63.94 y ± 10.17; 12 F, 19 M; with mean age at disease onset of 60.74 y ± 10.93) satisfied eligibility criteria and were enrolled in the study.

They were compared to 26 control subjects similar for age and sex (mean age 62.19 y ± 13.93; 13 M). Among the 26 control subjects, only 23 underwent to complete CAP and HRV assessment due to recordings artifact in the other 3.

Demographic and clinical data are reported in Table 3.

Clinical evaluation

ALS patients presented a mean ± std ALS-FSR score of 32.38 ± 8.86, and a mean ± std ALSSS score of 29.17 ± 5.42. At disease onset 4 patients had a predominantly bulbar localization, 4 patients had a generalized localization, and the other ones had a predominantly spinal localization. Patients and controls had similar ESS score (5.39 ± 3.45 vs 5.04 ± 2.25; p=0.6604), and PSQI (8.9 ± 4.4 vs 8.46 ± 2.30; p=0.6492), nineteen patients had high risk of OSAS according to BQ, while no patients received a clinical diagnosis of RLS or RBD.

Sleep architecture

Sleep parameters found in the two groups are reported in Table 4.

Compared to control subjects, ALS patients presented an impaired sleep structure characterized by decreased SE – sleep efficiency (p<0.0001), due to a shorter TST – total sleep time (p=0.0025) and to an increased amount of WASO – wake after sleep onset (p<0.0001). The distribution of sleep stages was also altered with respect to the control subjects, with increased percentage of sleep stage N1 (p<0.0001) and a decreased percentage of REM sleep (p<0.0001). Finally, the N1/N3 ratio (the ratio between the percentage of N1 and N3 sleep stages) was inverted and about four times higher in patients, mostly due to an increased amount of sleep stage N1 in patients (p<0.0001).

The hypnogram of a representative patient is illustrated in Fig 1a, showing an irregular sleep macrostructure characterized by repetitive stage shifts, numerous awakenings of different duration, increased N1 sleep stage and decreased REM sleep.

Respiratory and movement scoring parameters are reported in Table S1.

Heart rate and breathing rate of patients and control subjects are reported in Table 4-bis.

Sleep microstructure (CAP)

CAP parameters are reported in Table 5.

Compared to control subjects, patients affected by ALS had a significant reduction of CAP time ($p=0.0096$) and CAP rate ($p=0.011$), a decreased number of total A phases ($p=0.0015$), a decreased percentage of A1 phases ($p<0.0001$), an increased percentage of A3 phases ($p<0.0001$), and an inverted A1/A3 ratio (the ratio between the percentage of A1 and A3) ($p<0.0001$), which was three times lower in ALS patients than in controls. (see Fig 1b).

Quantitative Analysis of Heart Rate Variability

Table 6 reports the p-values from the Kruskal-Wallis test for features calculated for the whole night and using the median of 5-min windows for wakefulness, NREM, REM, light sleep and deep sleep. Concerning the full night analysis, pNN20, LF and LF/HF were significantly lower in ALS patients compared to controls.

State-specific analysis showed that during wakefulness, SDNN, SDNN index, RMSSD, pNN50, pNN20, total power, VLF, LF and HF were all lower in ALS patients. During NREM sleep, SDNN, pNN20, total power, VLF, LF and HF were lower in ALS patients compared to controls. Similar results, with the exception of SDNN, were found for light sleep, while in deep sleep, only HF was significantly lower in the ALS cohort compared to controls. During REM sleep, SDNN, RMSSD, pNN50, pNN20, total power, LF, HF, and LF/HF were significantly lower in ALS compared to controls.

There were no significant differences in the comparison between HRV features among sleep stages for patients and for controls except for VLF power in patients and for SDNN, total power and LF/HF ratio in control subjects (see Tables S2 and S3).

Correlation analysis

Correlation analysis between clinical parameters (ESS, PSQI, disease duration) and sleep scoring parameters (TST, SE, SL, WASO%, N1%, N2%, N3%, REM%) showed a positive correlation between ESS scores and the percentage of N3 sleep stage, while a negative correlation emerged

between PSQI and the percentage of N2 sleep stage. No correlations were found between both ESS and PSQI and TST and SE. Moreover, a positive correlation was found between disease duration and the percentage of WASO as well as a negative correlation between disease duration and TST.

A positive correlation was found between ALSFRS score and percentage of N2 sleep stage and a negative correlation between ALSFRS score and percentage of N3 sleep stage (Table 7).

In ALS patients, correlation analysis between ALSFRS score and HRV features showed a positive correlation between ALSFRS score and VLF, by contrast, no correlations were observed between HRV parameters and disease duration.

In ALS patients, no correlations were found between CAP parameters and clinical parameters (ALSFRS and disease duration) or HRV parameters.

