

# 1 **Regularity of cardiac rhythm as a marker of** 2 **sleepiness in sleep disordered breathing**

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## 24 **Abstract**

25

26 The present study aimed to analyse the autonomic nervous system activity using heart  
27 rate variability (HRV) to detect sleep disordered breathing (SDB) patients with and  
28 without excessive daytime sleepiness (EDS) before sleep onset.

29

30 Two groups of 20 patients with different levels of daytime sleepiness -sleepy group,  
31 SG; alert group, AG- were selected consecutively from a Maintenance of Wakefulness  
32 Test (MWT) and Multiple Sleep Latency Test (MSLT) research protocol. The first  
33 waking 3-min window of RR signal at the beginning of each nap test was considered for  
34 the analysis. HRV was measured with traditional linear measures and with time-  
35 frequency representations. Non-linear measures -correntropy, CORR; auto-mutual-  
36 information function, AMIF- were used to describe the regularity of the RR rhythm.  
37 Statistical analysis was performed with non-parametric tests.

38

39 Non-linear dynamic of the RR rhythm was more regular in the SG than in the AG  
40 during the first wakefulness period of MSLT, but not during MWT. AMIF (in high-  
41 frequency and in Total band) and CORR (in Total band) yielded sensitivity > 70%,  
42 specificity >75% and an area under ROC curve > 0.80 in classifying SG and AG  
43 patients.

44

45 The regularity of the RR rhythm measured at the beginning of the MSLT could be used  
46 to detect SDB patients with and without EDS before the appearance of sleep onset.

## 47 **Introduction**

48

49 Sleep-disordered breathing (SDB) is a common disorder with a range of harmful  
50 sequelae [1]. One of the most important symptoms is excessive daytime sleepiness  
51 (EDS) which has been related to an increase of driving accidents, psychosocial  
52 morbidity and poor quality of life [2-4]. Despite its relevance in clinical management,  
53 evaluation of EDS is hindered by the lack of a simple objective method.

54 Subjective sleepiness scales are easy to fill out but correlate poorly with objective  
55 measures [5, 6] because patients sometimes are unaware of their sleepiness or it is  
56 confounded with fatigue or depression [7]. In contrast, the multiple sleep latency test  
57 (MSLT) [8] and the maintenance of wakefulness test (MWT) [9] which are accepted as  
58 the gold standards to objectively assess EDS, are relatively complex and expensive to  
59 perform on daily routine. Thus, there is a pressing need to develop simplified objective  
60 methods that could be used broadly in clinical and real-life scenarios.

61

62 Recent studies suggest that changes in the level of sleepiness are associated with  
63 changes in autonomic nervous system (ANS) activity [10,11]. For instance, somnolent  
64 SDB patients have an abnormal sympatho-vagal balance during sleep [11] and an  
65 increased sympathetic tone during daytime wakefulness that normalizes after  
66 continuous positive airway pressure (CPAP) treatment [12]. This suggests that the  
67 structural alterations and dysfunction in central autonomic regulatory regions occurring  
68 in SDB might contribute to EDS [13].

69

70 In this context, ANS activity could be a potential candidate to measure EDS in SDB.

71 The simplest way to monitor ANS activity is by measuring the heart rate variability

72 (HRV), which describes fluctuations in autonomic inputs to the heart over time. It is  
73 measured by the variation in the beat-to-beat (RR) interval in the electrocardiogram  
74 (EKG) [14]. Different methods have quantified HRV. From the traditional linear  
75 measures to the more sophisticated time-frequency representation and non-linear  
76 techniques.

77

78 *Mean heart rate (HR)*, a simple time-domain measure, gradually decreases as sleep  
79 begins and achieves its lower value when stable N2 sleep stage appears [15-18]. In  
80 preadolescents a significant decreasing in heart rate even occurs as earlier as 30 seconds  
81 before the appearance of stage N1 [15]. It has also been described that subjects with  
82 longer sleep latencies in the MSLT and MWT present an increased HR at the beginning  
83 of each test [19-21]. Moreover, using some *frequency-domain measures*, Bonnet et  
84 Arand also found an increased sympatho-vagal balance in the non-sleepy subjects,  
85 without changes in the parasympathetic nervous system activity [22]. These findings in  
86 healthy adults suggest that measurements of ANS activity during wakefulness periods  
87 could help to study the EDS associated to SDB.

88

89 *Non-linear methods* have been developed recently to describe non-linear fluctuations in  
90 heart rate and inform about the regularity of heart rate time series [23]. It has been  
91 reported that non-linear dynamics of EEG signal during the first wakefulness period at  
92 the beginning of the MSLT is more regular (i.e. lower complexity) in SDB patients with  
93 objective EDS than in those without EDS [24]. However, little is known about the non-  
94 linear dynamics of cardiac activity related to EDS.

