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Research article

Gender differences of heart rate variability in patients with ischemic stroke

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Abstract: Background: Heart rate variability (HRV) is a parameter useful in monitoring post stroke cardiac dysautonomia. Our study is aimed at investigating HRV modifications in patients with stroke; (2) Methods: We investigated HRV parameters in 35 men and 35 women with stroke, data compared to 70 age and sex similar healthy subjects. We collected and interpreted HRV data related to resting state, deep breathing, and standing situations; (3) Results: The variables identified by classification methods to be discriminative in stroke groups for men and women classes are VLFms2, LFms2, LFnu, HFnu, and ApEn in resting state conditions, mean RR, HR, pNN50, VLFms2, LFnu, HFnu, ApEn and SampEn in deep breathing conditions, and mean RR, HR, HFnu, ApEn, and DFAα1 in standing conditions; (4) Conclusions: Monitoring of HRV in patients who have suffered an ischemic stroke is essential, as it is known that stoke contributes to an increased risk of cardiac autonomic dysfunction and consequently to a second unwanted vascular event.

Keywords: heart rate variability, resting heart rate, ischemic stroke, autonomic dysfunction

Introduction

Stroke represents the leading cause of long-term disability and the leading preventable cause of disability in the adult population worldwide [1]. In industrialized countries, it represents the third most common cause of death and a major burden with increasing clinical, economic, and social impact. Many patients have severe disabilities after stroke and only a few gain full recovery and independence. The level of disability can vary from weakness, and paralysis to cognitive impairment, including vascular dementia.

Stroke affects both men and women. However, the incidence rates and outcomes differ between the 2 genders. Stroke prevalence is much higher in women, with 4.1 million women currently living with stroke compared to 3.1 million men [2]. The incidence of stroke is higher in women because of their long life expectancy, and high stroke incidence at older ages [3-5].

Data from the REGARDS study (REasons for Geographic And Racial Differences in Stroke) highlights the fact that fewer women than men have prevalent stroke at young ages (45–54 years) and with increasing age this difference is eliminated. Among those aged 85 years and older there are almost three times more black women than men with stroke, and double in whites [6].

A lot of factors were considered to explain gender differences in stroke such as: sex hormones, lifestyle, coagulation status, genetic backgrounds, anatomical differences, etc. [7, 8].

The Warfarin-Aspirin Symptomatic Intracranial Disease study, which was performed on 569 patients with symptomatic intracranial arterial stenosis, showed that women had a significantly higher risk for intracranial arterial stenosis and for the combined endpoint of stroke and vascular death [8].

Associated autonomic dysfunction is frequent in both acute and chronic stages of cerebrovascular pathology. Heart rate variability (HRV) is a non-invasive assessment method of the autonomic nervous system activity that regulates heart rate (HR). Time-domain, frequency-domain, and nonlinear analysis of the HRV can establish the autonomic tone balance. Linear parameters are commonly used in clinical trials but nonlinear parameters such as the Poincaré plot, Detrended fluctuation analysis (DFA), Approximate Entropy (ApEn), and Sample Entropy (SampEn) are rarely analyzed despite there are proven they are more sensitive than linear parameters to smaller heart rate (HR) modulations [9].

The decreased HRV is persistent over time in stroke patients, as well as the alteration of the sympathetic/ parasympathetic tonus. These act as both mortality predictors and functional outcomes [10]. Both heart rate and blood pressure are modifiable parameters within stroke manifestations, especially related to cerebral edema, increased risk for the hemorrhagic transformation of ischemic stroke or expansion in case of an intracerebral hematoma, as well as atrial fibrillation, myocardial ischemia and sudden death, respectively [11, 12].

The main aim of the study was to evaluate cardiac autonomic function in patients with monofocal ischemic stroke in the right middle cerebral artery (MCA) territory, using HRV analysis during supine, deep breathing and active standing in comparison with healthy control individuals. We searched for correlation between sex and HRV parameters.

2. Patients and methods

2.1. Participant recruitment and inclusion criteria

In our study, we investigated 140 participants in evidence 70 (35 men and 35 women) patients with monofocal ischemic stroke in the right middle cerebral artery (MCA) territory and 70 (35 men and 35 women) age-related healthy subjects. The ischemic stroke patients were recruited from the Department of Neurology. The study was approved by our institutional ethics committee and all the patients gave consent in accordance with ethical principles. The study was carried out in accordance with the Helsinki Declaration.

Inclusion criteria were as follows: clinical assessment for stroke, evaluated in the first 6 months after the acute event, computed tomography (CT) or magnetic resonance imaging (MRI) showing a single ischemic lesion within the right hemisphere (superficial and/or profound MCA territory). We excluded patients treated with anti-psychotics, tricyclic antidepressants, and beta blockers because of their interference with HRV parameters. Also, there were excluded the study, patients with myocardial infarction, acute brain injury, arrhythmias, atrioventricular block or bundle branch blocks, seizures in the past 6 months, or diabetes mellitus.

The inclusion criteria for the controls were: normal cognitive status and two normal consecutive electrocardiograms in the course of one month.

2.2. Clinical autonomic function tests and measurement of heart rate variability

For this study short-term ECG recording was acquired during supine, deep breathing and standing position for 10 min each using the BIOPAC MP 150 data acquisition system. AcqKnowledge Software version 4.1.1. (BIOPAC Inc., Goleta, CA, USA) was used to analyze and remove from the recorded ECG all artefacts and ectopic beats. Kubios HRV® Analysis Software 2.0 for Windows (The Biomedical Signal and Medical Imaging Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used to generate the HRV parameters.

