Baroreflex Sensitivity with Different Lags for the Evaluation of Cardiovascular Autonomic Neuropathy in Subjects with Diabetes

Daiana Petry (daianapetry@gmail.com)
Universidade Federal de Santa Catarina https://orcid.org/0000-0003-0541-3253
Claudia Mirian de Godoy Marques
Universidade do Estado de Santa Catarina
Jefferson Luiz Brum Marques
Universidade Federal de Santa Catarina

Research

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Baroreflex Sensitivity with Different Lags for the Evaluation of Cardiovascular Autonomic Neuropathy in Subjects with Diabetes

Daiana Petry[1,2*], Claudia Mirian de Godoy Marques[3], Jefferson Luiz Brum Marques[1]

[1]Institute of Biomedical Engineering, Department of Electrical and Electronic Engineering, Federal University of Santa Catarina, Florianópolis-SC, Brazil

[2]Department of Environmental Engineering, State University of Santa Catarina, Lages-SC, Brazil

[3]Department of Health Sciences, State University of Santa Catarina, Florianópolis-SC, Brazil

Corresponding Author:
Daiana Petry
Avenida Luis de Camões, 2090. 88520-000. Lages, SC, Brazil.
+55 49 32899245
daiana.petry@udesc.br

ABSTRACT

Background: Impaired Baroreflex sensitivity (BRS) may indicate cardiovascular autonomic neuropathy (CAN), which often remains undiagnosed during the initial course of diabetes mellitus. The baroreflex mechanism can be considered negative feedback because of baroreflex delay, the time delay between a change in blood pressure, and the counteracting heart rate response. This work sought to analyze BRS through the sequence method, but establishing delays in checking the RR interval, from 1 to 10 RR intervals lag after systolic blood pressure change. We hypothesized that diabetic patients with subclinical CAN would have a detectable delay in autonomic nervous system activity and that it would differ from other patients.

Results: The study included 30 subjects with diabetes mellitus. Eleven patients had established CAN (mean ± SD age 37 ± 8 years), 9 patients had subclinical CAN (age 35 ± 10 years), and 10 patients did not have CAN (age 35 ± 6 years). Indexes related to the delay in response of the BRS were proposed and obtained. The three variables that showed potential to separate patients with and without CAN were highest BRS index, BRS with the largest number of sequences, and lag of the largest number of sequences. Several variables were observed to distinguish between individuals with subclinical and established CAN, including the number of sequences of the highest BRS, lag of the highest BRS, and the highest number of sequences. **Conclusions:** Thus, analysis of BRS and the reaction delay in the heart rate variability signal may contribute to the detection of CAN in its asymptomatic stage.

KEYWORDS: cardiovascular autonomic neuropathy, heart rate variability, baroreflex sensitivity, blood pressure variability, diabetes mellitus.

BACKGROUND

Chronic impairment of the sympathetic and parasympathetic branches of the autonomic nervous system can be caused by poor management of diabetes mellitus (DM) and can lead to diabetic autonomic neuropathy, a condition that can affect different organs and systems including the cardiovascular and respiratory systems. Cardiac autonomic neuropathy (CAN) occurs when autonomic control of the cardiovascular system is impaired, after exclusion of other causes of dysautonomia [1, 2, 3].

CAN is one of the most severe complications of DM and is associated with a low quality of life and poor prognosis [4]. Despite its potential negative impact on the quality of life of patients, CAN is among the least understood and diagnosed diabetic complications and is sometimes even ignored in individuals with DM [4, 5].

According to Fisher and Tahrani, approximately 17% to 66% of patients with type 1 DM (DM1) also have CAN and between 31% and 73% of patients with type 2 DM (DM2) also have CAN. This wide range of values is due to different criteria used for the diagnosis, differences in populations in the studies, and the different risk factors of CAN considered [6].

CAN is associated with an increased risk of mortality [7]; that was described in longitudinal studies as early as the 1990s, showing a 50% increase in the 5-year mortality risk in patients with DM and autonomic neuropathy compared to those with only DM [8, 9, 10,11,12,13].

CAN is also associated with an increased risk of sudden cardiac death due to the increase in the rate of fatal cardiac arrhythmias because of the imbalance between sympathetic and parasympathetic autonomic function as well as cardiac sympathetic denervation [14, 3]. Several studies have shown an association between prolonged QT interval and autonomic neuropathy, which may also make CAN patients more susceptible to life-threatening cardiac arrhythmias [15, 16, 17,18, 19, 20, 21, 22, 23, 24].

Cardiac autonomic neuropathy can be subdivided into subclinical CAN (SCAN), where changes are predominantly functional and reversible, and clinical CAN, where changes are advanced and structural. Heart rate responses to deep breathing, standing, and the Valsalva maneuver, as well as blood pressure response to

standing, also known as cardiovascular autonomic reflex tests, are considered the gold standard in clinical testing for autonomic neuropathy [25]. The majority of diabetic patients with CAN have a subclinical or asymptomatic disease, rendering the diagnosis and assessment of CAN in clinical practice rather tricky [5].