95

96 Using HRV measures, we aimed to find possible markers of ANS activity that could  
97 anticipate sleep onset in SDB patients and, therefore, detect patients with and without  
98 objective EDS. We analysed the first 3-min waking periods of the MWT and the MSLT  
99 to perform the study.

## 100 **Materials and methods**

101

### 102 **Subjects**

103 From a series of 98 consecutive patients with suspected SDB evaluated at the  
104 Multidisciplinary Sleep Disorders Unit of the Hospital Clinic of Barcelona, two groups  
105 of 20 consecutive patients each were selected, based on mean sleep latencies from a  
106 MWT-MSLT research protocol. The sleepy group (SG) consisted of the most somnolent  
107 patients who have both low MSLT ( $< 8$  min) and low MWT ( $< 20$  min) sleep latencies  
108 while the alert group (AG) represented the least somnolent patients with the higher  
109 MWT ( $\geq 20$  min) and MSLT ( $\geq 8$  min ) sleep latencies. Patients with discordance  
110 between MWT and MSLT scores (patients with MWT  $\geq 20$  min and MSLT  $< 8$  min or  
111 MWT  $< 20$  min and MSLT  $\geq 8$  min) were considered partially sleepy and were not  
112 included in the analysis. Exclusion criteria were age under 18 years, major medical or  
113 psychiatric disorders, use of beta-blockers or medications affecting wakefulness or  
114 sleep, and working in shifts or with irregular sleep-wake schedules during the four  
115 weeks before the sleep study. Nocturnal polysomnography (PSG) excluded any  
116 concomitant sleep disorder other than SDB.

117

118 The study was approved by the Hospital Clinic of Barcelona ethics committee (Comité  
119 Ètic Investigació Clínica (CEIC)) and written informed consent was obtained from all  
120 participants.

121

122

123

## 124 **Design**

125 Patients arrived to the sleep lab at 6 pm and underwent a 24-hour sleep study.  
126 Subjective daytime sleepiness and mood disorders were assessed using the Epworth  
127 Sleepiness Scale and the Hospital Anxiety and Depression Scale. After nocturnal PSG,  
128 a MWT-MSLT research protocol was conducted to quantify EDS throughout the day.  
129 An overview of the protocol is shown in **Table 1**.

130

131

132 Nocturnal PSG was performed according to standard practice parameters and diagnostic  
133 criteria [25, 26]. We recorded EEG (O2-A1, O1-A2, C4-A1, C3-A2, F4-A1, F3-O2),  
134 electrooculography, EKG, chin and right and left anterior tibialis surface  
135 electromyography, and synchronized audiovisual recording. Cannula, thermistor,  
136 abdominal and thoracic strain gauges, and finger pulse oximeter were used to measure  
137 respiratory variables. Apnea was defined as a complete cessation of airflow, measured  
138 using thermistor, for  $\geq 10$  sec. Hypopnea was defined as  $\geq 30\%$  reduction in nasal  
139 pressure signal excursions from baseline and associated  $\geq 3\%$  desaturation from pre-  
140 event baseline or an arousal. The apnea-hypopnea index (AHI) was the number of  
141 apneas plus hypopneas per hour of sleep. Sleep stages were manually scored according  
142 to Rechtschaffen and Kales criteria using 30-s epochs.

143

## 144 **MSLT and MWT protocol**

145 Patients underwent 5 trials of MWT followed by a research version MSLT [8, 27],  
146 every two hours starting at 8:30 am. We recorded in total 200 MSLT and 200 MWT  
147 naps. The order in which the tests took place was the same for all subjects. Between  
148 naps, patients were allowed to leave their rooms and stay in the waiting area,

149 performing routine activities or interacting with other patients in a quiet way. They were  
150 advised to avoid sleep between naps and technicians ensured this. Caffeine beverages  
151 were not allowed.

152

153 For the MWT, patients were seated at 45°, and were instructed to “*remain awake for as*  
154 *long as possible*”. For the MSLT, patients were instructed to “*lie quietly in a*  
155 *comfortable position and try to fall asleep*”. Test conditions, light intensity and  
156 temperature followed the standard recommendations from AASM 2005 [28]. Additional  
157 EKG was recorded using a 2-channel bipolar monitoring system with one electrode  
158 placed 2 cm below the right clavicle and the other 2 cm below the left clavicle.