In control subjects, correlation analysis between CAP and HRV features showed a positive correlation trend, although not statistically significant, between some CAP features, such as A1% and A3% and A1/A3 ratio, and some HRV parameters, such as total power, VLF and LF both during light sleep (N1 and N2 sleep stages) and during the entire NREM sleep.

Discussion

Although motor dysfunction is predominant in ALS, involvement of other systems including ANS has been documented.[3–6,8–10] Sleep is the ideal condition for studying the ANS, but only few overnight PSG studies have been carried out in patients affected by ALS[13,15] none of which evaluating sleep microstructure. Moreover, to our knowledge, this is the first extensive evaluation of ANS during sleep, with regard to sleep stages (N1, N2, N3, and REM), nocturnal wakefulness and CAP parameters.

The ALS patients of our study showed a marked disruption of sleep architecture, characterized by repetitive stage transitions (Fig. 1a), decreased SE and TST, reduced percentages of REM sleep, and increased amounts of WASO and stage N1. Enhancement of N1 led to an inversion of the N1/N3 ratio ($p<0.0001$), that is approximately four-fold higher compared to healthy controls,

indicating a predominance of light sleep in ALS patients, while deep sleep appeared statistically unaffected in percentage but highly fragmented and randomly distributed throughout the night. These results are in accordance with previous findings in ALS patients.[35,36] Another interesting finding is the unusual distribution of the N3 sleep stage along the night, that in normal conditions N3 is predominant in the first part of the night, conversely, in these patients, it is spread throughout the sleep recording time, being almost totally missing in some cases (Fig 1a). The abnormal behavior of the homeostatic sleep process may be related to the increased amounts of wakefulness and stage shifts in the first part of the night and to the reduction of CAP A1 subtypes, conditions that hamper the build-up and consolidation of slow wave sleep in the first part of the night.[37] Accordingly, our preliminary results[38] showed that patients affected by ALS with the most severe pathology had the most unstable sleep patterns, with % of high frequency band (HFC) < 4%, evaluated by means of cardio-pulmonary coupling (CPC),[39] indicating a more arousable sleep.

Impairment of sleep microstructure was another interesting finding in our ALS patients. Compared to healthy controls, ALS patients presented a significant reduction of CAP time and CAP rate and an inversion of A1/A3 ratio due to the reduced amounts of A1 subtypes and the increased amounts of A3 phases. The strong relationship between ANS activity and sleep microstructure,[40] the "inverted" CAP-related A1/A3 ratio and the predominance of light sleep indicate a shift toward an increased sympathetic tone and a reduction of parasympathetic activity during sleep in ALS patients. In addition, a subclinical involvement of cardiac ANS, mostly of vagal nerve efferents, and was demonstrated by HRV assessment during both sleep (NREM and REM) and wakefulness. In fact, compared to healthy controls, ALS patients presented a reduction of HRV features, both in time and frequency domain, influenced by sympathetic and vagal systems. In particular, full-night analysis revealed a reduction of pNN20 (a feature dependent on vagal activity, correlated to the HF component)[28], and of LF component (a feature that reflects baroreceptor, vagal and sympathetic influences)[41], and of LF/HF ratio, which is considered a marker of the sympatho-vagal balance

(although this assumption is not totally accepted, mostly because LF power is not a pure index of the ANS drive)[41].

State-specific analysis showed that wakefulness, NREM light sleep and REM sleep were characterized by a reduction of several time- and frequency-domain features, with alteration of the LF/HF ratio during REM sleep, indicating a global impairment of cardiac ANS in patients affected by ALS. In deep sleep, only LF was significantly lower in ALS group, reflecting the reduced amounts of subtypes A1, which physiologically dominate the SWS. Interestingly, the total-power, that mirrors the global reactivity of the HR, was significantly lower in patients compared to controls during both wakefulness and sleep and during both light NREM sleep and REM sleep, indicating a reduction of the variability of HR. Accordingly, SDNN, pNN20 and HF power, which are influenced by vagal activity, were decreased in patients during wakefulness, light NREM sleep and REM sleep.

Overall, these findings indicate that patients affected by ALS present a subclinical dysfunction of the cardiac autonomic function and a reduction of HRV likely due to vagal impairment, in accordance with previous reports on the involvement of parasympathetic system in ALS patients.[4,42] It is well known that decreased HRV is associated with increased risk in mortality for cardiac sudden arrest,[5,6] and low HRV is a strong predictor of increased mortality for coronary events, which occurs more frequently at night.[5] Therefore, impairment of ANS in ALS patients might represent a possible risk factor for sudden death during sleep.

