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Heart rate variability for assessing comatose patients with different Glasgow Coma Scale scores

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HIGHLIGHTS

- We study comatose patients classified into two subgroups according to the GCS, and compared with a normal control group, by calculating HRV indices in time, frequency and informational domains.
- In general, a notable reduction of HRV variability was found in patients, compared to normal subjects, and significant statistical differences between the subgroups of patients, classified according to GCS scores, were found for HRV indices in all domains.
- Our results are discussed regarding the progressive dysfunction the ANS networks when coma deepens.

ABSTRACT

Objective: To assess the autonomic nervous system (ANS) in coma by heart rate variability (HRV).

Methods: Sixteen comatose patients and 22 normal subjects with comparable ages and genders were studied. Patients were classified in two subgroups according to the Glasgow Coma Scale (GCS). Time, frequency, and informational HRV domain indices were calculated.

Results: A notable reduction of HRV was found in patients. Regarding the time domain indices, the triangular index, and the Delta_RRs, were significantly reduced in the subgroup with GCS = 3. Absolute power for the whole frequency spectrum decreased whenever GCS scores were lower. A significant decrement was found for absolute power of the VLF and LF bands in the subgroup of GCS = 3, and although it was lower for the HF band in these patients, those changes were not statistically significantly different. The LF/HF ratio and the Shannon's entropy indices were significantly reduced in the subgroup with GCS = 3. Our results are discussed regarding the progressive dysfunction the ANS networks when coma deepens.

Conclusions: The HRV procedure is a powerful tool to assess the ANS in comatose patients.

Significance: HRV is a minimally invasive, low-cost methodology, suitable for assessing the ANS in coma.

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1. Introduction

Several papers have appeared in the last few decades on the assessment of heart rate variability (HRV) in comatose patients

and in brain death (BD) (Biswas et al., 2000; Conci et al., 2001; Cooke et al., 2006; Freitas et al., 1996; Gujjar et al., 2004; Kahr-aman et al., 2010; Leipzig and Lowensohn, 1986; Li et al., 2003; Machado et al., 2005; Mejia and Pollack, 1995; Rapenne et al., 2000; Ryan et al., 2011; Shimomura et al., 1991; Su et al., 2005; Vakilian et al., 2011). Schwarz et al. (1987) using a time domain analysis found that the distribution of HRV and of heart rate showed a discrimination of the group of comatose patients from brain dead subjects and healthy subjects.

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Biswas et al. (2000) found that children with a Glasgow Coma Scale (GCS) score of 3–4 had a lower low-frequency/high-frequency (LF/HF) ratio compared with those who had a GCS score of 5–8, and that patients who progressed to BD had a markedly lower LF/HF ratio, with a significant decrease after the first 4 h of hospitalisation. Other patients with more favourable outcomes had significantly higher LF/HF ratios. These authors concluded that the LF/HF ratio may be helpful not only in identifying those patients who will progress to BD but also in predicting which patients will have favourable outcome.

Su et al. (2005) studied sympathetic and parasympathetic indices after severe head trauma, correlating HRV and GCS. However, they erred in stating that there had been no previous analysis of HRV in comatose patients grouped according to GCS (Machado et al., 2005). These authors correlated the parameters derived from spectral analysis of HRV with the GCS in five groups of patients with brain damage of various severities. They reported that an increase in severity of the brain-stem damage was accompanied with an augmentation of a sympathetic and a decrement of parasympathetic drives, as indicated by increasing of the LF band expressed in normalised units (%) and the ratio LF/HF and decreasing the HF band, respectively. Both LF and HF indices were nearly abolished in BD.

In early 1990s, we ran a protocol assessing comatose patients with different GCS scores, some progressing to BD (García et al., 1995). Thirty-five comatose patients with acute stroke were studied serially. We found that GCS scores increased or decreased ranging from 3 to 15, with either improvement or deterioration of coma. Patients were divided into four groups (GCS from 10 to 15; GCS from 7 to 9; GCS from 3 to 7; and BD). HRV was calculated using the variation coefficient (VC) calculated as $VC = (SD/M) \times 100$, where SD is the standard deviation of R–Rs in the sample and M the mean value for the time series of R–R intervals. Forty healthy subjects were also studied. We found that HRV did not progressively decrease when GCS scores diminished from 15 to 3 following a linear relationship, according to patients' evolution. HRV mean values and dispersion were higher in the group with GCS score ranging from 7 to 9 (García et al., 1995; Machado et al., 2005). A GCS score of about 8 has been related to a diencephalic level of consciousness impairment (Plum and Posner, 1980).

Nonetheless, in that study (García et al., 1995) we only analysed HRV indices in the time domain, and hence in this article we study comatose patients classified into two subgroups according to the GCS scores, compared with healthy subjects, assessing the autonomic nervous system (ANS) by calculating HRV indices in time, frequency and informational domains.

2. Methods

2.1. Subjects

A total of consecutive 21 comatose patients admitted in the intensive care unit of “Manuel Fajardo Rivero” Hospital (Havana) were initially enrolled in this study. Nonetheless, five patients were excluded according to the following criteria: patients with diabetes mellitus; cardiac arrhythmia; electrocardiographic signs of ischaemia; and antecedents of long-term use of drugs such as hypnotics, autonomic stimulants or alpha- or beta-blockers that may affect the autonomic system. It was not possible to elude those cases in which the use of inotropics and vasoconstrictors, to stabilise the arterial pressure was necessary. Hence, the final sample included 16 patients, nine females (56.25%) and seven males (43.75%), with an average age of 72.13 years and an SD of 10.94 years. Patients were also divided into two subgroups according to the GCS score: subgroup with GCS = 3, and subgroup with

the GCS score from 4 to 8. A group of 22 healthy subjects, 12 females (54.5%) and 10 males (45.5%), with a mean age of 71.20 and an SD of 9.8 years, was considered the control group. Informed written consent was obtained from patients' relatives and normal volunteers. The Ethical Committees of the Institute of Neurology and Neurosurgery, and of the “Manuel Fajardo Rivero” Hospital, approved the study.

All patients were neurologically examined and the GCS was applied to all cases. Only patients with a GCS score of 8 or less were enrolled in this study. A computed tomography (CT) scan of the brain was performed in all cases. Brain-dead patients were not included in the final sample, according to the Cuban Criteria for BD diagnosis (Machado, 2003, 2005, 2010; Machado et al., 2004).