159

160 If no sleep occurred MWT and MSLT trials were ended after 40 and 20 minutes  
161 respectively, or after unequivocal sleep, defined as three consecutive epochs of stage 1  
162 sleep, or one epoch of any other stage of sleep. Objective daytime sleepiness was  
163 measured from sleep latency defined as time from lights out to the first epoch of  
164 unequivocal sleep in each test.

165

## 166 **Assessment of Heart Rate Variability**

167 The RR series, intervals between consecutive beats, were obtained from each EKG nap  
168 recording with a sampling frequency of 256 Hz. After removing artifacts and ectopic  
169 beats, RR signals were resampled at 4 Hz. Naps with sleep latencies shorter than two  
170 minutes or EKG artifacts could not be analysed and were excluded. Then, the first  
171 waking 3-min window of RR signal at the beginning of each nap test was considered for  
172 the analysis whenever possible; otherwise we decided to fix a minimum window size of  
173 2-min.



174 Heart rate variability was described by measures obtained from traditional time-domain  
175 analysis (mean and standard deviation of RR interval), power spectral analysis in  
176 frequency-domain (individual low frequency and high frequency spectral power and  
177 low frequency to high frequency spectral powers ratio) [14] and Time-Frequency  
178 Representations (TFR) based on Choi-Williams Distribution [29]. Non-linear measures  
179 - correntropy (CORR) and auto-mutual-information function (AMIF) - were used to  
180 describe the regularity of the RR signal since they are suitable to be constructed based  
181 on short-term series [30, 31]. The applied methodology, the parameters involved in the  
182 calculation of TFR, CORR and AMIF are shown in the **Supporting Information File**.  
183 All these measures were calculated in the following frequency bands: low frequency  
184 (LF: 0.04-0.15 Hz), high frequency (HF: 0.15-0.4 Hz) and total band (TB: total  
185 frequency band). The analysis in the very low frequency band (<0.04 Hz) was not  
186 performed because 5-min of RR signal is the minimal window recommended for this  
187 purpose [14].

188

189 Since the present study was carried out analyzing only one short-length window of RR  
190 for each nap, the stationarity does not represent a significant problem [14]. A final  
191 check by visual inspection was carried out in order to ensure the analysis of artifact-free  
192 RR epochs.

193

## 194 **Data and statistical analysis**

195 Mean values of HRV measures of all MWT and all MSLT naps for each patient were  
196 considered for the analysis. They could be calculated if at least 3 MSLT and 3 MWT  
197 naps had available data.

198

199 Heart rate variability measures were compared between AG and SG using Mann-  
200 Whitney  $U$  test and within each group (between the MWT and the MSLT) with  
201 Wilcoxon signed-rank test. Bonferroni correction was applied and a significance level  
202  $p$ -value  $< 0.004$  was taken into account. Those HRV parameters that significantly  
203 differed between groups were evaluated throughout the day to confirm the results  
204 obtained in the average analysis. Associations between HRV measures and mean sleep  
205 latencies were evaluated with Spearman rank-order test, with a statistical significance  
206 assumed for  $p < 0.05$ .

207

208 A discriminant function was built with those HRV parameters that significantly differed  
209 between groups. The leaving-one-out method was performed as a validation method.  
210 Sensitivity ( $Sen$ ) and specificity ( $Spe$ ) were calculated for testing the performance of the  
211 measures. The proportion of SG patients correctly classified was counted by  $Sen$  and the  
212 proportion of AG patients correctly classified by  $Spe$ . The area under the ROC curve  
213 ( $AUC$ ) was also used to test the performance of the measures. The ROC curve was  
214 computed for the results of the predictions calculated with a logistic regression  
215 classification using a generalized linear model. The model was built by fitting a  
216 generalized linear regression of the predicted classes on the measures, using a normal  
217 distribution [32].

## 218 **Results**

219 Patient's characteristics and PSG results are shown in **Table 2**. Most patients were male  
220 and overweight. The SG was slightly younger and tended to have more subjective  
221 complaints of daytime sleepiness in comparison to the AG. All subjects slept well, with  
222 mean sleep efficiency higher than 80% and more than 6 h of sleep. Sleep structure was  
223 similar in both groups, but the longer stage 2 sleep latency in the AG. There was a wide  
224 spectrum of disease severity in both groups but the mean AHI and the associated  
225 oxygen desaturation index tended to be higher in the SG than in the AG, without  
226 achieving statistical significance. As expected by selection criteria, SG had shorter sleep  
227 latencies than the AG: MWT ( $11.5 \pm 4.54$  min *versus*  $35.3 \pm 6.33$  min,  $p$ -value < 0.001)  
228 and MSLT ( $4.4 \pm 1.96$  min *versus*  $11.66 \pm 2.41$  min,  $p$ -value < 0.001).