When CAN becomes clinically evident, the disease has likely reached its advanced stage, and its management is even more difficult. Early screening for CAN is therefore considered a good clinical practice and should be performed at the time of DM2 diagnosis and within 5 years of DM1 diagnosis [23, 25, 26].

The first detectable signs of SCAN are a decrease in heart rate variability (HRV) and abnormal baroreflex sensitivity (BRS), which may already be present at the time of DM diagnosis [5]. Impaired BRS may be a sign of autonomic diabetic cardiovascular neuropathy, which often remains unknown during the initial course of diabetes. BRS may be an additional marker for SCAN, as several studies have shown that BRS abnormalities occur before abnormalities are detected by conventional autonomic function tests [27, 28].

One of the most widely used methods for the analysis of BRS is the sequence method. In the sequence method, BRS is assessed as the slope of the regression line between spontaneous increases or decreases in systolic blood pressure (SBP) (1 mmHg) linearly related to increases or decreases in the RR interval (5 ms), in sequences of at least four consecutive heartbeats (three RR intervals). The mean slope of all sequences generates the BRS index of the signal [27, 29, 30, 5, 31].

The baroreflex mechanism is anatomically and physiologically complex and can be considered a closed-loop negative feedback system. The baroreflex delay is the time delay following a change in blood pressure before the counteracting RR response takes place [32, 33]. The traditional sequence method does not take the baroreflex delay into account.

The delay between increased SBP and baroreflex bradycardia is increased when the vagal tone is low [34] and when the sympathetic tone is high [35, 34]. This delay alteration, therefore, affects patients with SCAN. Once neuropathy develops, DM increasingly affects cardiac autonomic neurons, with pathological changes initially more evident in long nerve fibers. The longest autonomic nerve is the vagus nerve,

responsible for approximately 75% of all parasympathetic activity (that reflects mainly the high-frequency components of the baroreflex), and which tends to be involved early in the course of CAN development. The early stages of CAN, therefore, involve a reduction in parasympathetic activity, which results in a compensatory increase of the sympathetic tone. This increase in sympathetic tone continues until the end stage of CAN, when sympathetic denervation ensues, which spreads gradually from the apex to the base of the heart [36, 37, 7].

The delay is positively related to heart rate and age, but negatively related to baroreflex slope [34, 35]. These authors suggest that the delay of BRS response may signify that the autonomic nervous system takes longer than usual to sense and react to SBP increases or decreases, and also slows the reaction from the beginning of a stimulus, characterized by the low baroreflex slope. This behavior could be related to an impairment of the autonomic nervous system due to DM complications like CAN.

One of the most significant challenges with diabetic patients is to detect CAN in its subclinical stage, which could be seen as a transitory phase between diabetes without CAN and diabetes with established CAN (ECAN). CAN detection at its early stage carries out a better prognosis with a better quality of life and fewer complications [13, 23, 38].

As changes in BRS analysis may reflect the behavior of the autonomic nervous system in the maintenance of homeostasis, this work sought to analyze the BRS through the traditional sequence method (with lag 1), as well as establishing delays in checking the RR interval (from 0 to 10 RR intervals) to obtain the sequences for each of these lags. The hypothesis of this study focuses on the possibility that diabetic patients with SCAN present a delay in autonomic nervous system activity, differently from patients without CAN and patients with established CAN. In these cases (i.e., SCAN), the heart rate response to SBP change will not occur promptly.

It is expected that minimal lag with the highest BRS value (indicating faster response of the autonomic nervous system), and the highest number of sequences (indicating autonomic response to environmental stimuli in the body) are directly related to CAN stage; patients without CAN may have the highest values of

these two variables and lags smaller than those compared to any stage of CAN. The interaction of these variables may provide a biomarker for the early detection of CAN, particularly at its subclinical stage.

RESULTS

Table 1 shows the one-way ANOVA results for each variable, with a p-value of 0.05 or lower considered to be significant; p-value results indicated that only one of the studied variables, the number of sequences with the highest BRS value, did not present a significant difference between groups (Tukey post-hoc test comparisons). All other variables show a significant difference when comparing patients without CAN and patients with ECAN. For three variables, it was possible to differentiate the patients without CAN and those with SCAN, and for two variables, it was possible to differentiate patients with SCAN from patients with ECAN. None of the variables alone could distinguish all three of the groups.

The mean of RR intervals was not significantly different between patients with SCAN and ECAN, reinforcing the idea that for diseased patients at any stage of CAN, HRV is significantly reduced.