2.2. ECG recording

Electrocardiography (ECG) was recorded with the MEDICID-05 with disposable electrodes placed on the chest in positions CM₂ and V₅ and using a sampling frequency of 200 Hz. Filters were set for a band spectrum of 0.5–50 Hz. The ECG was recorded in every session for 30 min.

2.3. ECG analysis

Bipolar ECG recordings were offline exported as ASCII files to a software tool developed by our staff written in Delphi version 7.0 (MultiTools version 3.1.2, 2009–2012), for visual inspection, detection of the ‘R’ wave peaks and enabling manual editing. Accurate ‘R’ peak automatic detections obtained with the software's algorithms were always visually checked and properly corrected, when it was necessary, by a member of our staff. To transform the ordinal sequential R–R interval (RRI) series into proper temporal series, the original RRI sequences were interpolated using the piecewise cubic Hermite interpolation method obtaining a sampling period of 205.078 ms. The entire consecutive sequences of RRI obtained using this procedure were stored digitally for further instrumental analysis.

2.4. Pre-processing of RRI sequences

The duration of the RRI temporal series used in this study was of 420 s. The recommended duration (Task Force, 1996) has been 300 s, but to study the very-low-frequency (VLF) spectral band, it was necessary to increase this duration. We decided at last to use 420 s considering that it allowed us measuring nine valid discrete spectral frequencies (DFi) from the spectra, after discarding the first 10 DFi, also a mandatory requisite stated by this Task Force (1996). These nine DFi were a reasonable amount of values for assessing a particular recently identified sub-band of the VLF range, from 0.027344 to 0.03125, particularly sensitive to predict the risk of occurrence of cardiac disturbances, such as ventricular tachyarrhythmia (Bilgin et al., 2010).

The first 2 min in the RRI series were discarded, and the subsequent 420 s were submitted to a stability control test of the frequency pattern of those segments, using a time–frequency method, as will be later described. If the selected segment did not show the necessary spectral stability, the commencement of the sample was displaced for periods of 30 s, and was newly tested. Only when the required stability was achieved, the segments were accepted for subsequent processing. In the group of patients it was not necessary to use alternative segments, but in the control group it was needed in three subjects, in whom displacements were required to relocate the optimal segments from 30 to 90 s. Stability was defined when power and frequency indices did not show ostensible changes for at least 80% of the whole analysed time of 420 s.

The temporal series finally selected for the spectral analysis were submitted to a pre-processing sequence of actions, as has been suggested by several authors (de Souza Neto et al., 2007), including (a) subtraction of the mean value from all the items in the RRI series included in the window to diminish the effect of the DC value and reduce the spectral zero-frequency; (b) application of a median filter to replace outliers or abnormal values; (c) standard linear detrending to avoid any possible drifts in the RRI series; and (d) high-pass digital filtering (low cut-off frequency 0.02 Hz) using a sixth-order Butterworth infinite impulse response filter, but using a zero-phase shifting algorithm to avoid the distortion of original phase components.

2.5. Calculations of HRV indices

2.5.1. Time domain HRV indices

HRV indices calculated in the time domain were also calculated as detailed elsewhere (Montes-Brown et al., 2010, 2012). Standard recommended HRV indices were considered: the mean RRI value (M), the SD and the square root of the mean squared differences of successive RRI intervals. It was also calculated the mode (Mo) for a bin of 5 ms, the amplitude of the mode (AMo), as the number of RRI in the series with values equal to the mode expressed as percent of the total number of RRI, the delta range (Delta_RRs), as the difference between the maximal RRI value and the minimal RRI value observed in the series, the stress index and the triangular index (Task Force, 1996).

2.5.2. Frequency domain HRV indices

Pre-processed temporal RRI series were submitted to spectral analysis using the Welch periodogram method using a Hann window with 2048 samples and the fast Fourier transform (FFT) algorithm. The spectral resolution was $1/420 = 0.00238095$ Hz. Selected processing parameters allowed the study of spectral frequencies from 0.02 to 0.4 Hz, including the VLF band from 0.023 to 0.04 Hz, the LF band from 0.04 to 0.085 Hz, the mid-frequency (MF) band from 0.085 to 0.15 Hz and the HF band from 0.15 to 0.40 Hz. Absolute and normalised (%) values were considered for each spectral band. The ratio LF/HF was also calculated.

2.5.3. Informational domain HRV indices

Shannon's entropy (ShaEn), an informational domain HRV index, following the new classification proposed by some authors (Bravi et al., 2011), was also calculated for every RRI interpolated temporal series. The classic expression used for developing the algorithm was

$$H = -\sum_{i=1}^N p_i \log_2 p_i \quad (1)$$

where P_i is the probability of every possible value of the duration of an RRI, and N is the total quantity of samples. If the events observed would be all equiprobable, then the maximal ShaEn would be 11 ($\log_2 2048$), and if all the RRIs would have the same value, ShaEn should be 0 ($(-1) \times (2048/2048) \times (\log_2 2048/2048) = 0$) (Clark et al., 2012; Takahashi et al., 2012; Viola et al., 2011).

2.5.4. HRV time-varying spectral analysis

To assess the stability of the spectral indices in every selected window of the RRI time series of 420 s a time-varying spectral method using one of the most used methods was carried out: the short time Fourier transform (STFT) (Elsenbruch et al., 2000). It can be considered as one of the most conventional and intuitive, among the different methods used for time–frequency analysis of temporal series (Mainardi, 2009; Martinmaki et al., 2006; Martinmaki and Rusko, 2008; Seely and Macklem, 2004). A sliding

window of 256 s with displacement steps of 2 s was used, yielding 83 consecutive spectra, which confirm a compressed spectral matrix that can be quantitatively analysed and graphically presented using 3-D surface spectrograms. Only the segments which verified stability over time were selected for the spectral analysis.

All digital signal data processing in this study was carried out using standard custom-tailored programs developed by our staff with MATLAB (MathWorks Inc. Version 7.10.0.499, R2010a).

2.6. Statistical analysis

Comparisons between the control group and the group of patients were achieved using the Mann–Whitney test. Differences were considered statistically significant for $p < 0.05$. The Spearman rank correlation test was used to analyse relationships between different HRV variables and the GCS items. The package STATISTICA, version 8.0 (Statsoft Inc. 2007), was used for statistical analysis.