229  
230 Of the 400 naps recorded, thirty naps (7.5%) had sleep latencies shorter than 2 minutes  
231 or had EKG artefacts that did not allow interpreting the RR signal. Three subjects from  
232 the SG did not have the minimal HRV measures required (at least 3 MSLTs and 3  
233 MWTs naps with available data) and were excluded from the analysis. Regarding the  
234 window size of the RR signal, 344 from the remaining 370 available naps (93%) were  
235 analysed using three minutes and in the other 26 out naps with latencies between 2 and  
236 3 minutes the window size equalled the length of sleep latency.

237  
238 Differences between groups occurred exclusively during the MSLT (**Table 3**). We  
239 found that AMIF (in Total and HF band) and CORR (in Total Band) showed a more  
240 regular RR rhythm in the SG than in the AG ( $p < 0.004$ , after Bonferroni correction).  
241 This behaviour was confirmed in each of 5 MSLT naps throughout the day ( $p < 0.004$   
242 after Bonferroni correction). During the MWT, the RR rhythm was similar in both

243 groups. Differences between nap tests mainly occurred in the SG, showing a more  
244 regular RR rhythm during MSLT than during MWT in AMIF (in all frequency bands,  $p$ -  
245 range  $<0.001 - 0.002$ ) and CORR (in Total band,  $p < 0.001$ ). In the AG, no differences  
246 were observed between MWT and MSLT except for the AMIF in HF band, which  
247 showed an increased regularity of the RR rhythm during the MSLT ( $p < 0.001$ ). **Figure**  
248 **1(A)** shows the evolution of AMIF in HF band in both groups throughout the whole nap  
249 protocol.

250

251 No differences between groups were observed in any traditional linear and TFR  
252 measures, either in MSLT or MWT. However, we found that mean RR interval was  
253 longer (i.e. slower heart rate) during the MSLT than during the MWT, independently of  
254 the sleepiness group: the SG ( $985.2 \pm 149.6$  ms and  $937.9 \pm 138.2$  ms,  $p < 0.001$ ) and  
255 the AG ( $964.4 \pm 94.9$  ms and  $921.3 \pm 97.3$  ms,  $p < 0.001$ ). **Figure 1(B)** shows the  
256 evolution of mean RR interval in both groups throughout the nap protocol.

257

258 Correlation analysis showed that patients with shorter MSLT sleep latency had higher  
259 regularity of the RR rhythm. The best correlations were found with AMIF in HF band  
260 ( $\rho -0.49$ ,  $p$ -value = 0.002) and AMIF in Total band ( $\rho -0.47$ ,  $p$ -value = 0.003),  
261 followed by CORR in Total band ( $\rho -0.41$ ,  $p$ -value = 0.01) (**Figure 2**).

262

263 Each of the three measures yielded a  $Sen \geq 70\%$ ,  $Spe > 75\%$  and  $AUC > 0.80$   
264 discriminating the AG from the SG. However, AMIF in Total and HF bands achieved  
265 slightly better results than CORR in Total band (see **Table 4**).

266

267

## 268 **Discussion**

269

270 To our knowledge, this is the first study that uses linear and non-linear measures applied  
271 to RR signal in order to detect SDB patients with and without objective EDS. Using this  
272 approach we have demonstrated that the regularity of the RR rhythm during the first 3-  
273 min of wakefulness at the MSLT allows differentiating the sleepy from the alert  
274 patients. In contrast, in a situation where sleep latencies were much longer as occurs  
275 during the MWT, non-linear dynamics did not differ between groups.

276

277 We have observed that both AMIF (in HF and Total band) and CORR (in Total band)  
278 functions showed an increased regularity of the RR rhythm in sleepy patients, in  
279 comparison to alert patients during the first wakefulness period at the beginning of the  
280 MSLT. In a previous study, Melia U et Al. evaluated the regularity (conversely, the  
281 complexity) of the EEG signal using CORR functions and found that sleepy patients  
282 had a more regular EEG signal (analyzed in the  $\beta$  band) in the occipital region than alert  
283 patients also during the same nap test [24]. The findings obtained with the analysis of  
284 RR interval go in the same direction than those from the EEG, suggesting a common  
285 mechanism. We hypothesize that the increased regularity observed in the SG during the  
286 first waking 3-min could reflect the autonomic changes occurring with the proximity of  
287 sleep onset. The SG had shorter sleep latencies than the AG ( $4.4 \pm 1.96$  minutes *versus*  
288  $11.66 \pm 2.41$  minutes, respectively) and, therefore, even at the beginning of the test they  
289 were much closer to the EEG sleep onset than the AG. We confirmed these association  
290 with the correlation analysis showing that the RR signal was more regular when MSLT  
291 sleep latency was shorter (i.e. when sleep onset was closer). The lack of differences  
292 between groups at the MWT may also support our hypothesis. During this test, patients

293 are instructed to remain awake and sleep latencies are expected to be longer, as we  
294 observed in our study (MWT sleep latencies were longer than 10 minutes in both  
295 groups). However, we cannot exclude other factors that characterize MWT, such as the  
296 body position, the open eyes or the environmental dim-light that could have conditioned  
297 our results during this test.