Data acquisition was performed in a quiet room with temperatures between 20 and 22^{0}C. The data were recorded between 9 and 10 am, after an adaptation period of 15 minutes. All individuals were asked to avoid caffeine and alcohol 24 hours before the tests and to abstain from smoking for 12 hours.

A deep breathing test was used for the assessment of the parasympathetic function and the standing test was used for the assessment of the sympathetic function [13, 14].

We used the time domain analysis that calculates the Standard Deviation of Normal-to-Normal beat (SDNN), the square root of the mean squared differences of successive NN intervals (RMSSD), the proportion of differences in consecutive, the so-called normal-to-normal RR intervals that are longer than 50 ms and reflects the percentage of such intervals in comparison to the total number of analyzed intervals (pNN50). Using Fast Fourier Transform we analyzed frequency domain indices, such as very low frequency (VLF), low-frequency (LF, 0.04-0.15 Hz, ms^2), and high-frequency (HF, 0.15-0.4 Hz, ms^2) powers, LF in normalized units (LF nu= LF/ (TP-VLF), HF in normalized units (HF nu = HF/ (TP-VLF) and the ratio between LF/HF (considered an index of cardiac sympathetic/ parasympathetic tone balance) [15].

For the non-linear Poincaré plot, Approximate Entropy (ApEn), Sample Entropy (SampEn), and the Detrended Fluctuation Analysis (DFA) parameters were reported. Plotting the RR values of N on the x-axis, and the RR values of N+1 on the y-axis we obtained the Poincaré plot. Indice SD_1 obtained from the Poincaré plot was used to determine the short-term variability of a nonlinear system [16]. SD1 was correlated with RMSSD and high frequency [17]. We also used short-term $\alpha 1$ (calculated from 4–16 beats) scaling exponents of DFA to reveal short-term fluctuation [18]. Normal values of α around 1 are found in healthy subjects; lower values indicate a reduced fractal property of heart rate and have been correlated with mortality in elderly subjects [19]. ApEn was used to quantify the overall complexity and predictability of the signal. A large value of ApEn indicated a regular signal and high values indicate a more irregular signal. This parameter was demonstrated to be influenced by record length; therefore Richman and Moorman developed SampEn which is not recorded length-dependent [20].

3. Results

The analysis was conducted on two groups of subjects, stroke, and control, of 35 women and 35 men each, on whom time domain, frequency domain, and nonlinear measures were performed, in three different conditions: resting state, deep breathing, and standing.

Some descriptive statistics analyses were performed for all these variables, including means, standard deviations, confidence interval of means, the amplitude of the variation interval, medians, interquartile intervals, variations of interquartile intervals, coefficients of variation, kurtosis, and skewness.

The subject's ages are 62.7±6.25 for men in the stroke group, and 61.2±7.62 for the control group. Women's ages are respectively 63.7±10.14 in the stroke group and 61.5±8.1 in the control group. Only the confidence intervals were summarized, for all the enumerated situations, in Table 1. The largest coefficients of variation, with values greater than 1, corresponding to large confidence intervals of means, were found for variables pNN50, for both men and women, and LF ms^2, for men, for resting state. pNN50, for men, for women, for deep breathing, pNN50, for both men and women, for the standing test conditions, which correspond in both stroke and control groups, although with different values. These observations refer to the homogeneity of the samples being studied.

Because of the small volumes of the samples, n=35, the Shapiro-Wilk test was used to attest to the normality of their distributions, and correspondingly, univariate similarity analysis tests which were performed were the T-test and Kruskal-Wallis, the choice between the parametric or non-parametric test being based on the p-value of the Shapiro-Wilk test. There were performed such tests between subjects with stroke, and those from the Control group, men and women, in all of the three different testing conditions, resting state, deep breathing, and standing, for about 18 variables with numerical continuous values. The tables also include p-values of the T-tests, or Kruskal-Wallis, if the variable isn't normally distributed, stating if there are significant differences between the stroke group and the control samples. Variables with significant differences, from the point of view of their means, between men and women, from stroke groups or from control groups have their p-values <0.05 bolded. There are also bolded p-values <0.05 of the tests performed between stroke and control groups over men and, respectively, women.

Table 1. Descriptive statistics analysis results (confidence intervals of means), for both Stroke and Control groups, each with 35 women and 35 men subjects, in three distinctive conditions: (a) resting state, (b) deep breathing, and (c) standing.

Variable name	Women N=35 (Stroke)	Women N=35 (Control)	Men N=35 (Stroke)	Men N=35 (Control)
Mean RR	783.08 ± 35.33	717.42 ± 35.6	827.15 ± 45.68	738.19 ± 40.06
SDNN	44.06 ± 7.41	51.15 ± 7.54	47.99 ± 6.4	59.67 ± 10.31
HR	77.95 ± 3.33	82.46 ± 3.69	79.16 ± 3.81	74.11 ± 2.74
RMSSD	39.04±11.65	45.43±9.99	32.1 ± 5.81	56.26 ± 10.69
pNN50	4.37±1.93	8.25±2.62	4.73±1.87	13.21±4.03
VLF ms^2	215.91±70.61	223.46±78.2	401.29±88.86	368.91±96.62
LF ms^2	622.26±118.35	922.23±249.48	552.99±224.9	981.69±291.37
HF ms^2	281.14±81.59	845.23±301.8	231.83±66.26	864.4±269.39
LF/HF	2.76±0.41	1.3±0.2	2.29±0.4	1.19±0.16
LF nu	69.42±4.33	56.25±3.37	66.32±3.36	54.38±3.17
HF nu	30±4.15	43.05±3.38	33.53±3.37	45.2±3.2
SD1	26.37±6.56	36.7±7.42	22.75±4.11	39.75±7.66
ApEn	1.55±0.37	0.97±0.08	0.92±0.08	0.92±0.07
SampEn	1.14±0.12	1.32±0.17	1.09±0.13	1.13±0.15
DFA α1	1.22±0.13	1.02±0.07	1.18±0.1	0.96±0.08