3. Results

Brain injury aetiology was intracerebral haemorrhage in eight patients, ischaemic cerebral infarct in four, post-anoxic encephalopathy in two and metabolic encephalopathy in two cases. The subgroup with GCS = 3 included six patients, and the subgroup with GCS score ranging from 4 to 8, 10 cases (three cases with 4, three with 6, one with 7 and three with GCS = 8).

In order to stabilise the arterial pressure in patients, dopamine infusion ($5.5 \mu\text{g kg}^{-1} \text{min}^{-1}$) was used in the six patients with GCS = 3, and in two cases from the subgroup with GCS from 4 to 8 (two patients with GCS = 4).

Heart rate (beats per minute) was significantly higher in the patients, compared to the control group (Table 1). In our subgroup of patients with GCS = 3, we found two cases with heart rate greater than 110 beats/min, meanwhile the rest (four patients) showed a heart rate lower than 65 beats/min. These four patients also presented arterial blood pressure less than 100/60 mmHg.

The results of statistical comparisons between patients and the control group for HRV indices calculated for time, frequency and informational domains are shown in Table 1. HRV showed an overall decrement in these cases, with reduced PSD values for VLF and LF bands, and a significant decrement of the LH/HF ratio. Time-domain indices of HRV, such as SD and Delta_RRs, expressing global variability, and others showing short-time variability (RMSSD, pNN50 and pMean2%), were also statistically significantly lower in the group of patients. It was interesting to note that the Shannon's entropy index was significantly lower in patients. Absolute PSD for all frequency bands were also significantly reduced in the group of patients. Meanwhile, relative PSD only showed statistical differences between control subjects and patients for P_MF (significantly decreased in patients), and for P_HF (significantly incremented in patients). The LF/HF ratio was significantly diminished in the group of patients.

In Table 2, the same HRV indices from Table 1 are shown, but now comparing both subgroups of patients, classified according to the GCS scores. Regarding the HRV indices in the time domain, Delta_RRs, and the Triangular indices were significantly decreased in the subgroup with GCS = 3. Overall, absolute power for the whole frequency spectrum decreased whenever GCS scores were lower. Nonetheless, significant decrements were only found for absolute PSD of the VLF and LF bands in the subgroup with GCS = 3. The LF/HF ratio was significantly lower in the subgroup with GCS = 3. Concerning the normalised relative power indices, nu_VLF and nu_MF were significantly lower, and the nu_HF was significantly higher in the subgroup with GCS = 3. Shannon's entropy index was significantly lower in the subgroup with GCS = 3.

Table 1
Comparative analysis between the group of patients and the control group for time, frequency and informational HRV domains indices. O/N, order number of items in the table; results of items from 9 to 15 are expressed as mean [Quartile range] (Min value and Max values). SD, standard deviation of R–R sequence; Delta_RRs, difference between R–R maximum and R–R minimum; RMSSD, root mean square of squared differences of R–R series; pNN50, proportion of R–R intervals exceeding the precedents by 50 ms; pMean2%, proportion of R–R intervals exceeding the precedents for at least 2% from the mean value of the R–R series; Strl, stress neurovegetative index; Ln, natural logarithm; nu, normalised units; nd, non-dimensional units; cu, conventional units; SEM, standard error of the mean; *U*, value of the *T* score of Mann Whitney's test; *p*, associated probability to the value of *T* scores. Bold values indicate significant statistical differences.

O/N	Indices	Control group (N = 22)	Patients (N = 16)	<i>U</i>	<i>p</i>
1	Heart rate (beat/min) (M ± sem)	70.1 ± 1.63	86.97 ± 5.87	96.0	0.018
2	Mean of RRs (ms) (M ± sem)	866.6 ± 21.29	737.6 ± 48.7	96.0	0.018
3	Delta_RRs (ms)	232.77 ± 63.3	69.48 ± 50.55	9.0	0.000...
4	SD (ms) (M ± sem)	37.2 ± 2.31	13.3 ± 3.32	35.0	0.000...
5	RMSSD (ms) (M ± sem)	22.2 ± 1.37	7.6 ± 1.41	29.0	0.000...
6	pNN50 (%) (M ± sem)	3.6 ± 0.90	0.00 ± 1.19	80.0	0.000...
7	pMean2% (%) (M ± sem)	39.8 ± 2.77	13.0 ± 62.29	63.0	0.000...
8	Strl (cu) (M ± sem)	8.294 ± 1.01	908.9 ± 351.80	18.0	0.000...
9	Tril (cu) (M ± sem)	38.79 ± 2.71	4.47 ± 0.77	0.0	0.000...
10	Shannon's entropy (cu)	4.743 [0.62] (3.54–5.44)	3.038 [2.25] (0.903/4.31)	38.0	0.000...
11	Ln (P_MBF) ms ²	10.6490 [1.29] (9.22–11.68)	7.7233 [3.54] (–0.26–9.67)	5.0	0.000...
12	Ln (P_BF) ms ²	11.2950 [1.40] (9.48–12.91)	7.7559 [3.84] (2.18–10.87)	12.0	0.000...
13	Ln (P_MF) ms ²	10.8695 [2.00] (8.57–13.05)	7.5288 [3.71] (2.33–10.10)	13.0	0.000...
14	Ln (P_AF) ms ²	11.0190 [1.22] (9.68–12.20)	8.2469 [2.96] (5.36–11.07)	27.0	0.000...
15	Ln (P_Tot) ms ²	12.6975 [1.11] (11.08–13.93)	9.9066 [3.35] (5.60–11.90)	9.0	0.000...
16	LF/HF (nd)	2.059 [3.24] (0.59–8.82)	1.064 [1.20] (0.02–4.69)	95.0	0.017
17	nu_VLF (%) (M ± sem)	18.423 ± 8.26	14.601 ± 13.73	120.0	0.098
18	nu_LF (%) (M ± sem)	21.893 ± 10.43	23.186 ± 14.84	126.0	0.139
19	nu_MF (%) (M ± sem)	31.482 ± 10.28	12.838 ± 6.94	100.0	0.024
20	nu_HF (%) (M ± sem)	28.198 ± 14.59	49.323 ± 27.68	97.0	0.019

Table 2
Comparative analysis between the subgroups of patients with GCS = 3, and the subgroup with GCS score ranging from 4 to 8, for time, frequency and informational HRV domains indices. O/N, order number of items in the table; results of items from 9 to 15 are expressed as mean [Quartile range] (Min value and Max values). Delta_RRs, difference between R–R maximum and R–R minimum; SD, standard deviation of R–R sequence; RMSSD, root mean square of squared differences of R–R series; pNN50, proportion of R–R intervals exceeding the precedents by 50 ms; pMean2%, proportion of R–R intervals exceeding the precedents for at least 2% from the mean value of the R–R series; Strl, stress neurovegetative index; Ln, natural logarithm; nu, normalised units; nd, non-dimensional units; cu, conventional units; M, mean value; SEM, standard error of the mean; *U*, value of the *T* score of Mann Whitney's test; *p*, associated probability to the value of *T* scores; GCS, Glasgow Coma Scale. Bold values indicate significant statistical differences.