298

299 We failed to observe differences between groups in traditional linear and TFR  
300 measures. Our results contrast with a study performed in healthy young adults that  
301 showed an increased heart rate and a higher sympatho-vagal balance in the alert subjects  
302 during MSLT (MSLT sleep latency < 7min), compared to the sleepy subjects (MSLT  
303 sleep latency < 7min) [22]. However, the sample, subject's age, nap protocol and  
304 window size of our work differ from that study and may have influenced the results. In  
305 another study performed in SDB patients with and without EDS during nocturnal sleep,  
306 Lombardi et al. [11] showed that the sleepy group had an increased cardiac sympatho-  
307 vagal balance (low frequency to high frequency spectral power ratio) throughout the  
308 whole night. We assumed that during wakefulness, confounding factors that may  
309 influence the cardiac rhythm such as sleep-related respiratory events are not to be  
310 expected. However, we cannot discard that breathing instability typical of the transition  
311 from wakefulness to sleep may have occurred in some patients during the measurement  
312 of HRV and, therefore, conditioned the results. Another work by Donadio et al. [13],  
313 who evaluated the muscle sympathetic nerve activity by microneurography, determined  
314 that severity of EDS in sleepy SDB patients was related to daytime sympathetic  
315 hyperactivity. In our study, we failed to find similar results due in part to the different  
316 protocols and techniques used to measure ANS activity, since Donadio et al. had not an  
317 alert SDB group to compare with.

318

319 Despite the mean RR interval was unable to differentiate between the AG and SG, it  
320 varied between MSLT and MWT regardless the level of sleepiness. The mean RR  
321 interval was reliably shorter (i.e. heart rate was faster) during the MWT, without  
322 associated changes in other traditional linear measures. It has been argued that the  
323 differences between tests are related to changes in physiological level of arousal. In fact,  
324 specific methodological conditions distinguish each test, promoting to remain awake in  
325 MWT and to fall asleep in MSLT. From this point of view, the effect of upright tilt-up  
326 [33, 34] and its combination with the instruction to remain awake could explain the  
327 increase in heart rate, accompanying the longer sleep latencies typically observed during  
328 the MWT [21]. We have also corroborated the influence of the time of day on heart rate,  
329 with special emphasis during the digestion in the 4<sup>th</sup> block of MSLT/MWT [21]. At that  
330 moment, heart rate was almost identical for MSLT and MWT in both groups and  
331 achieved its highest values of all day (i.e. lowest RR interval) (see **Figure 1(B)**).

332

333 This study has several inherent limitations. First, we could not analyze HRV with the  
334 recommended 5-min windows of RR signal because of the nature of our investigation  
335 [14]. The SG, which was characterized by very short periods of wakefulness before the  
336 appearance of sleep onset, showed sleep latencies shorter than 5 minutes in 65 of 100  
337 MSLT naps that prevented the calculation of mean HRV values in this group and the  
338 comparison with the AG. The selection of window size of 3-min increased the number  
339 of available naps to evaluate HRV but, again, the most somnolent patients did not show  
340 enough RR signal in 40 of 100 MSLT naps. Therefore, we decided to find a  
341 compromise between the window length analyzed and the number of naps excluded  
342 from the analysis. To maximize the number of available MSLT naps in the SG, we

343 included those naps with sleep latencies between 2 and 3 minutes and we evaluated  
344 HRV with a window size that equaled the length of sleep latency. In this way, we could  
345 get more information about the HRV associated to sleepiness in the SG and thus, to  
346 calculate the mean HRV values and compare the groups. A second limitation is that we  
347 did not monitor breathing during naps and thus, we cannot exclude the inclusion of  
348 occasional windows containing sleep-related respiratory events that could have  
349 influenced our results. This cannot be, however, a major problem because the MWT and  
350 MSLT protocols that we used were ended as soon as the patients fall asleep. Finally,  
351 care must be taken with the discrimination ability of the AMIF and CORR functions to  
352 identify each group, considering the small number of subjects and the lack of validation  
353 set.