(a)

Variable name	Women N=35 (Stroke)	Women N=35 (Control)	Men N=35 (Stroke)	Men N=35 (Control)
Mean RR	755.89±34.28	715.61±31.68	830.46±41.09	738.57±41.58
SDNN	74.57±10.97	69.87±12.15	62.15±9.51	63.93±13.58
HR	81.58±3.55	75.12±2.67	74.16±3.74	82.28±5.32
RMSSD	64.45±12.89	69.31±13.97	58.35±14.01	60.32±11.81
pNN50	13.59±4	12.47±3.49	12.71±9.94	13.05±3.7
VLF ms^2	418.46±125.99	254.17±77.19	527.4±129.23	719.86±122.74
LF ms^2	641.34±157.25	474.09±93.86	581.31±149.95	418.2±82.23
HF ms^2	351.71±76.55	379.29±78.61	427.83±84.7	451.89±108.13
LF/HF	2.09±0.47	1.33±0.15	1.53±0.33	1.07±0.15
LF nu	67.71±6.32	56±5.69	55.59±4.45	51.43
HF nu	35.1±6.31	44.01±5.73	43.86±4.62	48.35±5.02
SD1	45.66±9.14	50.13±9.5	42.01±9.66	44.28±8.66
ApEn	1.49±0.39	0.75±0.06	0.82±0.1	0.82±0.08
SampEn	0.84±0.11	0.81±0.11	0.94±0.14	1.02±0.16
DFA α1	0.98±0.11	1.09±0.24	1±0.1	1.05±0.14

(b)

Variable name	Women N=35 (Stroke)	Women N=35 (Control)	Men N=35 (Stroke)	Men N=35 (Control)
Mean RR	739.63±41.34	666.99±36.34	813.97±35.42	660.97±41.14
SDNN	55.69±8.4	51.28±9.71	60.48±9.36	45.43±8.01
HR	83,05±4.28	80.07±2.7	75.42±3.55	83.31±2.78
RMSSD	39.04±11.65	45.43±9.99	32.51±7.83	37.6±8.44
pNN50	4.37±1.93	8.25±2.62	4.85±2.56	6.36±1.9
VLF ms^2	341.91±66.42	222±69.36	386.57±83.13	186.09±68.23
LF ms^2	731.14±167.67	747.97±150.57	723.03±202.12	545.43±122.53
HF ms^2	302.51±85.08	382.51±106.26	259.26±79.77	222.06±61.36
LF/HF	3.28±0.65	2.36±0.43	3.68±0.99	2.82±0.35
LF nu	70.01±5.99	63.93±3.65	71.9±4.33	68.48±3.79
HF nu	29.81±5.91	35.92±3.64	27.93±4.31	31.38±3.76
SD1	27.65±8.25	31.78±6.6	23.58±5.6	29.04±7.47
ApEn	1.53±0.43	0.84±0.08	0.82±0.07	0.9±0.17
SampEn	0.82±0.09	0.93±0.15	0.91±0.1	0.96±0.15
DFA α1	1.18±0.13	1.15±0.12	1.29±0.09	1.23±0.1

(c)

Classification tests were also performed using Machine Learning Classification Toolbox from Matlab 2019. Over 1000 classification tests were performed, the best results being summarized along with the probability test results in Table 2. The table includes the name of the classifier model that produced the result, and eventually the learner type or distance involved. Parameters were finely tuned to produce the classification accuracies and ROC-AUC (Receiver Operating Characteristic - Area Under the Curve) mentioned in the adjacent columns. The best results (AUC >=0.7) have those values bolded.

Table 2 Hypothesis tests for significance, T-tests or Kruskal-Wallis, depending on the relevance of Shapiro-Wilk tests results for normality, for attesting significant differences between groups: Stroke and Control - women versus men, as well as women and men, Stroke versus Control, emphasizing the three analysis conditions: (a) resting state, (b) deep breathing and (c) standing.

Variable name	Classification model type	Accuracy	AUC	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	(Stroke) Shapiro-Wilk p-value (women)	(Stroke) Shapiro-Wilk p-value (men)
Mean RR	Ensamble-subsp. discr.	0.6	0.61	0.126	0.433	**0.01**	**0.004**	0.78	0.27
SDNN	KNN-Euclidean	0.6	0.58	0.418	0.18	0.18	0.055	0.32	0.22
HR	SVM-Gaussian	0.629	0.61	0.628	0.0004	0.07	0.033	0.33	0.27
RMSSD	KNN-Mahalanobis	0.6	0.59	0.542	0.72	**0.004**	**0.0002**	0.59	0.31
pNN50	KNN-cosine	0.53	0.5	0.495	0.988	**0.02**	**0.0003**	0.63	0.18
VLF ms^2	SVM-cubic	**0.76**	**0.76**	**0.001**	0.69	0.44	0.31	**<<0.05**	**0.02**
LF ms^2	KNN-Euclidean	**0.71**	**0.78**	0.42	0.47	0.36	0.47	**0.014**	**<<0.05**
HF ms^2	Ensambe-bagging	0.55	0.54	0.344	0.924	**0.0007**	**<<0.05**	0.74	0.34
LF/HF	SVM-cubic	0.62	0.64	0.094	0.485	**<<0.05**	**<<0.05**	0.41	0.12
LF nu	SVM-Gaussian	**0.71**	**0.74**	0.39	0.36	0.4	**<<0.05**	**<<0.05**	0.49
HF nu	SVM-Gaussian	**0.67**	**0.7**	0.61	0.38	0.51	**<<0.05**	**<<0.05**	0.55
SD1	KNN-Euclidean	0.58	0.59	0.347	0.564	**0.04**	**0.0002**	0.27	0.94
ApEn	KNN-Euclidean	**0.67**	**0.7**	**0.002**	0.75	0.94	**0.001**	**<<0.05**	0.6
SampEn	SVM-Cubic	0.51	0.52	0.543	0.102	0.095	0.911	0.56	0.42
DFA α1	KNN-cosine	0.57	0.52	0.612	0.29	**0.006**	**0.0007**	0.87	0.37