Indices	GCS = 3 (N = 6)	GCS from 4 to 8 (N = 10)	<i>U</i>	<i>p</i>	
1	Heart rate (beat/min) (M ± sem)	77.7 ± 11.0	92.5 ± 6.5	17	0.16
2	Mean RRs (ms) (M ± sem)	834.8 ± 90.6	679.3 ± 50.7	17	0.16
3	Delta_RRs (ms)	51.94 ± 56.9	80.00 ± 46.2	5	0.007
4	SD (ms) (M ± sem)	15.71 ± 8.0	15.85 ± 2.86	22	0.38
5	RMSSD (ms) (M ± sem)	10.29 ± 2.5	9.95 ± 1.78	26	0.66
6	pNN50 (%) (M ± sem)	4.16 ± 3.05	0.31 ± 0.21	22	0.38
7	pMean2% (%) (M ± sem)	180.4 ± 166.0	16.7 ± 6.87	24	0.51
8	Srtl (cu) (M ± sem)	1729.7 ± 787.7	416.5 ± 226.5	24	0.51
9	Tril (cu) (M ± sem)	3.1 ± 1.23	6.04 ± 0.85	11	0.04
10	Shannon's entropy (cu)	1.497 [0.71] (0.90–2.53)	3.963 [0.81] (2.92–5.44)	2	0.01
11	Ln (P_VLF) ms ²	3.675 [5.98] (–0.27–8.88)	8.176 [1.60] (6.16–9.77)	7	0.01
12	Ln (P_LF) ms ²	4.246 [4.98] (2.18–9.93)	8.679 [2.18] (6.31–10.84)	11	0.04
13	Ln (P_MF) ms ²	4.845 [4.55] (2.33–10.1)	7.902 [2.57] (4.91–9.71)	17	0.15
14	Ln (P_HF) ms ²	6.657 [3.97] (5.36–10.63)	8.741 [1.94] (6.81–11.07)	16	0.13
15	Ln (P_Tot) ms ²	6.761 [3.43] (5.61–11.4)	10.157 [1.68] (7.69–11.90)	13	0.06
16	LF/HF (nd)	0.262 [0.94] (0.07–1.09)	1.27 [2.32] (0.11–4.69)	4	0.004
17	nu_VLF (%) (M ± sem)	4.57 ± 1.87	20.62 ± 4.42	6	0.009
18	nu_BF (%) (M ± sem)	11.26 ± 4.23	30.34 ± 3.95	25	0.59
19	nu_MF (%) (M ± sem)	7.859 ± 3.62	12.02 ± 1.86	7	0.01
20	nu_HF (%) (M ± sem)	69.83 ± 8.79	37.02 ± 7.56	12	0.05

In Table 3, the Spearman correlation analysis between HRV indices values and the GCS items (eyes, verbal, motor responses and total score) in the group of patients is shown. For 'eyes response' no significant correlations were found. Regarding verbal response, a positive significant correlation was found for indices RMSSD, and P_HF (classic parasympathetic indices). Concerning the motor response, a positive significant correlation was found for the Tril, Shannon's entropy, P_VLF, and P_Tot (classic sympathetic indices) and P_HF. The total GCS score showed a positive significant correlation with P_VLF, P_Tot and LF/HF, suggesting a main sympathetic link between the total score of GCS and the sympathetic branch of the ANS.

The grand average of non-dimensional PSD, calculated as the geometric means of every discrete frequency, is compared between

the control group (upper panel) and patients (bottom panel) in Fig. 1. A remarkable significant PSD reduction (almost 10 times lower) was found in the group of patients.

In Fig. 2 power spectra from the six patients with GCS 3 (A), from the 10 patients with GCS with values from 4 to 8 (B), and from the 22 healthy control subjects (C) are shown. The spectral range included frequencies from 0.026 to 0.4 Hz, with a sample resolution of 0.00238095 Hz. The obvious PSD decrement of the whole spectrum, comparing patients (A and B) with healthy subjects (C), is clearly noted. Moreover, PSD values in the subgroup of patients with GCS = 3 (A) are reduced for the whole spectrum, compared with the subgroup of patients with GCS score ranging from 4 to 8 (B).

In Fig. 3 compressed spectral matrices (CSMs) from a normal subject (panels A and A') and from a patient with GCS = 8 (panels

Table 3

Results obtained from the Spearman correlation among the HRV indices and the GCS items in the group of patients. SD, standard deviation; Delta_RRs, difference between R–R maximum and R–R minimum; RMSSD, root mean square of successive squared differences; pNN50, proportion of R–R intervals exceeding more than 50 ms from precedent ones; pMean2%, proportion of R–R intervals exceeding precedent ones for more than 2% from the mean value in the sequence; StrI, neurovegetative stress index; Tril, triangular index; ShaEn, Shannon's entropy; P_VLF, absolute PSD in the VLF spectral band; P_LF, P_MF, and P_HF, idem for the corresponding spectral bands; LF/HF, ratio between the absolute PSD of the LF and the HF bands; nu_VLF, values of the PSD in the VLF band expressed as normalised units (%); nu_LF, nu_MF, and nu_HF, idem for the corresponding spectral bands. Highlighted values indicate significant Spearman correlation indices for $p < 0.05$; GCS, Glasgow Coma Scale; GCS_Total, total GCS score. Bold values indicate significant statistical differences.