354

355 There is a clear need for a simple and practical tool that could be routinely administered  
356 during wakefulness to diagnose those individuals with EDS and to prevent the  
357 undesirable consequences related to EDS. In the clinical practice, MSLT is considered  
358 the reference test for objectively measure daytime sleepiness and is mainly based on  
359 EEG. However, it requires more than 10 electrodes correctly placed on the scalp and  
360 face and a trained technician to interpret the signals. In our study, we have evaluated  
361 EDS with a simpler an easier to record signal than EEG. Two EKG derivations and a 3-  
362 min window of waking RR signal recorded at the beginning of MSLT were enough to  
363 detect significant differences in regularity of cardiac rhythm between the AG and the  
364 SG. De Gennaro et al. have also measured the oculomotor activity (another easy to  
365 record biological signal) during the first waking 150-sec from MSLT and found that a  
366 decrease in spontaneous blinking and an increase in slow eye movements were  
367 associated with shorter sleep latencies [35] These two works reflect the potential



368 applicability of alternative biological signals to EEG for monitoring sleepiness in the  
369 clinical practice and diagnose EDS. Furthermore, the reliable detection of drowsiness in  
370 real-life scenarios such as driving has received increased interest in the last few decades,  
371 mainly for the purpose of preventing driving accidents and errors [36]. Although several  
372 automatic detection methods exist, those that employ biological signal processing are  
373 the most feasible because they inform about the body's response to drowsiness. Some  
374 high-risk professions such as professional drivers could benefit from these automatic  
375 detectors of sleepiness for preventing accidents at the wheel. We propose that the  
376 development of new EKG indexes based on AMIF and correntropy functions may allow  
377 the automatic detection of sleepiness in this setting. However, we are still far and further  
378 studies should be addressed.

379

380

381 In conclusion, non-linear dynamics of the RR rhythm may detect those SDB patients  
382 with and without EDS before the appearance of MSLT sleep onset. Larger studies  
383 including different degrees of daytime sleepiness, and different window sizes of RR  
384 signal analysis would be of interest to elucidate the importance of non-linear measures  
385 of HRV in the identification of EDS while the subject is awake. The evaluation along  
386 the entire wake-sleep transition during sleep latency tests and also during other  
387 scenarios (i.e. driving simulations) should also be tested to elucidate if the findings  
388 observed at the beginning of the test would remain stable or changed as sleep onset  
389 approaches.

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## 486 **Figure Legends**

487

488 **Figure 1. Representation of (A) AMIF-HF and (B) mean RR interval throughout**  
489 **the 5 blocks of MWT and MSLT.**

490 Sleepy group, in blue, alert group, in red. In graphic (A), SG shows an increased  
491 regularity of RR rhythm during almost all nap tests in comparison to AG, especially at  
492 the MSLT. Within each group, however, there is a type of nap test effect, with increased  
493 values at the MSLT in comparison to MWT. In graphic (B), both groups show a  
494 reliably longer mean RR interval (i.e. slower heart rate) during MSLT as compared to  
495 MWT, but there are no differences between groups in any test. The lowest values of all  
496 naps are seen during the 1<sup>st</sup> and 4<sup>th</sup> block, after breakfast and lunch time. Abbreviations:  
497 SG, sleepy group; AG, alert group; AMIF-HF, Auto-mutual information function in  
498 high frequency band; mean RR, mean RR interval; MWT, maintenance of wakefulness  
499 test; MSLT, multiple sleep latency test.

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502 **Figure 2. Correlation between (A) AMIF-TB, (B) AMIF-HF, and (C) CORR-TB**  
503 **with sleep latency from MSLT of all subjects, when using averages.**

504 In blue the SG, in red the AG. Regression lines are represented in black. Note that  
505 patients with shorter sleep latencies showed an increased regularity of the RR rhythm  
506 (*rho* in: (A) -0.47, (B) -0.49, (C) -0.41,  $p < 0.05$ ). Abbreviations: Auto-mutual  
507 information function (AMIF) in: total band (AMIF-TB), high-frequency band (AMIF-  
508 HF); Correntropy function in total band (CORR-TB); MSLT, Multiple Sleep Latency  
509 Test.