The negative correlation between severity of disease (ALSFRS score) and the power of VLF of the whole night, indicates that the worse the ALS severity the lower the VLF power. VLF power is strongly correlated with the time-measure SDNNI, but its uncertain underlying physiological mechanism seems to be generated by the cardiac intrinsic nervous system with a contribution from the parasympathetic nervous system and influences by the sympathetic nervous system. [41] This data is remarkable because VLF rhythms seem to be fundamental for health, and low VLF power has

been associated with all-cause mortality, including arrhythmic death. Basing on this result patients with the more severe ALS may have a higher risk of sudden death due to arrhythmias. I

In our study no other correlations have been found between HRV features and disease severity and disease duration, indicating that impairment of cardiac autonomic control in ALS may start early and in all stages of the disease.

The lack of correlation between curtailed PSG parameters, such as TST and SE, and daytime sleepiness (measured by ESS) and perceived quality of sleep (measured by PSQI) was another interesting finding. These results could be partially explained by the preservation of N3 percentages (similar to healthy controls) and by a cognitive impairment linked to the reduction of the A1 components of CAP, which are involved in sleep-related memory processes.[43,44]

In healthy controls, CAP and HRV features showed a positive correlation trend between A1%, A3% and A1/A3 ratio with total-power, VLF and LF. In contrast, no significant correlation between CAP metrics and HRV parameters was not found in ALS patients, suggesting a degeneration of the central neural network involved in the generation of CAP and in the control of the ANS, that seem to be functionally interconnected.[40] In particular, the thalamo-cortical pathways, which modulate the transient EEG oscillations that characterize the phases A of CAP,[40] are known to be linked to the limbic system and a large area extended from hypothalamus to medulla oblungata.[45] Involvement of hypothalamus in ALS has been documented by several studies showing hypothalamic TDP-43 inclusions or hypothalamus atrophy (also present in familial pre-symptomatic gene carriers) and appears to be correlated to early onset and disease progression.[7]

The impairment of vagal cardiac autonomic system, demonstrated by decreased HRV features, and the absence of correlation between HRV features and disease duration indicate that in our ALS patients the involvement of ANS is primitive, and not directly correlated with disease progression, although it may worsen with the latter stages of the disease, as shown by the correlation between ALSFRS and VLF power.

In conclusion, the disruption of interconnected networks due to a degenerative process conduct to an imbalance of the ANS which promotes a severe macro- and microstructural sleep fragmentation which in turn contributes, like a "vicious cycle", to an increased instability of the autonomic balance (Fig 2).

Our study confirms that ALS is likely a multisystem neurological syndrome since the early stages of the disease. Therefore, during the diagnostic process it is recommended to investigate, beside the motor system, also sleep, cognitive and autonomic functions.

Taking into account the risk of sudden death during sleep due to decreased HRV, the relationships between disturbed sleep and cognitive deficits[43,46] in neurodegenerative diseases, the well-known association between cognitive deficits and poor prognosis in ALS,[47] and the impact of nocturnal hypoventilation in anticipating respiratory failure,[11] it is recommended, during routine exams, to assess autonomic function and sleep, in order to early identify and adequately treat any comorbidity, aiming at improving the quality of life and possibly prolong life expectancy.

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List of abbreviations:

AHI: apnea-hypopnea index

ALS: amyotrophic lateral sclerosis

ALSFRS: amyotrophic lateral sclerosis functional rating scale

ALSSS: amyotrophic lateral sclerosis severity scale

ANS: autonomic nervous system

A1: phase A1 of cyclic alternating pattern

A2: phase A2 of cyclic alternating pattern

A3: phase A3 of cyclic alternating pattern

BQ: Berlin questionnaire

BR: breathing rate

CAP: cyclic alternating pattern

ECG: electrocardiogram

EEG: electroencephalogram

EMG: electromyography

ESS: Epworth sleepiness scale

HF: high frequencies

HR: heart rate

HRV: heart rate variability

IRLSRS: international restless legs syndrome severity rating scale

LF: low frequencies

NCAP: not CAP

NN: intervals between normal R peaks of QRS

NREM: not REM sleep

N1: N1 phase of NREM sleep

N2: N2 phase of NREM sleep

N3: N2 phase of NREM sleep

NS: not statistically significant

ODI<3%: oxygen desaturation index below 3%

pNN20: proportion of interval differences of successive NN intervals greater than 20 ms

pNN50: proportion of interval differences of successive NN intervals greater than 50 ms

PSG: polysomnography

PSQI: Pittsburgh sleep questionnaire index

RBDSQ: REM behavior disorder screening questionnaire

REM: rapid eye movement sleep stage

RLSDI: restless legs syndrome diagnostic interview

RMSSD: square root of the mean squared differences of successive NN intervals

RR: interval between R peaks of QRS

SDANN: standard deviation of the average NN interval

SDNN: standard deviation of the NN intervals

SE: sleep efficiency

SL: sleep latency

SpO2: peripheral saturation of O2

TDP-43: Trans-Activator Regulatory DNA binding protein 43 kDa

TL90: percentage of TST spent with SpO2 below 90%

TST: total sleep time

VLF: very low frequencies

WASO: wakefulness after sleep onset

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Tables legend

Table 1

Exclusion criteria for patients and control subjects

Table 2

Questionnaires and evaluation scales used for evaluating sleep and disability

Table 3: demographic and clinical data of the 31 patients enrolled for CAP and HRV analysis.