(a)

(b)

Variable name	Classification model type	Accuracy	AUC	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	(Stroke) Shapiro-Wilk p-value (women)	(Stroke) Shapiro-Wilk p-value (men)
Mean RR	Ensamble-subsp. discr.	**0.67**	**0.75**	**0.006**	0.375	0.084	**0.002**	**0.041**	0.26
SDNN	SVM-Gaussian	0.61	0.59	0.206	0.087	0.561	0.829	0.55	0.66
HR	SVM-cubic	**0.67**	**0.76**	**0.005**	0.181	**0.004**	**0.014**	0.77	0.24
RMSSD	Ensamble-GentleBoost	0.57	0.58	0.518	0.321	0.604	0.828	0.1	0.83
pNN50	Ensamble-GentleBoost	**0.64**	**0.7**	0.39	0.7	0.38	0.52	**0.007**	**<<0.05**
VLF ms²	KNN-Chebychev	**0.7**	**0.82**	0.33	0.47	0.28	0.47	**<<0.05**	**0.004**
LF ms²	KNN-Euclidean	0.55	0.52	0.576	0.365	0.069	0.829	0.83	0.73
HF ms²	KNN-Mahalanobis	0.6	0.65	0.18	0.274	0.611	0.723	0.52	0.62
LF/HF	Ensamble-Sunsp.discr.	0.64	0.63	0.37	0.53	0.46	0.44	**<<0.05**	**<<0.05**
LF nu	SVM-Gaussian	**0.67**	**0.71**	**0.018**	0.225	**0.041**	0.223	0.55	0.88
HF nu	KNN-Gaussian	**0.69**	**0.83**	**0.026**	0.251	**0.037**	0.186	0.52	0.92
SD1	Ensamble-GentleBoost	0.57	0.56	0.579	0.359	0.493	0.723	0.47	0.21
ApEn	Ensamble-bagging	**0.77**	**0.89**	**0.0016**	0.44	**0.0006**	0.938	**<<0.05**	0.67
SampEn	SVM-Gaussian	**0.74**	**0.81**	0.24	0.051	0.65	0.42	**<<0.05**	**0.011**
DFA α1	KNN-Jaccard	0.61	0.57	0.814	0.783	0.405	0.517	0.18	0.2

(c)

Variable name	Classification model type	Accuracy	AUC	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	T-test/ Kruskal-Wallis p-value (Stroke)	(Stroke) Shapiro-Wilk p-value (women)	(Stroke) Shapiro-Wilk p-value (men)
Mean RR	Ensamble-subsp.discr.	**0.71**	**0.79**	**0.0071**	0.824	**0.009**	**<<0.05**	0.27	0.66
SDNN	SVM-Gaussian	0.6	0.51	0.441	0.348	0.488	0.016	0.99	0.51
HR	Ensamble-bagging	**0.74**	**0.86**	**0.0068**	0.49	0.59	**0.0007**	0.17	**0.02**
RMSSD	KNN-Euclidean	0.54	0.55	0.349	0.228	0.4	0.372	0.52	0.51
pNN50	Ensamble-subsp.discr.	0.57	0.52	0.762	0.239	**0.018**	0.339	0.8	0.67
VLF ms²	KNN-Euclidean	0.55	0.61	0.397	0.456	**0.013**	**0.0003**	0.94	0.83
LF ms²	SVM-Gaussian	0.58	0.58	0.95	**0.038**	0.88	0.132	0.56	0.16
HF ms²	Linear discriminant	0.57	0.51	0.454	0.0103	0.236	0.455	0.31	0.34
LF/HF	Tree-TowingRule	0.58	0.55	0.497	0.097	**0.021**	0.107	0.34	0.03
LF nu	Tree-MaxDeviantReduct	0.62	0.6	0.605	0.083	0.084	0.23	0.5	0.19
HF nu	SVM-cubic	**0.67**	**0.74**	0.48	0.35	0.42	0.225	**<<0.05**	0.27
SD1	KNN-Euclidean	0.54	0.53	0.41	0.578	0.429	0.239	0.29	0.72
ApEn	Tree-TowingRule	**0.7**	**0.77**	**0.002**	0.5	0.78	0.376	**<<0.05**	0.29
SampEn	KNN-Hamming	0.57	0.53	0.164	0.704	0.197	0.519	0.6	**0.004**
DFA α1	KNN-Euclidean	**0.79**	**0.84**	0.32	0.45	0.711	0.54	0.11	**0.011**

Figure 1 shows some results concerning the distribution of three variables, one from each type of measurement performed over the subjects, time domain, frequency domain, and nonlinear, reflecting all of the three testing conditions, resting state, deep breathing, and standing, which provided good discrimination performances of data belonging to men or women, with classification models: mean RR, VLFms² and SampEn.