Indices	Eyes response	Verbal response	Motor response	GCS_Total
Heart rate	0.28	0.12	0.33	0.34
Mean R-Rs	-0.28	-0.12	-0.33	-0.34
Delta_RRs	0.28	0.29	0.38	0.43
SD	-0.04	0.33	0.39	0.28
RMSSD	0.15	0.57	0.23	0.25
pNN50	-0.23	0.36	-0.06	-0.15
pMean2%	-0.10	0.45	0.03	-0.02
StrI	-0.08	-0.16	-0.17	-0.20
Tril	0.09	0.24	0.53	0.47
ShaEn	0.02	0.25	0.55	0.45
P_VLF	0.30	0.24	0.60	0.61
P_LF	0.10	0.16	0.48	0.44
P_MF	-0.01	0.16	0.38	0.30
P_HF	0.15	0.53	0.51	0.45
P_Tot	0.20	0.45	0.59	0.53
LF/HF	0.43	-0.16	0.37	0.51
nu_VLF	0.01	-0.08	0.49	0.41
nu_LF	-0.39	-0.41	-0.18	-0.25
nu_MF	-0.15	0.37	-0.33	-0.36
nu_HF	-0.02	-0.33	0.28	0.25

B and B') are shown. In panel A, the whole frequency spectrum (0.04–0.4 Hz) is considered. A high PSD of about 12,000 ms^2 limits any possibility to study the HF range. Nonetheless, in panel A' when the frequency range from 0.15 to 0.40 Hz is only analysed, PSD fluctuations within the HF range can be determined. Considering the PSD scale, the PSD maximum value is 1500 ms^2 , and hence it is easy to understand that PSD values of about 12,000 ms^2 overlap any analysis of the HF band, when the whole HF range is assessed. In panels B and B', the same analysis is shown for a patient with GCS = 8, considering the whole and the HF ranges, respectively. PSD values within the HF band are better visualised in panel B'.

CSMs from the same normal subject of Fig. 3 are shown in Fig. 4 (panels A and A'). CSMs for the whole frequency (panel C), and the HF (panel C') ranges, corresponding to a patient with GCS = 3, are also presented. This Figure is very illustrative to demonstrate the PSD reduction in P_LF and P_MF values, with a relative preservation of PSD values within the 0.15–0.40-Hz frequency range in the patient with GCS = 3, but with very much lower values, compared to the healthy subject.

4. Discussion

To discuss our results it is necessary to exclude the possible effect of drugs used in patients. We already remarked that we carefully discarded those cases with a personal history of long-term use of drugs such as hypnotics, autonomic stimulants or blockers that may affect the autonomic system. Nonetheless, our patients with lowest GCS were under dopamine therapy.

Dopamine effect is dose dependent (Dandamudi and Chen, 2011; Elkayam et al., 2008; Tian, 2012). Low doses (from 2 to 5 $\mu\text{g kg}^{-1} \text{min}^{-1}$) induce an interaction with D1 dopaminergic receptors located in kidneys, mesenteric plexus and coronary arteries, dilating blood vessels, thus increasing overall renal perfusion.

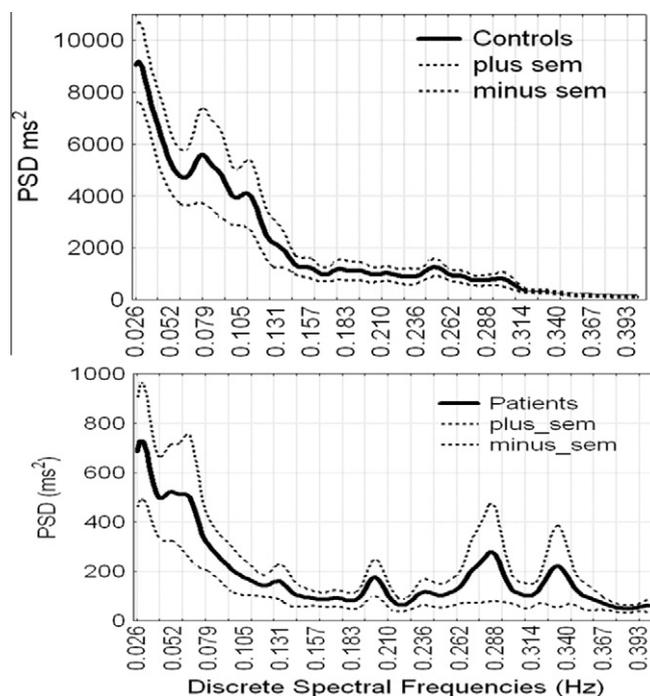


Fig. 1. The geometric mean values obtained from the individual spectra of the control group (upper panel), and for the group of patients (lower panel), are shown. The geometric mean values, representing the estimation of the power spectral density of the RRI intervals of both groups, are plotted with the corresponding plus and minus one standard error of the mean (SEM), in order to provide a graphical and a statistical measure of the mean values variability.

Nonetheless, with the dose we used in our patients (5.5 $\mu\text{g kg}^{-1} \text{min}^{-1}$), it rouses a sympathetic predominance, with a cardiac inotropic and positive chronotropic effect, raising the heart rate, and increasing the blood pressure (mainly systolic, and less affecting diastolic blood pressure), and producing a decrement of the global HRV. This physiological effect on HRV indices resembles that related to physical stress in healthy subjects, producing a clear sympathetic predominance, with an increment of LF and VLF, and the LH/HF indices (Chen et al., 2011; Hottenrott et al., 2006; Ng et al., 2009; Simoes et al., 2010). Our six cases with GCS = 3, and the other 2 with GCS = 4, on the contrary, showed significant lower values for P_VLF and P_LF, with a significant decrement of the LH/HF index. Moreover, the heart rate and arterial blood pressure values were not in the range of those produced by the effect of dopamine (Tian, 2012). Hence, our findings were related to the dysfunction of the ANS in deep coma, and not to the dopamine effect.