Legend: ALSFRS: Amyotrophic Lateral Sclerosis – Functional Rating Scale; ALSSS: Amyotrophic Lateral Sclerosis Severity Scale; ESS: Epworth Sleepiness Scale; PSQI: Pittsburgh Sleep Questionnaire Index; ns: not statistically significant

For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used. Differences were considered significant when p value were <0.05.

Table 4: sleep scoring parameters in patients and controls.

Legend: TST: Total Sleep Time; SL: Sleep latency; SE: sleep efficiency; WASO: wake after sleep onset. N1/N3: the ratio between the percentage of N1 and N3 sleep stages; AHI: apnea-hypopnea inex; ODI<3%: oxygen desaturazion below 3% index; mean SpO2: mean value of SpO2 recorded during the night; TL90%: percentage of time spent with SpO2 below 90%; [a]significance based on Student's t-test; [b]significance based on Mann-Whitney test; NS: not statistically significant.

For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used for data with Gaussian distribution, Mann-Withney test was used for data without

normal distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p value were <0.05.

Table 4-bis Heart rate and respiratory rate parameters in patients and scoring

Legend: HR: heart rate; LS: light sleep; N1: the phase N1 of NREM sleep; N2: the phase N2 of NREM sleep; DS: deep sleep equivalent to the phase N3 of NREM sleep; BR: breathing rate; NS: not statistically significant. For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used for data with Gaussian distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p value were <0.05.

Table 5: CAP (Cyclic Alternating Pattern) scoring parameters in patients and controls.

Legend: A1/A3: the ratio between the percentage of A1 and A3 phases of CAP; [a]: significance based on Student's t-test; [b]: significance based on Mann-Whitney test; NS: not statistically significant.

For comparison of parameters obtained by CAP scoring between patients and controls Student's t test was used for data with Gaussian distribution, Mann-Withney test was used for data without normal distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p value were <0.05.

Table 6: p-values from the Kruskal-Wallis test between ALS and controls. The top part of the table reports values of the HRV features calculated for the whole night, while the bottom part reports values computed on 5-min epochs and then grouped by sleep state using median values. P values below 0.05 are highlighted in bold and represent significant differences.

Legend: *Time Domain Features*: MeanNN: Mean of all the NN intervals; SDNN: standard deviation of the NN intervals over the entire recording; SDANN: standard deviation of the average

NN intervals calculated over 5 minutes; SDNN index: mean of the 5-minute standard deviations of NN intervals calculated over the entire recording; RMSSD: square root of the mean squared differences of successive NN intervals; pNN50: proportion of interval differences of successive NN intervals greater than 50 ms; pNN20: proportion of interval differences of successive NN intervals greater than 20 ms. *Frequency Domain Features*: Tot power: the total spectral power of the HRV between 0 Hz and its Nyquist frequency; VLF: amplitude of the HRV power spectrum in the very low frequency (<0.04 Hz) range; LF: Amplitude of the HRV power spectrum in the low frequency (0.04-0.15 Hz) range; HF: Amplitude of the HRV power spectrum in the high frequency (0.15-0.4 Hz) range; LF/HF: the ration between HF to LF.

Table 7: Assessment of correlation between HRV features in time- and frequency- domain of whole night recording and ALSFRS score of patients affected by ALS.

Legend: ALSFRS: Amyotrophic lateral sclerosis- functional rating scale; CI: confidence interval; NS: not statistically significant.

Parameters obtained by sleep scoring (not shown I the table), CAP scoring (not shown in the table) and HRV analysis were correlated with each other (not shown in the table) and with clinical features, namely ALSFRS score, PSQI and ESS by calculating the Spearman correlation coefficient (Spearman's r).

Figure Legend

Figure 1

Hypnogram (**a**) and graphic representation of Cyclic Alternating Pattern (**b1, b2**), and heart rate variability (**c**) of 1-night video-PSG of a patient affected by ALS.

A1, A2, A3: phases A of CAP; B: phase B of CAP; NCAP: brain electrical EEG without CAP

Figure 2

Graphic representation of relationship between sleep, autonomic nervous system and cyclic alternating pattern.