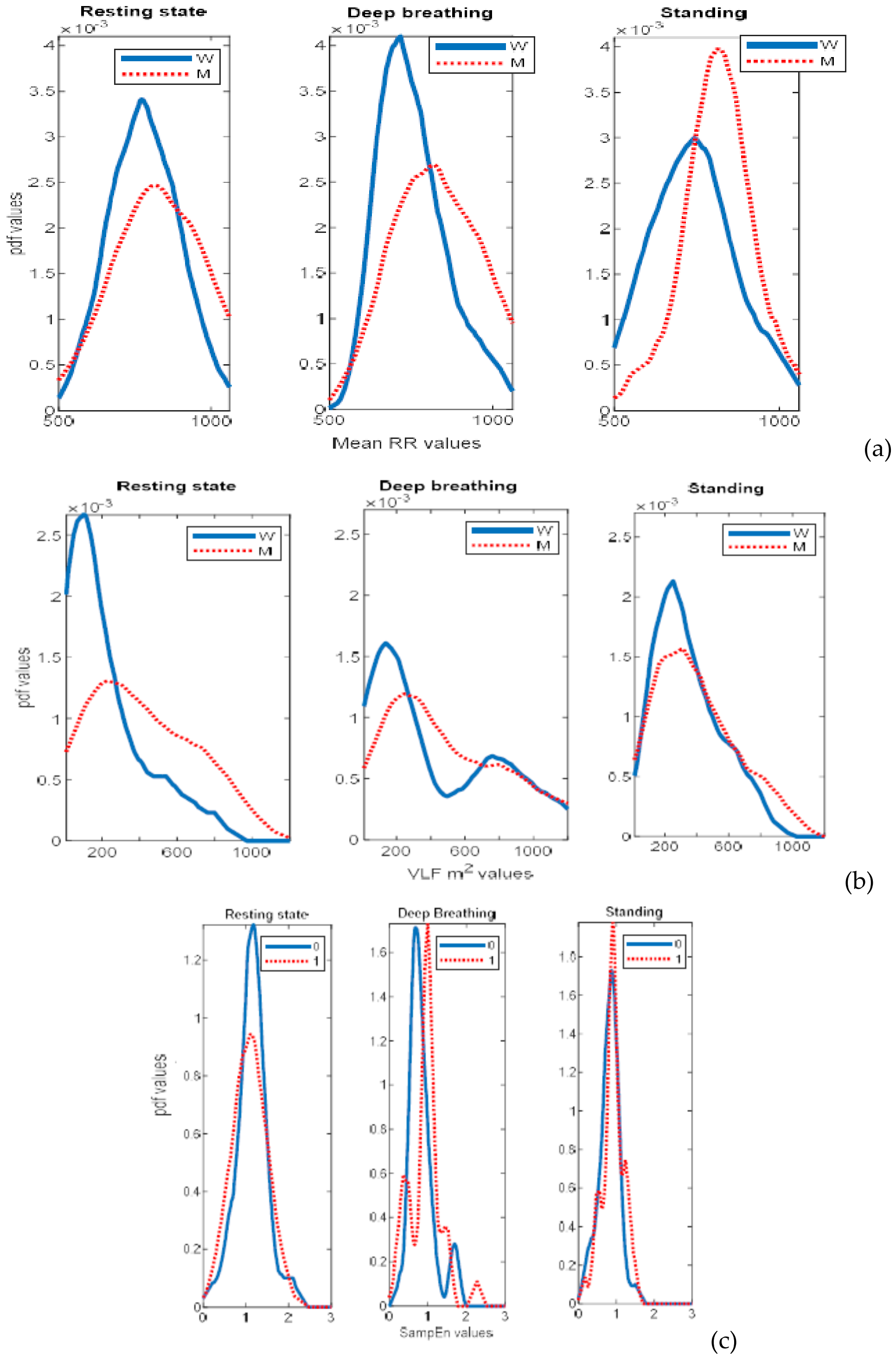
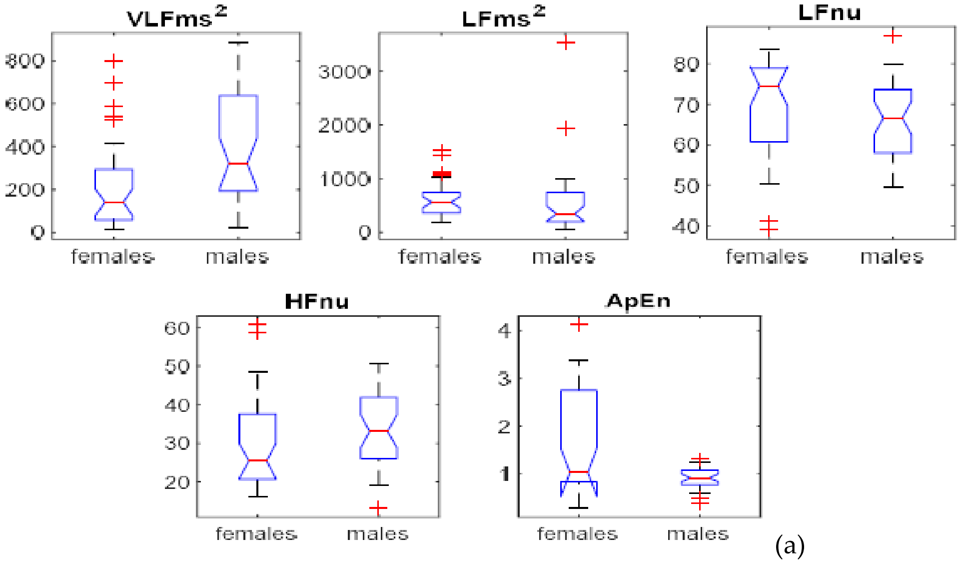
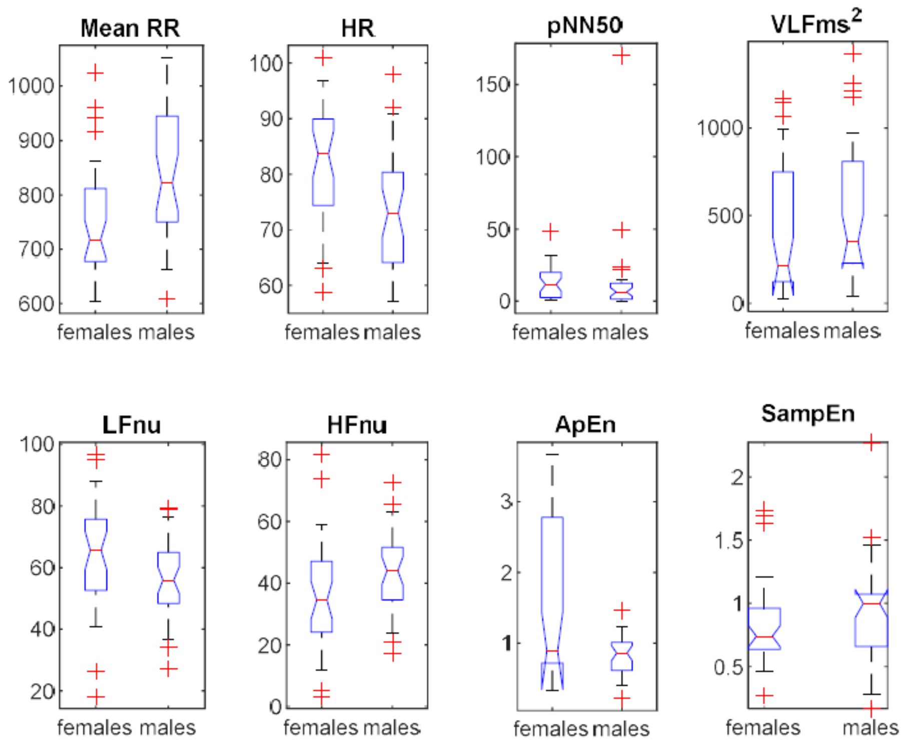