In our cases, we found in our cases a significant increment of heart rate compared to healthy controls. The significant increment of the heart rate in comatose patients may be considered as a consequence of the diminished control of the vagal centres in the brain stem over the pacemaker cells of the sinus node, and also due to an imbalance produced by the relative increment between the sympathetic influences, represented in the PSD of the VLF, LF and MF spectral bands, and the reduction in the vagal PSD in the HF band (Biswas et al., 2000; Baillard et al., 2002; García et al., 1995; Gujjar et al., 2004; Machado et al., 2005). It is clear that the heart frequency represents the integral effect of many factors (neural, hormonal, humoral and environmental) impeding over the cells of the auricular node (Montes-Brown et al., 2010, 2012). However, in these patients, where the majority of the independent variables were carefully controlled, the main factor must be ascribed to the ANS control (Baillard et al., 2002; García et al., 1995; Machado et al., 2005; Montes-Brown et al., 2010, 2012). In our subgroup of patients with GCS = 3, we found two cases with

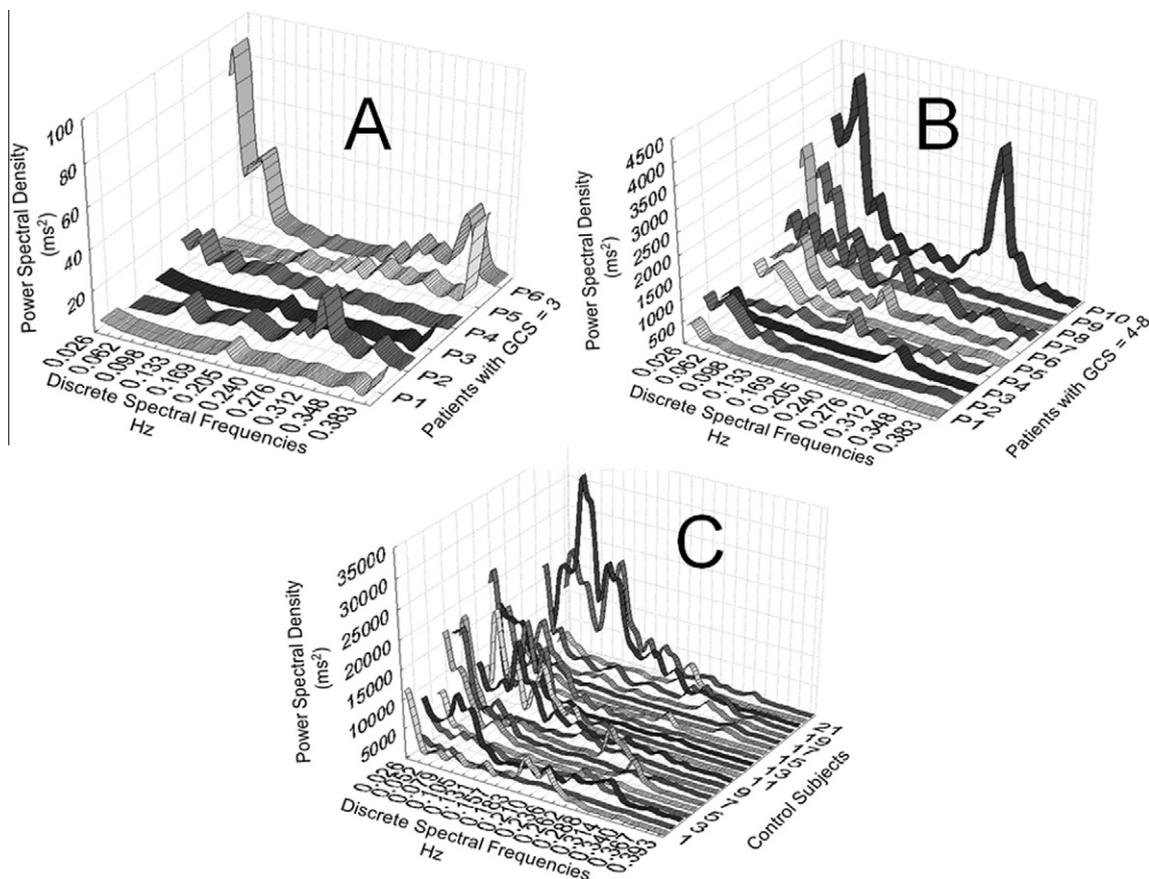


Fig. 2. Power spectra represented as ribbons in 3-D diagrams, obtained from the 6 patients with GCS = 3 (A), from the 10 patients with GCS score 4 to 8 (B), and from the 22 healthy control subjects (C), are shown. The spectral range was 0.026–0.4 Hz, with a frequency resolution of 0.00238095 Hz.

heart rate greater than 110 beats/min, meanwhile the rest showed a heart rate lower than 65 beats/min. These findings deserve special attention for future research.

In general, time, frequency and informational domain indices pointed out a notable reduction of HRV in comatose patients, compared to normal subjects. Nonetheless, the most important findings in this study came from the comparison of comatose patients, classified into two subgroups according to the GCS scores. Regarding the time domain, the triangular index and the Delta_RRs were significantly reduced in the subgroup with GCS = 3, indicating the potential efficacy of this indices for future studies of comatose patients.

Concerning the differences between both subgroups for HRV indices in the frequency domain, altogether, in our patients the PSD for the whole frequency spectrum decreased whenever GCS scores were lower. A significant decrement was found for both P_VLF and P_LF in the subgroup of GCS = 3, meanwhile although P_HF was lower in these patients, those changes were not statistically different, showing a remaining activity in the HF band, compared with the LF range indices. Then a question arises to explain why the slow bands tend to disappear when coma deepens with GCS = 3, remaining an activity within the HF range.

To understand these findings, it is necessary to remark on new functional areas of the ANS, recently described by Goodchild and Moon (2009). These authors demonstrated the existence of vasomotor sympathetic nuclei in the brainstem and cervical spine: the rostral ventrolateral medulla, the caudal ventrolateral medulla, the giganto cellularis depressor area, the caudal ventrolateral medullary vasodepressor area, the caudal pressor area, the intermediate pressor area and other neuronal structures extending from

the inferior pole of the inferior olive to the C1–C2 cervical spinal segments. Macefield and Henderson (2010) have shown the presence of almost all of these structures, using fMRI and recording muscle sympathetic neural activity.

Then, the loss of absolute power of the LF band, when coma deepens until a GCS = 3, might be explained by the destruction of brain-stem sympathetic structures, mainly those related to the rostral ventrolateral medulla oblongata area. Regarding the VLF band, there are still discussions about its origin, probably related to the renin–angiotensin system, to the thermoregulatory function mechanisms, and to central sympathetic activity (Takabatake et al., 2001). Nonetheless, other authors (Goodchild and Moon, 2009; Llewellyn-Smith, 2009; Macefield and Henderson, 2010) have affirmed that the VLF reflects a functional activity integrated in the lower part of the medulla, and the first two segments of the spine. Hence, this area is particularly involved in an adrenergic control of both vasomotor tone and heart rate, and then it should be particularly affected during the clinical evolution of coma, when the caudal region of medulla oblongata is progressively destroyed. We recently studied a brain-dead patient by HRV, and found the permanence of VLF waves during 10 min, vanishing after that moment (Machado, 2011). We considered those VLF waves as a probable correlate of residual vasomotor activity after BD.