In subjects with ALS the degeneration process conducts to an imbalance of the ANS which move towards (causing) a severe macro and microstructure sleep fragmentation that in turn contribute, like a "vicious cycle", to increase the sympathetic instability.

Supplementary material

Fig S1: Phases A of CAP

Method S1: HRV assessment

Table S1: Respiratory and movement data of PSG of patients and control subjects

Table S2: Comparison between time- and frequency-domain features among the different sleep stages in patients

Table S3: Comparison between time- and frequency-domain features among the different sleep stages in control subjects

Figure 1

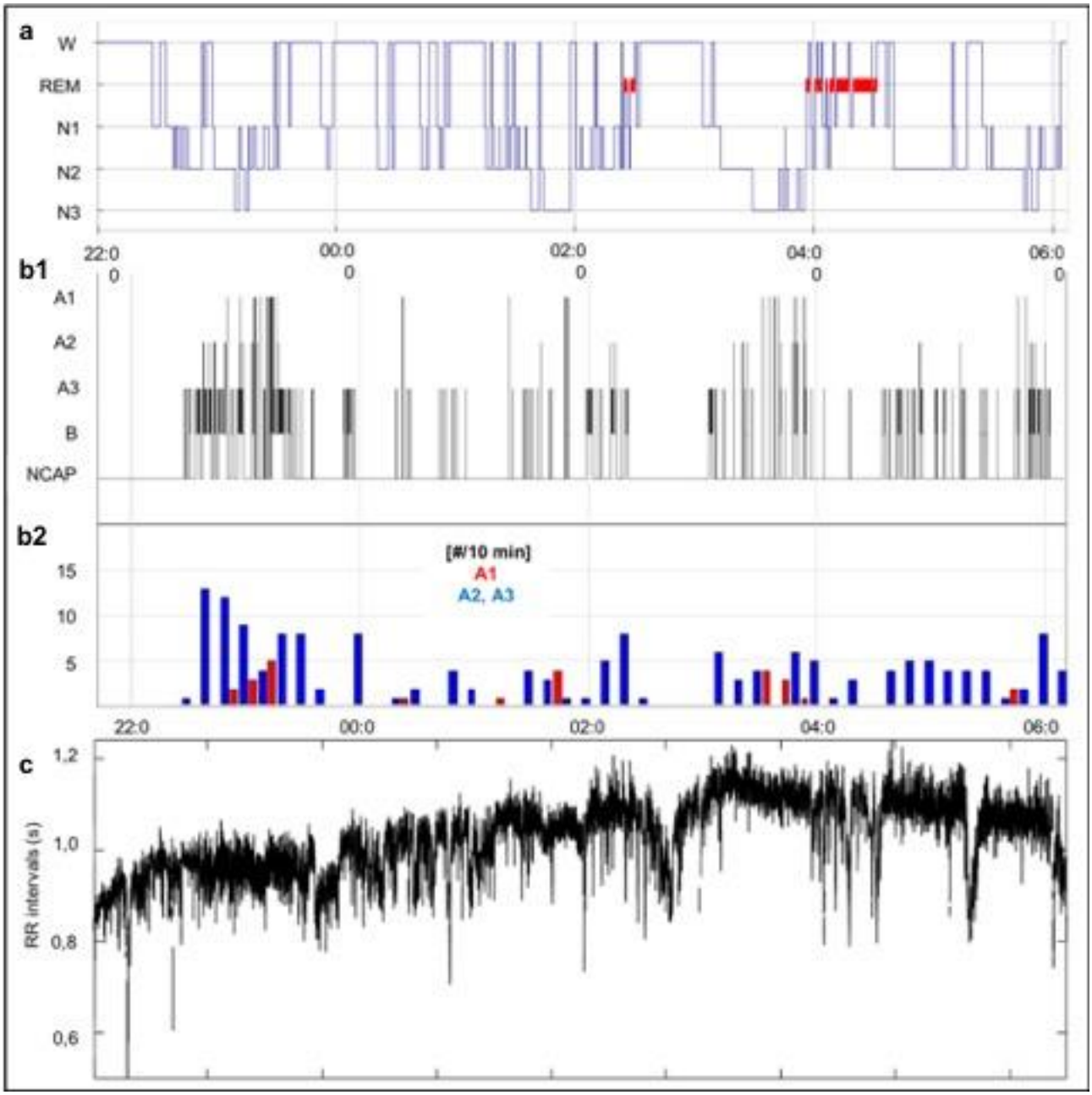

Figure 2

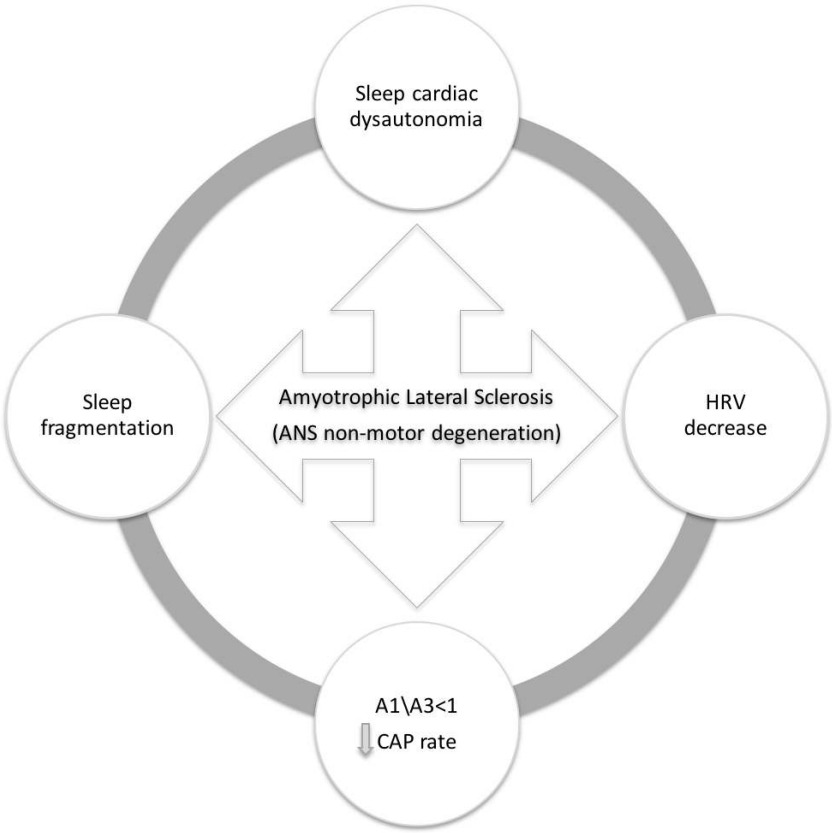

Table 1 Exclusion criteria for patients and control subjects

Exclusion criteria for patients	Exclusion criteria for control subjects
Age<18 years Pregnant female patients Patients who denied consent Comorbidities: cancer, alterations of heart rhythm, coronary artery disease, heart failure, diabetes Psychiatric disorders, kidney or liver disease, sleep disorders (Apnea-Hypopnea Index >15, Periodic Leg Movements Index> 15) Drugs and medicaments: cancer and cardioactive drugs (namely beta-blockers, beta-agonists, calcium channels blockers, antiarrhythmic drugs, antidepressants, antipsychotics, AchE inhibitors, alpha receptors agonists and antagonists). Patients under NIV (not invasive ventilation) Patients whose severe clinical conditions did not allow one night in the sleep lab	Age<18 years Pregnant females Denied consent Comorbidities: any cardiac disorders, diabetes or diagnosed psychiatric disorders, kidney or liver disease, cancer, any sleep disorders No intake of medication affecting the ANS and all psychotropic drugs affecting EEG and CAP.

Table 2 Questionnaires and evaluation scales used for evaluating sleep and disability