Figure 1. Graphical representations of pdf for Mean RR (a), VLF m2 (b), and SampEn (c) variables values, for three different conditions, resting state, deep breathing, and standing, for women (W) and men (M) in the Stroke groups. Examples are drawn from the three groups of variables: time domain, frequency domain, and nonlinear measures.

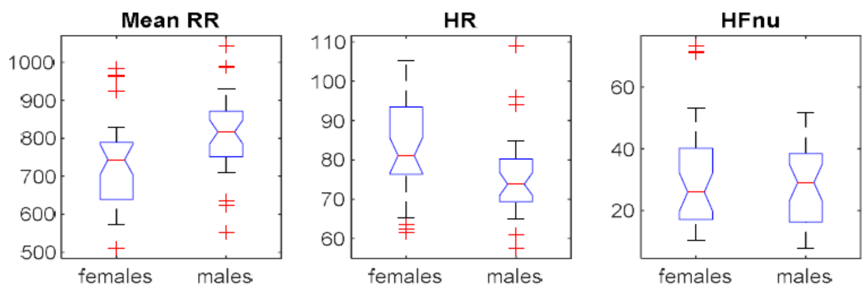
In Figure 2 one can see the boxplots of the most discriminating variables from the point of view of the Machine Learning Classification Toolbox of the Matlab 2019 software package. The probability tests emphasized significant differences between men and women in some of the measured variables, from the point of view of their means similarity. Some machine learning classifiers implemented in Matlab discovered additional variables with good discrimination performances over the two classes, men and women, which may be explained by the differences in medians and their associated notches of those variables, reflected in Figure 2. The variables may be easily identified in Table 2, where their classification accuracy and ROC-AUC values show fair and good discriminative values [21].

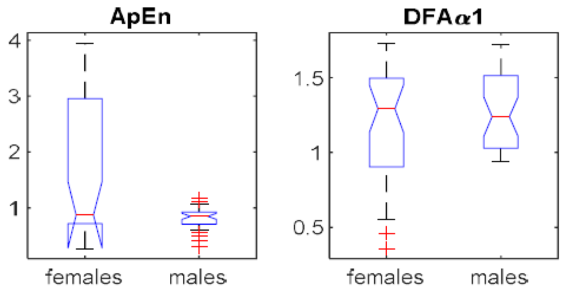


(a)



(b)





(c)

Figure 2. Box plots of the variables with the most elevated values of accuracies and AUC, from Stroke groups, for the three testing conditions: (a) resting state, (b) deep breathing, and (c) standing.

The variables identified by classification methods to be discriminative in Stroke groups for men and women classes are VLFms², LFms², LFnu, HFnu, and ApEn in resting state conditions, Mean RR, HR, pNN50, VLFms², LFnu, HFnu, ApEn and SampEn in deep breathing conditions, and Mean RR, HR, HFnu, ApEn, and DFA⊚1 in standing conditions.

In the resting state, the difference between men and women with stroke shows that men had a significant increase in VLF mean value, 401.29±88.86 ms² vs women 215.91±70.61 ms² (mean ± confidence), with $p=0.0007$, a result which was confirmed through the classification result expressed by an accuracy of 0.76 and AUC of 0.76, results obtained through a cubic SVM classifier. One can see this difference reflected in the form of the probability distribution values depicted in Figure 1 -b. The ratio LF/HF was increased in both stroke groups, men and women, when compared with the control ones, with $p<0.05$, but with no significant differences between their mean values for the stroke groups, $p=0.25$. The discrimination performance between men and women by means of this parameter, through an also cubic SVM classifier, which provided the best classification results in this case, was medium, with an accuracy of 0.63 and an AUC of 0.64. This means that there is a 64% chance to distinguish men vs. women with stroke by means of investigating this parameter.