Table 1: One-way ANOVA results of each variable for patients with NCAN, SCAN, and ECAN

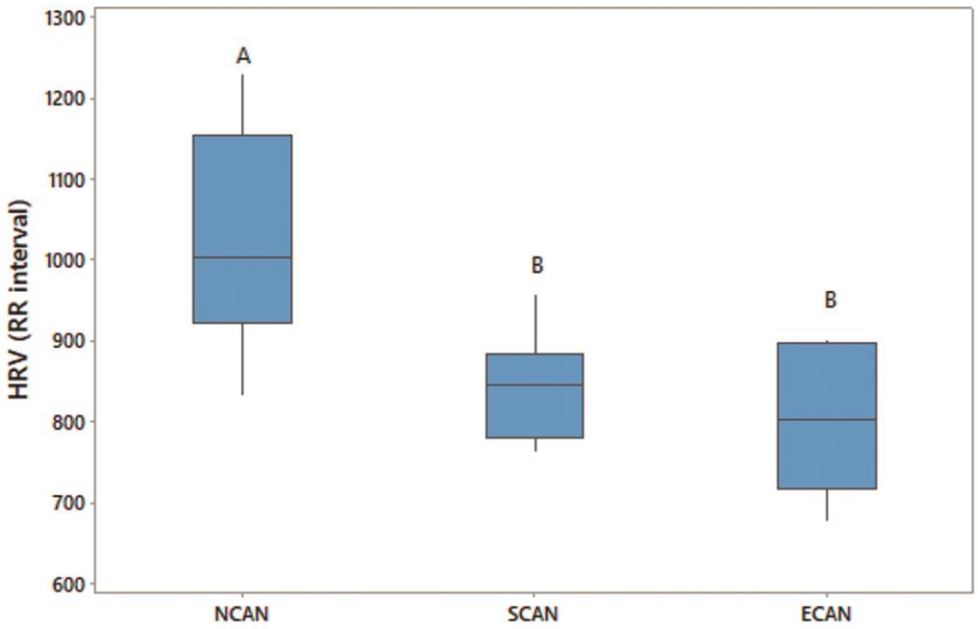
Index	NCAN mean ± SD	SCAN mean ± SD	ECAN mean ± SD
i1	31.8 ± 6.2 *^	17.8 ± 7.79 #	11.2 ± 4.7 #
i2	38.3 ± 18.3 ^	45.9 ± 11.3 ^	16.8 ± 7.5 #*
i3	7.3 ± 4.3	8.8 ± 5.9	7.4 ± 4.3
i4	21.9 ± 4.4 *^	10.8 ± 2.6 #	7.1 ± 2.5 #
i5	4.5 ± 2.4 *^	8.0 ± 2.7 #	7.4 ± 1.7 #
i6	0 ± 0.0 ^	0.11 ± 0.3 ^	2.1 ± 2.5 #*
i7	1029.9 ± 136.2*^	838.8 ± 64.3 #	801.3 ± 5.2 #
i8	124.4 ± 15	127.6 ± 12.8	124.0 ± 7.8

* Different from SCAN; ^ Different from ECAN; # Different from NCAN; $p < 0.05$ considered significant.
NCAN = No Cardiovascular Autonomic Neuropathy, SCAN = Subclinical Cardiovascular Autonomic Neuropathy, ECAN = Established Cardiovascular Autonomic Neuropathy.
i1 = Higher BRS, i2 = Greater number of sequences, i3 = Number of sequences of the highest value of BRS, i4 = BRS of the highest number of sequences, i5 = Lag of the highest BRS value, i6 = Lag of the highest number of sequences, i7 = RR interval, i8 = SBP (systolic blood pressure).

Figures 1, 2, and 3 present boxplots comparing the studied variables in patient groups. The A and B labels in each box indicate significant differences between groups. No boxplot was generated for SBP