Concerning the parasympathetic neural control of the heart rate, the so-called polyvagal theory (Porges, 2007), and other evidences point to a predominance of the neurons in the NA as the main source of the chronotropic cardiac activity, while the neurons of VDN innervate mainly the gastro-intestinal tractus (Berntson et al., 2007; Philbin et al., 2010). Nonetheless, these parasympathetic nuclei might be also destroyed when the medulla oblongata

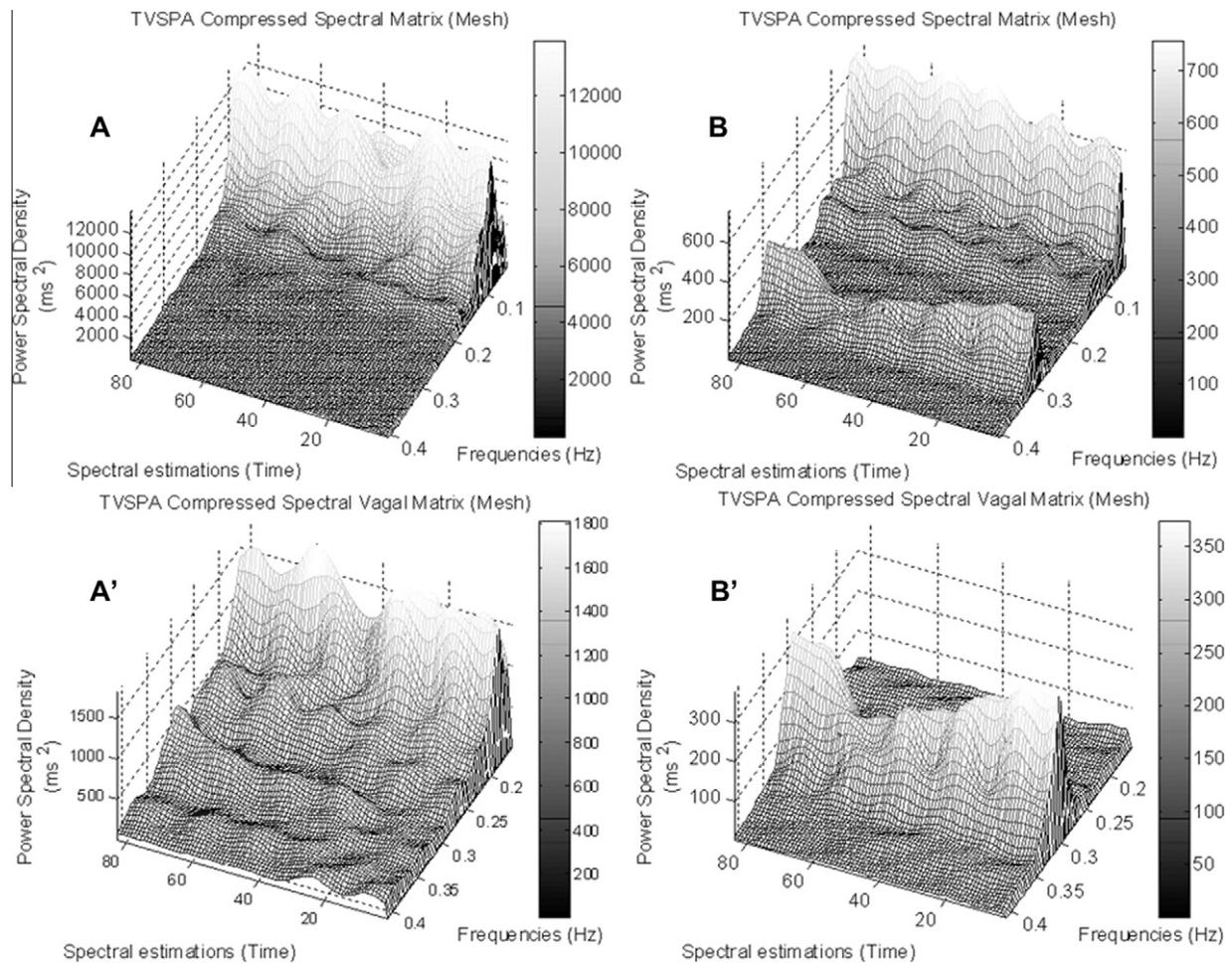


Fig. 3. In this figure compressed spectral matrices (CSM) from a normal subject (panels A and A') and from a patient with GCS = 8 (panels B and B'), are shown. The power spectral density (PSD) in the HF range is not clearly visualised in the panel A, because PSD in the very low (VLF) and low frequency ranges (LF), from 0.026 to 0.15 Hz, is very high, and overlaps the high frequency (HF) range components. Nonetheless, in panel A' when the frequency range from 0.15 to 0.40 Hz is only analysed, PSD fluctuations within the HF range can be determined, allowing to assess HF frequencies stability during the whole analysis time in this subject. The CSM in the panel B corresponds to a comatose patient with GCS = 8 and LF and HF components are clearly detected, because PSD within the LF range is not so high as in the control healthy subject, presented in panel A. Nevertheless, the diagram in B' allows a better analysis of the HF range in this patient.

function is gradually destroyed when coma becomes deeper. Nonetheless, the HF band is closely related to respiratory mechanisms, arising in pulmonary receptors, and others, which send information to respiratory centres, are closely anatomically related to the above-mentioned parasympathetic nuclei in the brainstem (Gujjar et al., 2004; Goodchild and Moon, 2009; Porges, 2007).

Hence, we consider that the remaining but reduced absolute power within the HF band in our cases might be explained by the effect of mechanical ventilation. An useful method in future research for testing if this remaining activity in the HF band is really due to the effect of mechanical ventilation would be to change the frequency of the ventilator during a short period of time. For example, changing the frequency of ventilator from 15 per min (the peak frequency would appear at 0.25 Hz) to 18 per min (the peak would appear at 0.3 Hz) would allow to confirm that the remaining activity is certainly due to the effect of the mechanical ventilation. It could be also a previous step before applying the apnoea test for BD diagnosis.

Our findings are similar to those reported by other authors (Biswas et al., 2000; Ryan et al., 2011; Su et al., 2005;), who have found a progressive disappearance of different frequency bands when clinical evolution in comatose patients deteriorates, despite that the aetiology of brain injury in our patients is different, because we did not include traumatic brain-injured patients.