Both the LF and HF (ms²) spectra were much lower compared to the control group, it can be observed that at rest the spectrum of the LF component, which represents both branches of the autonomic nervous system, didn't display significant differences in women with stroke compared to men with stroke, $p=0.29$. Despite this, the LF parameter showed good discriminatory properties in the classification attempt (accuracy=0.71 and AUC=0.78). Also, the LFnu parameter when tested by a one-tailed T-Test for significance in evaluating equality between mean values of men and women with stroke, provided p-value = 0.13, but the classification results were good, with accuracy = 0.71 and AUC = 0.74, due to significant differences in their median values: 557 ms² in women vs. 344 ms² for the LF values, and respectively 74.5 vs 66.5 for LFnu values. Although the LF/HF ratio attested a greater value for women than for men with stroke, $p = 0.42$, it showed modest discriminative performances regarding the classification of men vs women with stroke, with accuracy = 0.62 and AUC = 0.64.

The one-tail T-test confirmed a greater value of the mean ApEn for the women group (1.53±0.43) than for the men group (0.82±0.07) with stroke, with $p = 0.0008$. The parameter also provided good discriminative properties between men and women with stroke (accuracy = 0.67, AUC=0.7) with a Euclidean KNN classifier.

In deep breathing conditioning, the Mean RR parameter mean value of the men group with stroke (830.46±41.09) is significantly greater than the mean value of the women group with stroke (755.89±34.28), $p=0.003$, and also the HR mean value for women (81.58±3.55) is significantly greater than that for men (74.16±3.74) with stroke condition, $p = 0.0023$. Both of these results were confirmed by the parameters classification performances. which are mentioned in table 2 – deep breathing, which also reveals the classifier to provide the respective performances, the best obtained in a classification task where multiple classifiers, with various parameter values, were tested.

In what concerns frequency domain measures, comparing women and men stroke groups, women's mean value of LF/HF ratio, 2.09±0.47, was found significantly greater than that of the men's mean, 1.53±0.33, with p = 0.027. Also, the LFnu parameter provided a likewise result, with p = 0.009. The HFnu parameter attested for men's mean value, 43.86±4.62, greater than that of women.s, 35.1±6.31, with p = 0.013. For these parameters, only LFnu and HFnu have good discriminative properties between men and women with stroke, as can be seen in table 2 – deep breathing.

From the nonlinear measures, only ApEn validated the hypothesis that women's mean value, 1.49±0.39, is significantly greater than that of men's, 0.82±0.1, with p = 0.0008. The best classification result, obtained with an ensemble bagging tree classifier, was very good, with accuracy = 0.77 and AUC = 0.89, although, in this case, the median values, women = 1.024, men = 0.912, are not significantly different, as can also be seen in Figure 2-b.

In a standing position situation, there are fewer parameters to indicate significant differences between men and women with stroke. For time domain measures Mean RR proved that men's mean value is greater than the women's (values are mentioned in Table 1 – Standing), with p = 0.0036. Figure 1 – also argues this statement. Their respective median values are also significantly different, 816.7 for men and 743.9 for men, as depicted in Figure 2-c. Consequently, good discrimination properties were emphasized through an ensemble classifier with a random subspace learning algorithm: accuracy 0.71, AUC = 0.79 (Table 2 – Standing). Alternatively, women's HR mean value proved to be greater than men's (Table 1 – Standing), with p = 0.0035. As for the previous measure, women's median value of HR, 81.08, is significantly greater than that of men's, 73.86, which is emphasized through boxplot representation with notches for a = 0.05 significance level, in Figure 2 – c. Classification accuracy = 0.74 and AUC = 0.86 validate the significant differences between the two values through discrimination capacity (Table 2 – Standing). The nonlinear measure AppEn is also able to discriminate between men and women with stroke, with accuracy = 0.7, and AUC = 0.77, and set order between mean values, women's greater than men's, with p = 0.001. Median values, 0.874 for women with stroke and 0.848 for men with stroke, don't differ significantly (Figure 2 – c).

4. Discussion

An acute ischemic lesion involving the cortical network controlling the activity of the autonomic nervous system may imbalance autonomic responses at cardiac level and lead to an increased risk of arrhythmia [22].

Graff and collaborators [23] underlined the contribution of HRV in-depth analysis to stroke prognosis and stated that while HRV assessed by linear methods may provide long-term prognostic value, complex, non-linear measures of HRV may rather assess the impact of the neurological state on temporary patterns of heart rate post stroke [23]. In the same line of evidence, it has been recently shown that acute ischemic stroke patients had a significant reduced complexity of HRV. Early assessment of HRV by non-linear methods can be a potential predictor of stroke-in-evolution in newly admitted non- atrial fibrillation ischemic stroke patients [24, 42].

Greater SNS activation and PNS withdrawal during and after exposure to stroke cause an increase in oxidative stress and inflammatory processes, also alter the modulation of the renin–angiotensin system, nitric oxide (NO), and endothelin production. SNS is part of the artery–brain circuit and the increased activity of sympathetic nerve fibers disturb endothelial function and can increases the growth of atheroma's [25]. PNS known as the cholinergic anti-inflammatory pathway has important roles in neuroimmune interactions [26]. Exaggerated SNS activity may impair endothelial function and since blood vessels provide nutrients for neurons and synapses, endothelial dysfunction has a major impact on the autonomic nervous system [27].