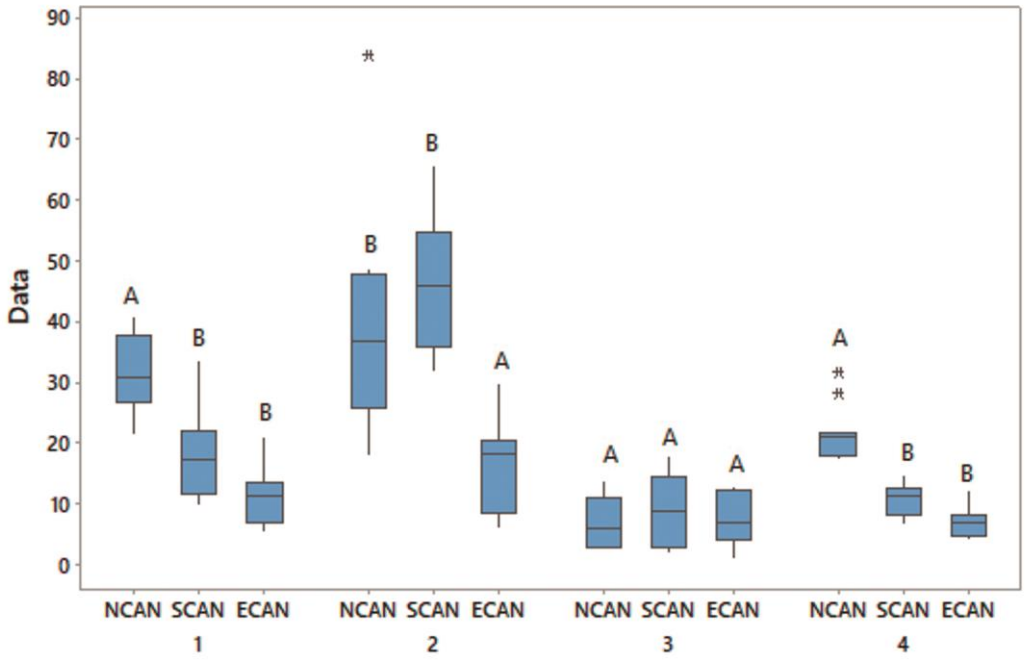
because this variable showed no significant difference between any of the patient groups (p>0.05).

Figure 1: Boxplot of HRV values, defined as RR intervals, for different patient groups.



The "A" and "B" labels indicate that HRV values of NCAN patients are significantly different from those in SCAN patients (p = 0.001) and those in ECAN patients (p = 0.000). There was no significant difference in HRV/RR intervals between patients with SCAN and patients with ECAN.

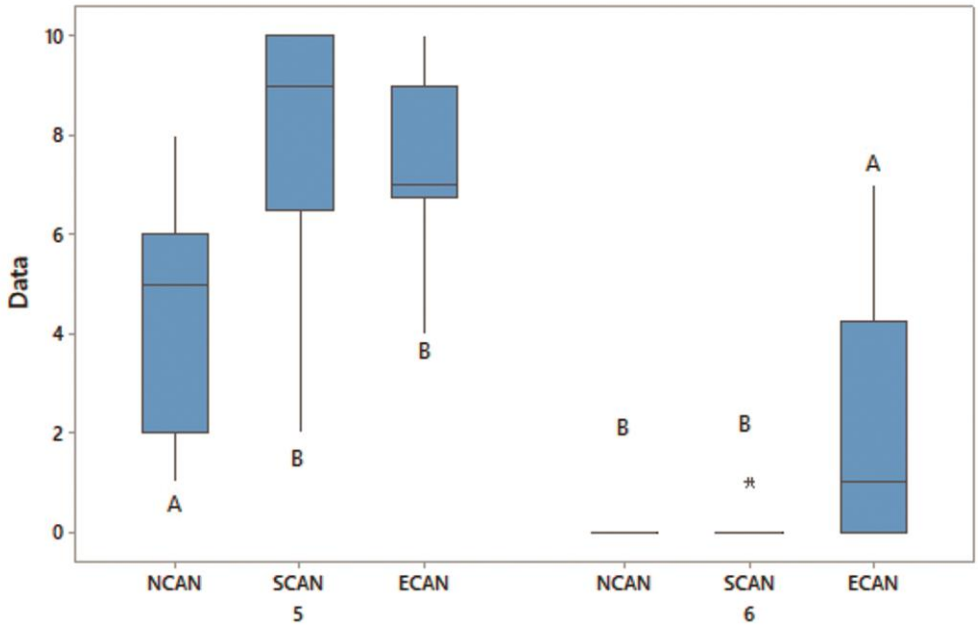
Figure 2: Boxplot depicting values of the variables 1, 2, 3 and 4 for different patient groups.



"1": higher BRS, "2": greater number of sequences, "3": number of sequences of the highest value of BRS, and "4": BRS of the highest number of sequences. The "A" and "B" labels indicate statistical significance. For (1), NCAN patients are significantly different from SCAN (p = 0.000) and ECAN patients (p = 0.000).

For (2), ECAN patients are significantly different from SCAN (p = 0.000) and NCAN patients (p = 0.003). For (3), there are no significant differences between groups. For (4), NCAN patients are significantly different from SCAN (p = 0.000) and ECAN patients (p = 0.000). The symbols * indicate three outliers samples that were considered in the study.

Figure 3: Boxplot depicting values of the variables 5 and 6 for different patient groups.