Su et al. (2005) affirmed that the absolute power of the LF and HF bands decreased stepwise when GCS was diminishing until a GCS = 4. These authors could not technically consider the VLF band, because they only studied a time segment of 288 s. Ryan et al. (2011) demonstrated that the VLF band is an independent predictor of mortality and morbidity in haemodynamically stable traumatic brain-injury patients. Gujjar et al. (2004) found that the LF and VLF spectral PSD correlated with mortality. A significant reduction in LF power has been associated with traumatic brain injury, BD and sympathetic blockade (Goldstein et al., 1998; Fathizadeh et al., 2004).

We found in our cases a significant decrement of the LF/HF ratio in the subgroup of GCS = 3, in relation to the subgroup with GCS ranging from 4 to 8. This finding is related to our previous discussion about the progressive loss of P_LF, meanwhile some remaining activity persists in the HF band. Several authors have affirmed that the LF/HF index mirrors the sympathovagal balance (Appel et al., 1989; Montano et al., 1994; Sands et al., 1989), and others affirm that it reflects sympathetic modulations (Malliani et al., 1991; Malliani and Pagani, 1991; Montano et al., 1994). Biswas et al. (2000) reported that their patients with a GCS of 3 or 4 was associated with a lower LF/HF. Ryan et al. (2011) found that decrements in VLF and LF/HF distribution were the only indices significantly correlated with mortality in traumatic brain-injury patients.

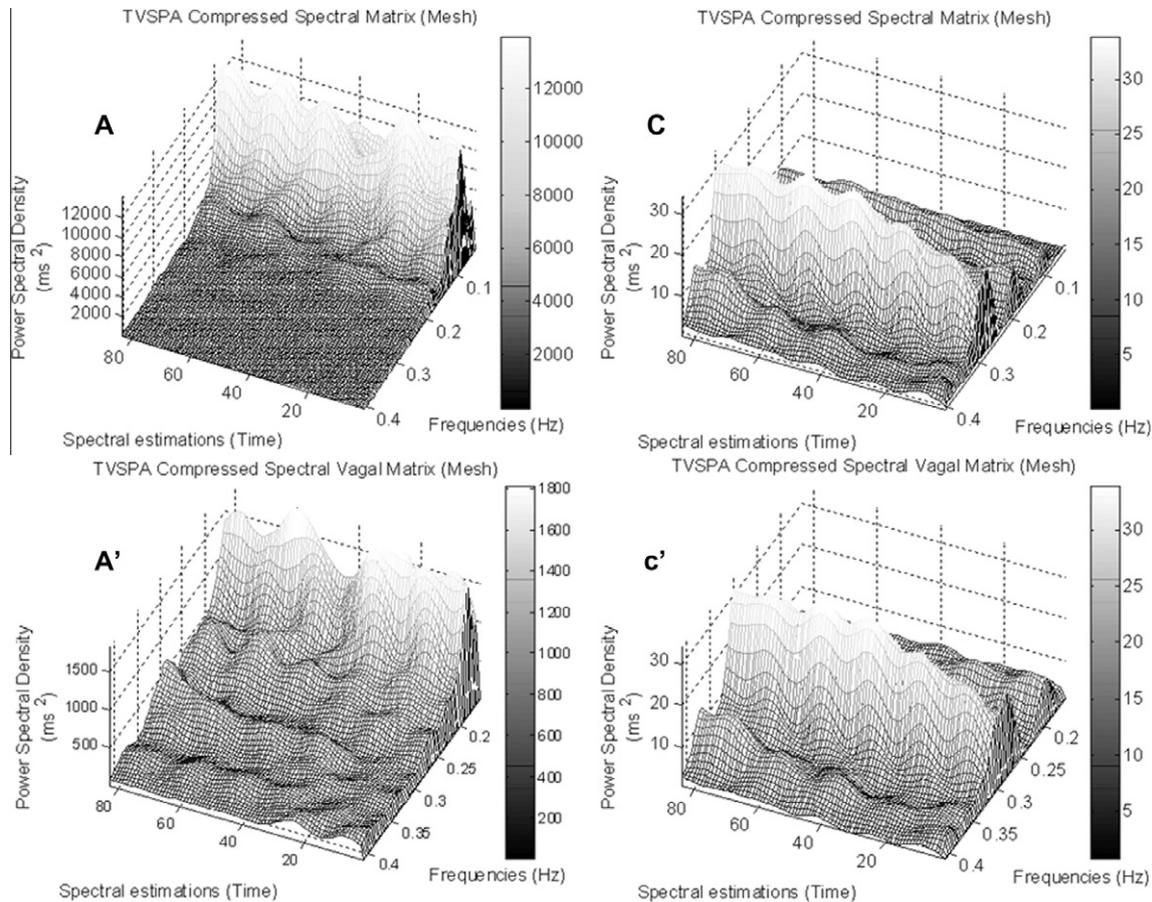


Fig. 4. In A, and in A', the compressed spectral matrices (CSM) of the same control subject of the Fig. 3 are shown. The CSM from a patient with GCS = 3, corresponding to the whole spectrum range (panel C), and to the high frequency (HF) range (from 0.15 to 0.40 Hz), are presented. CSM from the patient (C and C') only show components in the HF range, with a very low power density value. Nonetheless, the estimated spectra were stable regarding frequency and PSD for the entire period of 1200 s of ECG recording, so assuring the righteous decision of using segments of 420 s from the whole RRI series.

We used in this study an informational domain HRV index, the Shannon's entropy and two other time-domain indices: the triangular index and the Delta_RRs. These indices, assessing an overall HRV, also showed a significant positive correlation with the GCS, and were significantly reduced in those patients with GCS = 3. Then, not only the LF/HF ratio should be considered studying comatose patients, because the Shannon's entropy, the triangular index, and the Delta_RRs, with known physiological relationship, were also very useful for differentiating our two subgroups of comatose patients.

Nonetheless, for future studies including patients with GCS = 3, showing a very reduced HRV, sampling frequency should be no less than 1000 Hz, which methodologically might give the opportunity of detecting very reduced variability of HRV indices.

Therefore, we conclude that HRV is a minimally invasive, low-cost methodology particularly suitable for assessing the ANS in coma (García et al., 1995; Machado et al., 2005; Ryan et al., 2011).

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