Questionnaire/scale	Significance
Epworth Sleepiness Scale – ESS	A self-administered questionnaire that assesse diurnal sleep propensity
Pittsburgh Sleep Questionnaire Index – PSQI	A self-administered questionnaire that evaluates sleep habits of the previous month
Berlin Questionnaire – BQ	A screening test for OSAS (obstructive sleep apnea syndrome)
Restless Legs Syndrome Diagnostic Interview – RLSDI	A 4-questions interview required for diagnosing RLS
Restless Legs Syndrome Severity Rating Scale – IRLSRS	A 10-items interview for assessing the presence and the severity of the RLS
REM behavior Disorder Screening Questionnaire – RBDSQ	A self-administered questionnaire that evaluates the presence of RBD
Revised ALS Functional Rating Scale – ALSFRS-revised	A 12- or 13-items scale (depending on the presence of respiratory insufficiency) that evaluates the severity of the disability due to pregressive motor neurons degeneration ALS
ALS Severity Scale – ALSSS	A 4-items scale that evaluates the severity of the disability due to pregressive motor neurons degeneration ALS

Table 3 demographic and clinical data of the 31 patients enrolled for CAP and HRV analysis.

	Patients (n=31)	Controls (n=26)	
	mean ± S.D.	**mean ± S.D**	**p value**
Age (years)	63.94 ± 10.17	62,19 ± 13.93	0.2740 (ns)
ESS	5.39 ± 3.45	5.04 ± 2.25	0.6604 (ns)
PSQI	8.90 ± 4.44	8.46 ± 2.30	0.6492 (ns)
Age at disease onset (years)	60.74 ± 10.93		
Disease duration (years)	3.26 ± 4.78		
ALSFRS	32.38 ± 8.86		
ALSSS	29.17 ± 5.42		

Legend: ALSFRS: Amyotrophic Lateral Sclerosis – Functional Rating Scale; ALSSS: Amyotrophic Lateral Sclerosis Severity Scale; ESS: Epworth Sleepiness Scale; PSQI: Pittsburgh Sleep Questionnaire Index; ns: not statistically significant

For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used. Differences were considered significant when p value were <0.05.