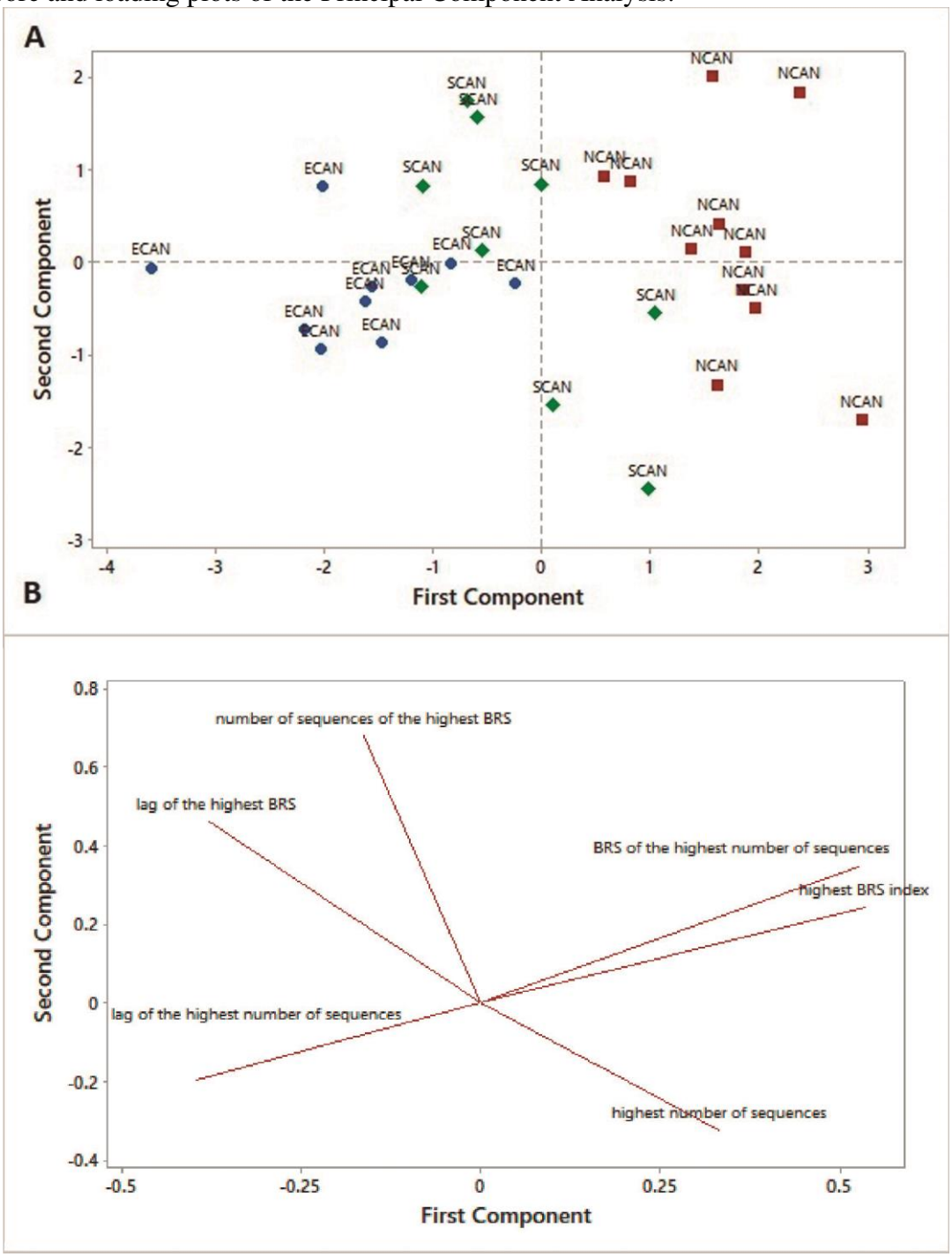
"5": lag of the highest BRS value and "6": lag of the highest number of sequences. The "A" and "B" labels indicate statistical significance. For (5), NCAN patients are significantly different from SCAN (p = 0.007) and ECAN patients (p = 0.024). For (6), ECAN patients are significantly different from SCAN (p = 0.000) and NCAN patients (p = 0.000). The symbol * indicate one outlier sample that was considered in the study.

In multivariate tests, all variables are considered together to account for interactions between variables to differentiate between patient groups. Using the MANOVA multivariate test, Wilks' λ was significant, approximately zero. Also, PCA was applied to understand the structure and dimensions of the data, to emphasize variation and to reveal patterns in the dataset, and ultimately to differentiate between patient groups.

Figures 4 and 5 show the PCA results. Figure 4A shows the two-dimensional dispersion of patients relative to the first two principal component scores, explaining approximately 70% of the data variance. Figure 4B shows the loading graph, identifying the variables with the most significant effect on each component. Figure 5 (parts A and B) shows the dispersion of patients concerning the first three principal component scores, explaining, all together, approximately 80% of the data variation. Panel B shows the same

graph of Panel A, just rotated for better visualization. The third principal component was included only to show three-dimensional dispersion of the patients, as it was already possible to group patients according to their CAN status with only two principal components.