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# Tables

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**Table 1. Sleep Study Design**


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<b>18:00</b>	<b>Enter to Sleep Lab</b>
<b>18:30</b>	<b>ESS, HAD</b>
<b>19:00</b>	<b>Electrodes placement</b>
<b>20:00</b>	<i>Dinner</i>
<b>23:00</b>	<b>Start PSG</b>
<b>7:30</b>	<b>End PSG</b>
<b>7:30 – 8:15</b>	<i>Breakfast</i>
<b>8:30 – 9:10</b>	<b>1<sup>st</sup> MWT</b>
<b>9:30 – 9:50</b>	<b>1<sup>st</sup> MSLT</b>
<b>10:30 – 11:10</b>	<b>2<sup>nd</sup> MWT</b>
<b>11:30 – 11:50</b>	<b>2<sup>nd</sup> MSLT</b>
<b>12:30 – 13:10</b>	<b>3<sup>rd</sup> MWT</b>
<b>13:30 – 13:50</b>	<b>3<sup>rd</sup> MSLT</b>
<b>13:50 – 14:15</b>	<i>Lunch</i>
<b>14:30 – 15:10</b>	<b>4<sup>th</sup> MWT</b>
<b>15:30 – 15:50</b>	<b>4<sup>th</sup> MSLT</b>
<b>16:30 – 17:10</b>	<b>5<sup>th</sup> MWT</b>
<b>17:30 – 17:50</b>	<b>5<sup>th</sup> MSLT</b>

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ESS, Epworth Sleepiness Scale; HAD, Hospital Anxiety and Depression Scale PSG, Polysomnography; MWT, Maintenance of Wakefulness Test; MSLT, Multiple Sleep Latency Test.

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**Table 2. Clinical and PSG characteristics: descriptive data and differences between groups.**

	<b>SLEEPY GROUP</b>	<b>ALERT GROUP</b>	<b>p-values</b>
<b>CLINICAL VARIABLES</b>			
Sex (Male/Female)	14/6	14/6	NS
Age (years old)	53.4 ± 6.0	57.5 ± 7.8	*0.04
Body Mass Index (kg/ m <sup>2</sup> )	29,9 ± 4,5	29,2 ± 4.8	NS (0.48)
Epworth Sleepiness Scale	12.8 ± 3.9	10.9 ± 4.6	NS (0.09)
HADS-A	5.9 ± 2.8	4.9 ± 3.0	NS (0.24)
HADS-D	4,4 ± 3,3	2,9 ± 2.9	NS (0.09)
<b>SLEEP QUALITY</b>			
Time in Bed (min)	463.6 ± 29.0	468.0 ± 28.5	NS (0.75)
Total Sleep Time (min)	381,2 ± 75,7	372,5 ± 48.8	NS (0.34)
Sleep Efficiency (%)	81,1 ± 14,6	79,8 ± 8.9	NS (0.15)
Wake After Sleep Onset (min)	70,8 ± 54,2	69,5 ± 34.1	NS (0.36)
<b>SLEEP STRUCTURE</b>			
Stage 2 sleep latency (min)	18,0 ± 23,3	25,2 ± 19.9	*0.01
REM sleep latency (min)	132,1 ± 83,9	102,7 ± 67.3	NS (0.32)
Stage 1 (%)	17.7 ± 10.6	17.4 ± 9.0	NS (0.98)
Stage 2 (%)	59,3 ± 8,7	56,8 ± 9.2	NS (0.81)
Stage 3 (%)	8.4 ± 6.7	11.4 ± 7.9	NS (0.28)
REM sleep (%)	14.7 ± 6.8	14.4 ± 6.0	NS (0.86)
Number of REM episodes	3,6 ± 1,6	3,7 ± 1.5	NS (0.99)
Number of Phase Changes	181.5 ± 68.5	171.8 ± 58.3	NS (0.83)
PLM Index (events/h)	9,0 ± 22,8	6,2 ± 11.8	NS (0.98)
<b>RESPIRATORY PARAMETERS</b>			
Arousal Index (events/h)	39,7 ± 22,9	32,4 ± 21.1	NS (0.21)
Apnea-Hypopnea Index (events/h)	40,1 ± 28,0	27,7 ± 26.9	NS (0.06)
Mean SaO <sub>2</sub> (%)	93,3 ± 2,1	93,2 ± 3.0	NS (0.61)
Cumulative time spend below a SaO <sub>2</sub> of 90%	10,5 ± 13,5	9,7 ± 16.7	NS (0.19)
Oxygen desaturation Index 3%	31,1 ± 25,0	22,0 ± 29.0	NS (0.08)
Nadir of Sa O <sub>2</sub>	77,8 ± 8,9	81,2 ± 12.0	NS (0.18)

With the exception of sex proportion all results are expressed as mean ± SD. Level of significance was for  $p < 0.05$ . NS, non-significant.