Table 4 sleep scoring parameters in patients and controls.

	Patients (n=31)	Controls (n=26)	
	mean ± S.D.	**mean ± S.D.**	**p value**
TST (min)	346.73 ± 101.31	417.56 ± 69.01	**0.0025**[a]
SL (min)	31.5 ± 23.57	49.66 ± 133.48	NS (0.1416)[b]
SE (%)	68.27 ± 16.81	84.77 ± 13.83	**<0.0001**[b]
WASO (% of SPT)	25.78 ± 14.08	7.24 ± 4.66	**<0.0001**[a]
N1 (% of TST)	20.83 ± 9.59	7.60 ± 4.71	**<0.0001**[a]
N2 (% of TST)	41.23 ± 10.68	43.97 ± 10.50	NS (0.6070)[a]
N3 (% of TST)	22.38 ± 11.22	26.22 ± 8.64	NS (0.2326)[a]
REM (% of TST)	15.55 ± 8.09	21.49 ± 5.88	**0.0065**[a]
N1/N3 %	1.52 ± 2.06	0.36 ± 0.36	**<0.0001**[b]

Legend: TST: Total Sleep Time; SL: Sleep latency; SE: sleep efficiency; WASO: wake after sleep onset. N1/N3: the ratio between the percentage of N1 and N3 sleep stages; AHI: apnea-hypopnea inex; ODI<3%: oxygen desaturazion below 3% index; mean SpO2: mean value of SpO2 recorded during the night; TL90%: percentage of time spent with SpO2 below 90%; [a]significance based on Student's t-test; [b]significance based on Mann-Whitney test; NS: not statistically significant.

For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used for data with Gaussian distribution, Mann-Withney test was used for data without normal distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p value were <0.05.

Table 4-bis Heart rate and respiratory rate parameters in patients and scoring

	Patients (n=31)	Controls (n=26)	
	mean ± S.D.	**mean ± S.D.**	**p value**
HR whole night	68.33 ± 9.32	64.95 ± 6.48	NS (0.1489)
HR NREM	66,73 ± 8.58	64.01 ± 6.60	NS (0.2011)
HR LS (N1+N2)	66.60 ± 8.62	63.40 ± 6.19	NS (0.1263)
HR DS	67.37 ± 8.52	65.53 ± 7.85	NS (0.4157)
HR REM	66.91 ± 9.45	65.38 ± 5.85	NS (0.4857)
HR wake	74.07 ± 8.40	71.27 ± 7.60	NS (0.2044)
BR whole night	20.76 ± 3.81	16.93 ± 2.28	<0.0001
BR NREM	20.39 ± 3.64	16.77 ± 2.28	<0.0001
BR LS (N1+N2)	20.55 ± 3.80	16.76 ± 2.24	<0.0001
BR DS (N1+N2)	19.86 ± 3.60	16.75 ± 2.28	<0.001
BR REM	21.26 ± 4.75	17.18 ± 3.13	<0.001
BR wake	21.89± 4.06	18,77 ± 3.13	<0.01

Legend: HR: heart rate; LS: light sleep; N1: the phase N1 of NREM sleep; N2: the phase N2 of NREM sleep; DS: deep sleep equivalent to the phase N3 of NREM sleep; BR: breathing rate; NS: not statistically significant. For comparison of parameters obtained by sleep scoring between patients and controls Student's t test was used for data with Gaussian distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p values were <0.05.

Table 5 CAP (Cyclic Alternating Pattern) scoring parameters in patients and controls.

	Patients (n=31)	**Controls (n=23)**	
	mean ± S.D	**mean ± S.D**	**p value**
CAP time (min)	100.52 ± 65.21	141.29 ± 36.97	**0.0096**[a]
CAP rate	32.07 ± 18.02	43.10 ± 10.23	**0.011**[a]
A phases – total (n°)	206.58 ± 145.33	291.26 ± 79.67	**0.0015**[b]
A1 %	28.78 ± 16.51	51.59 ± 13.65	**<0.0001**[b]
A2 %	20.15 ± 8.32	19.03 ± 665	NS (0.5958)[a]
A3 %	51.07 ± 19.40	29.38 ± 12.03	**<0.0001**[b]
A1/A3 %	0.80 ± 0.72	2.53 ± 1.33	**<0.0001**[b]

Legend: A1/A3: the ratio between the percentage of A1 and A3 phases of CAP; [a]: significance based on Student's t-test; [b]: significance based on Mann-Whitney test; NS: not statistically significant.