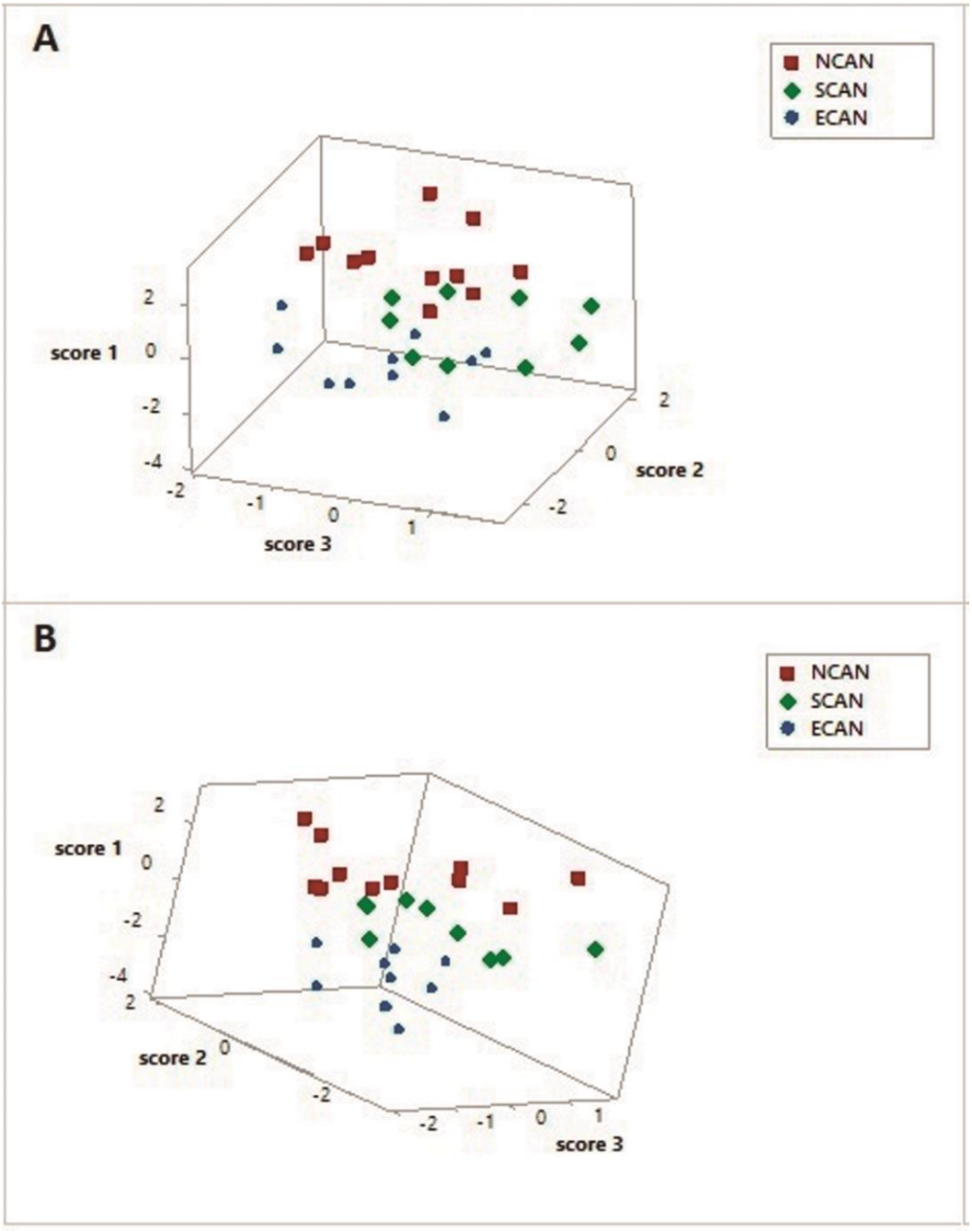
Figure 4: Score and loading plots of the Principal Component Analysis.



(A) Two-dimensional dispersion of patients regarding the first two principal component scores, explaining approximately 70% of the data variance. (B) Loading graph identifying variables with the greatest effect on each component or the variables responsible for data clusters. NCAN patients shown by red squares are positioned in the second and fourth quadrants and ECAN patients shown by blue circles are positioned in the

first and third quadrants. SCAN patients shown by green diamonds are positioned between NCAN and ECAN patients.

Figure 5: Three-dimensional dispersion of patients concerning the scores of the first three principal components.



Explaining approximately 80% of the data variance. Part B shows the same graph of part A, rotated for better visualization.

The variables with the most significant effect on the first principal component, accounting for 49% of the variation between patients without CAN and with ECAN, were highest BRS index, BRS of the highest

number of sequences, and lag of the highest number of sequences. The variables with the greatest effect on the second principal component, accounting for 21% of the variation between patients, were the number of sequences of the highest BRS and lag of the highest BRS. The variables with the most significant effect on the third principal component, accounting for 10% of the variation between patients, were number of sequences of the highest BRS and highest number of sequences.