Abbreviations: HADS-A, hospital anxiety and depression scale – Anxiety; HADS-D, hospital anxiety and depression scale – Depression; PLM Index, periodic limb movements index; Sa O<sub>2</sub>, oxygen desaturation.

**Table 3. Linear, Time-Frequency Representation and Nonlinear measures at the beginning of MSLT and MWT: descriptive data and differences between groups.**

	MSLT			MWT		
	SG	AG	<i>p</i>	SG	AG	<i>p</i>
<b>LINEAR MEASURES</b>						
MeanRRi (ms)	985,23 ± 149,61	964,39 ± 94,85	NS (0,915)	937,90 ± 138,16	921,32 ± 97,26	NS (0,796)
STDRRi (ms)	47,22 ± 18,69	36,29 ± 10,16	NS (0,091)	40,91 ± 18,52	38,56 ± 10,61	NS (0,819)
LF (a.u)	58,25 ± 11,85	56,98 ± 7,35	NS (0,532)	56,71 ± 10,00	57,56 ± 8,58	NS (0,915)
HF (a.u)	41,75 ± 11,85	43,02 ± 7,35	NS (0,532)	43,29 ± 10,00	42,44 ± 8,58	NS (0,915)
LF/HF ratio (a.u)	1,87 ± 0,88	1,73 ± 0,73	NS (0,615)	1,65 ± 0,71	1,82 ± 0,65	NS (0,410)
<b>TFR MEASURES</b>						
TFR-TB (ms <sup>2</sup> )	4582,56 ± 3914,34	2624,44 ± 1398,56	NS (0,156)	4417,47 ± 4366,42	3126,50 ± 2300,46	NS (0,522)
TFR-LF (a.u)	1,75E-05 ± 7,22E-06	1,48E-05 ± 2,82E-06	NS (0,532)	1,52E-05 ± 3,49E-06	1,36E-05 ± 2,69E-06	NS (0,120)
TFR-HF (a.u)	8,29E-06 ± 4,97E-06	5,79E-06 ± 1,76E-06	NS (0,065)	5,35E-06 ± 1,71E-06	4,90E-06 ± 1,99E-06	NS (0,353)
<b>NON-LINEAR MEASURES</b>						
AMIF-TB (a.u)	0,39 ± 0,04	0,35 ± 0,03	0,001*	0,36 ± 0,04	0,35 ± 0,02	NS (0,855)
AMIF-LF (a.u)	0,48 ± 0,04	0,44 ± 0,03	NS (0,004)	0,46 ± 0,04	0,45 ± 0,03	NS (0,474)
AMIF-HF (a.u)	0,34 ± 0,04	0,32 ± 0,02	0,001*	0,32 ± 0,02	0,30 ± 0,02	NS (0,075)
CORR-TB (a.u)	0,36 ± 0,05	0,31 ± 0,04	0,001*	0,30 ± 0,04	0,31 ± 0,07	NS (0,749)
CORR-LF (a.u)	0,28 ± 0,05	0,27 ± 0,03	NS (0,419)	0,25 ± 0,04	0,26 ± 0,04	NS (0,512)
CORR-HF (a.u)	0,36 ± 0,09	0,33 ± 0,05	NS (0,532)	0,34 ± 0,06	0,32 ± 0,06	NS (0,139)

Results are expressed as mean ± SD. After Bonferroni correction, level of significance was \*:  $p < 0.004$ . NS, non-significant.

Abbreviations: MSLT, multiple sleep latency test; MWT, maintenance of wakefulness test; meanRRi, mean RR interval; SDVRRi, standard deviation of the RR interval; LF(nu), low-frequency spectral power; HF(nu) high-frequency spectral power; LF/HF ratio, low-frequency to high-frequency spectral power ratio; TFR measures, Time-frequency representation in: total band (TFR-TB), low-frequency band (TFR-LF), high-frequency band (TFR-HF); Auto-mutual information function (AMIF) in: total band (AMIF-TB), low-frequency band (AMIF-LF), high-frequency band (AMIF-HF); Correntropy (CORR) in: total band (CORR-TB); low-frequency band (CORR-LF); high-frequency band. Units of measurement: ms, milliseconds; a.u, absolute units; ms<sup>2</sup>, square milliseconds.

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**Table 4. Discrimination between the AG and SG at the MSLT.**

	Sen (%)	Spe (%)	AUC
AMIF-TB	71	80	0,83
AMIF-HF	76	85	0,81
CORR-TB	71	75	0,81

Abbreviations: Sen, sensibility; Spe, specificity, AUC, area under the curve; Auto-mutual information function (AMIF) in: total band (AMIF-TB), ) and high-frequency band (AMIF-HF); Correntropy in total band (CORR-TB).