For comparison of parameters obtained by CAP scoring between patients and controls Student's t test was used for data with Gaussian distribution, Mann-Withney test was used for data without normal distribution. Gaussian distribution of data was evaluated by means of D'Agostino & Pearson Normality test. Differences were considered significant when p value were <0.05.

Table 6 p-values from the Kruskal-Wallis test between ALS and controls.

The top part of the table reports values of the HRV features calculated for the whole night, while the bottom part reports values computed on 5-min epochs and then grouped by sleep state using median values. P values below 0.05 are highlighted in bold and represent significant differences.

Whole night analysis

	MeanNN	SDNN	SDANN	SDNNi	RMSSD	pNN50	pNN20	TotPWR	VLF	LF	HF	LF/HF
	0.178	0.184	0.461	0.068	0.236	0.173	**0.027**	0.194	0.073	**0.049**	0.665	**0.010**

5 min epochs - median analysis (Sleep state analysis)

	MeanNN	SDNN	SDANN	SDNNi	RMSSD	pNN50	pNN20	TotPWR	VLF	LF	HF	LF/HF
wake	0.276	**0.002**	0.074	**0.001**	**0.034**	**0.039**	**0.012**	**0.002**	**0.004**	**0.001**	**0.011**	0.120
NREM	0.361	**0.033**	0.095	0.081	0.120	0.097	**0.033**	**0.036**	**0.023**	**0.005**	**0.049**	0.962
LS	0.184	**0.015**	**0.047**	**0.033**	0.073	0.064	**0.010**	**0.017**	**0.009**	**0.003**	**0.019**	0.962
DS	0.381	0.152	0.106	0.354	0.259	0.540	0.109	0.162	0.157	**0.040**	0.152	0.919
REM	0.728	**0.017**	0.244	0.065	**0.012**	**0.011**	**0.017**	**0.016**	0.055	**0.000**	**0.014**	**0.027**

Legend: *Time Domain Features*: MeanNN: Mean of all the NN intervals; SDNN: standard deviation of the NN intervals over the entire recording; SDANN: standard deviation of the average NN intervals calculated over 5 minutes; SDNNi: mean of the 5-minute standard deviations of NN intervals calculated over the entire recording; RMSSD: square root of the mean squared differences of successive NN intervals; pNN50: proportion of interval differences of successive NN intervals greater than 50 ms; pNN20: proportion of interval differences of successive NN intervals greater than 20 ms. *Frequency Domain Features*: Tot power: the total spectral power of the HRV between 0 Hz and its Nyquist frequency; VLF: amplitude of the HRV power spectrum in the very low frequency (<0.04 Hz) range; LF: Amplitude of the HRV power spectrum in the low frequency

(0.04-0.15 Hz) range; HF: Amplitude of the HRV power spectrum in the high frequency (0.15-0.4 Hz) range; LF/HF: the ration between HF to LF.

Table 7 Assessment of correlation between HRV features in time- and frequency- domain of whole night recording and ALSFRS score of patients affected by ALS.

Whole night	ALSFRS vs meanNN	ALSFRS vs SDNN	ALSFRS vs SDANN	ALSFRS vs SDNNi	ALSFRS vs RMSSD	ALSFRS vs pNN50	ALSFRS vs pNN20	ALSFRS vs totPower	ALSFRS vs VLF	ALSFRS vs LF	ALSFRS vs HF	ALSFRS vs LF/HF
Spearman r	0.26	0.23	0.12	0.36	0.12	0.14	0.25	0.25	0.38	0.33	0.25	-0.03
95% CI	-0.12 to 0.58	-0.16 to 0.56	-0.27 to 0.48	-0.01 to 0.66	-0.27 to 0.47	-0.25 to 0.49	-0.14 to 0.57	-0.14 to 0.57	0.01 to 0.66	-0.05 to 0.63	-0.14 to 0.57	-0.40 to 0.35
P value (two-tailed)	NS (0.17)	NS (0.22)	NS (0.53)	NS (0.052)	NS (0.55)	NS (0.48)	NS (0.19)	NS (0.195)	**0.0414**	NS (0.08)	NS (0.19)	NS (0.86)

Legend: ALSFRS: Amyotrophic lateral sclerosis- functional rating scale; CI: confidence interval; NS: not statistically significant.

Parameters obtained by sleep scoring (not shown I the table), CAP scoring (not shown in the table) and HRV analysis were correlated with each other (not shown in the table) and with clinical features, namely ALSFRS score, PSQI and ESS by calculating the Spearman correlation coefficient (Spearman's r).