The purpose of our research was to underline the differentiated sex influence on the autonomic HR modulation in right MCA ischemic stroke patients. We considered in our analysis time domain parameters attributed to the parasympathetic activity as RMSSD and pNN50 and frequency domain parameters as HF. To evaluate de balance of autonomic nervous system we used LF/HF ratio values. We also used nonlinear analyses parameters such as: SD1, approximate entropy, sample entropy, detrended fluctuation analysis. SD1 is considered a parasympathetic index of sinus node control [28].

The variables identified by classification methods to be discriminative in Stroke groups for men and women classes are VLFms^2, LFms^2, LFnu, HFnu, and ApEn in resting state conditions, Mean RR, HR, pNN50, VLFms^2, LFnu, HFnu, ApEn and SampEn in deep breathing conditions, and Mean RR, HR, HFnu, ApEn, and DFAa1 in standing conditions.

The ratio LF/HF in resting condition was increased in both stroke groups, men and women, when compared with the control ones, with $p<0.05$, but with no significant differences between their mean values for the stroke groups.

Both the LF and HF (ms^2) spectra were much lower compared to the control group, which represents both branches of the autonomic nervous system which are affected by stroke.

The LF and LF nu parameters, which express the sympathetic activity, showed good discriminatory properties in the classification. Also ApEn provided good discriminative properties between men and women with stroke (accuracy = 0.67, AUC=0.7) with a Euclidean KNN classifier.

In deep breathing conditioning, women's mean value of LF/HF ratio, 2.09±0.47, was found significantly greater than that of the men's mean, 1.53±0.33, with p = 0.027. The HFnu parameter, which reflect parasympathetic activity, attested for men's mean value, 43.86±4.62, greater than that of women.s, 35.1±6.31, with p = 0.013. From the nonlinear measures, only ApEn validated the hypothesis that women's mean value, 1.49±0.39, is significantly greater than that of men's, 0.82±0.1, with p = 0.0008.

In a standing position situation, there are fewer parameters to indicate significant differences between men and women with stroke. Women's HR mean value proved to be greater than men's (Table 1 – Standing), with p = 0.0035., which reflect a greater sympathetic activity in women compare with men with stroke in standing position.

The majority of stroke incidence can be attributed to vascular risk factors such as hypertension, hyperlipidemia, diabetes mellitus, smoking, and atrial fibrillation. These factors occur in both men and women, but there are several stroke risk factors that are specific to women such as sex hormones, the use of oral contraceptives, menopause, migraine with aura, etc.

The risk of developing cardiac arrhythmia (atrial fibrillation, ventricular tachyarrhythmia) in post stroke patients is higher in right hemispheric involvement, which is associated to raised sympathetic activity and low HRV. Atrial fibrillation (AF) has become increasingly common around the world, with an estimated prevalence of 596 cases per 100,000 men and 373 cases per 100,000 women [29]. In the Framingham Heart Study, the adjusted risk for stroke was almost two-fold higher in women with AF than in men with AF (HR¼1.92; 95% CI, 1.2–3.07) [30].

Another influencing factor for cerebrovascular reactivity are sex steroid hormones. We know that Estradiol promote endothelial vasodilatation and stimulates blood flow whereas testosterone has the opposite effects [31]. Cerebrovascular reactivity in postmenopausal women is decreased compare with premenopausal women and with age-matched men [32]. In the Women Estrogen Stroke Trial, exogenous estrogens did not reduce the risk of stroke or mortality among postmenopausal women with a history of stroke or transient ischemic attack [33].

The comorbidity between ischemic stroke and cardiovascular disease with migraine is recognized already for decades [34, 35]. Both men and women with migraine with aura have an approximately two fold increased risk of ischemic stroke [34]. The association between migraine and stroke seem to be higher in women and the risk increased to sevenfold in women who use oral contraceptives and even nine fold when they are also smoking [36, 37].

Monitoring of cardiovascular parameters in patients who have suffered an ischemic stroke is essential, as it is known that there is an increased risk for secondary vascular events mediated by disrupting the autonomic nervous system, secondary to the brain injury [38].

HRV quantification is a useful, non-invasive method for evaluating the sympatho-vagal balance reflected on the heart rate. Recent studies showed that abnormal time-domain (SDNN, RMSS), frequency-domain (LF, HF) parameters and nonlinear entropy parameters (DFA, ApEn) are predictors for stroke severity, functional outcome, and mortality [39]. Reduce HRV and loss of parasympathetic tonus and altered HRV parameters during the sleep cycle are associated with worse prognosis [40, 41].

Early cardiac monitoring may change long-term prognosis, since sympathetic overactivity predisposes to secondary cerebro- and cardiovascular events. The brain-heart axis with bi-directional valences implies a multidisciplinary approach to reduce the mortality and morbidity related to cardiovascular diseases and stroke, the progress of diagnostic techniques and the understanding of pathogenic mechanisms being aimed at improving the prognosis and the quality of life of the stroke patient.

One limitation of our study is the low number of participants which may decrease the statistical power. Therefore, further studies with larger groups are needed in order to confirm and strengthen these results.

5. Conclusions

In conclusion, both linear and non-linear parameters of HRV measured in our study demonstrated a decreased vagal influence on heart rate and an increased sympathetic tone in stroke patients in comparison with control group. The variables identified by classification methods to be discriminative in stroke groups for men and women classes are HR, LFms^2, LFnu, HFnu, ApEn and SampEn in resting state. deep breathing and standing.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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