DISCUSSION

It is possible to separate patients into the three defined groups, as shown in Figures 4A, 5A, and 5B. The variables, highest BRS index, BRS of the highest number of sequences, and lag of the highest number of sequences were used to distinguish patients without CAN and with ECAN. The variables number of sequences with the highest BRS, lag of the highest BRS, and the highest number of sequences were used to distinguish patients with SCAN from other patients.

The three variables that showed potential to distinguish patients with CAN and patients without CAN (highest BRS index, BRS of the highest number of sequences, and lag of the highest number of sequences) can be physiologically validated. Reactions of the autonomic nervous system immediately following environmental stimuli represent a healthy response, as an individual is always receiving environmental stimuli that affect their blood pressure. Normal responses must also be rapidly terminated to restore homeostasis. Therefore, diabetic individuals without autonomic nervous system impairment should present with a high number of sequences, minimal delay before onset, and rapid termination of response, together contributing to a high BRS value.

As previously mentioned, the critical variables to distinguish between individuals with SCAN and ECAN included the number of sequences of the highest BRS, lag of the highest BRS, and the highest number of sequences. While patients with SCAN may still present autonomic nervous system reactions, delayed reactions are also confirmation of autonomic nervous system impairment. These variables differentiate patients according to the disease stage because, as the disease progresses, it is possible to identify autonomic

nervous system dysfunction.

According to the principal component analysis, there are two dimensions differentiated by the variable highest BRS index, BRS of the highest number of sequences, and lag of the highest number of sequences. The scatter plot (Figure 4) shows that these two dimensions correspond to the NCAN and ECAN patient groups. The variables highest BRS index, BRS of the highest number of sequences have vectors with similar direction and magnitude.

Thus, it is possible to consider that only one of these variables is sufficient to position NCAN and ECAN patients in the same positions as they are in the scatter plot (Figure 4). We can say that NCAN patients are grouped in regions where the BRS of the highest number of sequences are high and the lag of the highest number of sequences are low. In the opposite values to this logic were grouped the patients ECAN. It should be noted that the values of the largest number of sequences remained in lags 0 or 1, almost entirely.

The patients who had no correlation with this behavior coincided with the group of patients with SCAN. This group of patients (SCAN) showed the following behavior according to the principal component analysis: higher lags and number of sequences of the highest BRS, and higher number of sequences (Figure 4).

Some studies revealed that the sequence method can show an oscillatory pattern related to the respiratory influence on SBP and/or PI (pulse interval) variability, limiting the SAP ramps to 3 or 4 beats of length, and consequently quantifying mainly the high-frequency components of the baroreflex (vagal tone) [44]. Considering that in patients with SCAN the vagal tone is low, but the sympathetic tone may still be high [34, 35], the result is consistent with the hypothesis that the delayed BRS may reflect this condition and assist in the detection of CAN in the subclinical phase.

It is well known that care should be taken with the sequence method for estimating BRS with lags greater than 1, as this estimation is not only taking into account the rapid response of the autonomic nervous system but is being influenced by other different regulatory factors of systolic blood pressure and heart rate [45, 46, 47, 48, 49]. However, the results found in this paper demonstrate that, at least, we can consider that

patients with SCAN present a different behavioral pattern than the other two groups of patients when BRS analysis in different lags is studied. The pattern that differentiated NCAN patients from ECAN is characterized by results of significant variables centered on 0 and 1 lags, which are already validated for the study of autonomic nervous system response. On the other hand, patients with SCAN departed from this behavior, demonstrating a disorder, although still functional, in the action of the autonomic nervous system HRV and BP regulation.

CONCLUSION

In conclusion, this work demonstrates that BRS analysis and the delay of HRV reaction may be a promising approach to detect SCAN.

METHODS

The study included 30 diabetic male and female patients. Patients were divided into three groups presented in Table 2: 10 patients with ECAN (6 males and 4 females), 9 with SCAN (5 males and 4 females), and 11 without CAN (6 males and 4 females). From those data analyzed in Table 2, just Duration of Diabetes had any significant difference between the groups. The ECAN patients had more duration of diabetes than other groups. This data agrees with the idea that the risk of autonomic neuropathy increases with the duration of diabetes. ECAN and SCAN were diagnosed using Ewing tests [14], considering the composite score index. If the composite score was up to 1 the patients were NCAN, if the composite score was between 2 and 3 the patients were SCAN, and if the composite score was more than 3 the patients were ECAN. None patient was under medication during the study, and none was hypertensive or had any other comorbidity. All patients were no smoker and non-alcoholic. The institutional research ethics committee approved the study, and all patients provided informed consent.

Table 2: Subjects of the Study.

Data	NCAN	SCAN	ECAN
Age (years)	35.27 ± 6.0	35.77 ± 11.22	37.80 ± 8.01
Age on onset (years)	21.54 ± 10.27	21.89 ± 12.28	14.8 ± 9.17
Duration of diabetes (years)	13.73 ± 9.38 ^	13.89 ± 10.37 ^	23.0 ± 9.22 #*
BMI (units)	23.75 ± 2.74	25.18 ± 3.44	24.42 ± 2.91
HbA1c (%)	8.56 ± 1.85	8.64 ± 0.90	8.21 ± 0.93
Respiratory rate (resp/min)	12.94 ± 1.82	13.81 ± 1.16	14.94 ± 2.29

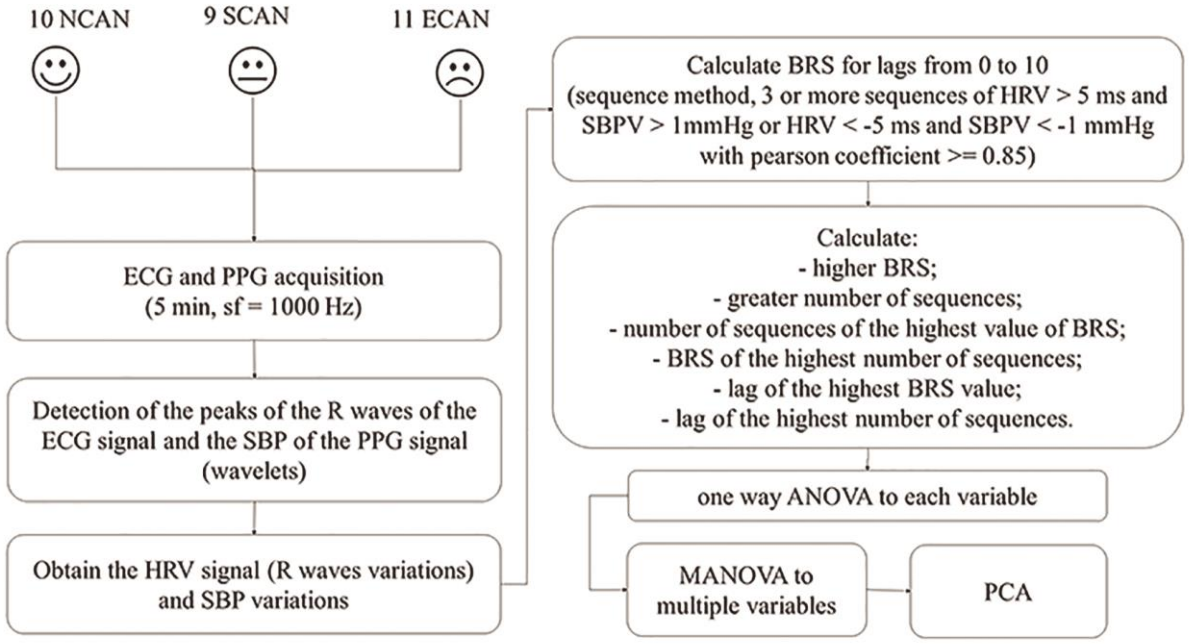
* Different from SCAN; ^ Different from ECAN; # Different from NCAN; $p < 0.05$ considered significant.
NCAN = No Cardiovascular Autonomic Neuropathy, SCAN = Subclinical Cardiovascular Autonomic Neuropathy, ECAN = Established Cardiovascular Autonomic Neuropathy, BMI = body mass index, HbA1c = glycosylated hemoglobin.

Electrocardiogram (ECG) and photoplethysmogram (PPG) signals were obtained over 5 minutes with subjects at rest in a dorsal supine position. Continuous non-invasive Photoplethysmography (PPG) was recorded using a validated non-invasive device (Finapres® NOVA) together with an ECG recording using a WinDaq data acquisition software (DATAQ Instruments, Inc.) with a sampling rate of 1000 samples/second. ECG signals without extrasystoles were evaluated. The methodology followed to the detection of the systolic (SBP) and diastolic (DBP) values of the PPG signal was: with the correct time alignment between the PPG and ECG signals, the time value of the R wave peak of the ECG is found and the same point in time in PPG is marked. This point will correspond to the value of the diastolic pressure. Similarly, the systolic pressure will be the time value in the PPG signal corresponding to the T-wave peak in the ECG [42]. Figure 6 shows the methodological flow chart of the study after signal acquisition and patients' classification.

To analyze the ECG and PPG data, MATLAB (MathWorks®) programming was used to develop a software tool. Signals were preprocessed with a fourth-order Butterworth low-pass filter with a cut-off frequency of 30 Hz and a third-order Butterworth high-pass filter with a cut-off frequency of 0.8 Hz [39] to improve the accuracy of ECG feature extraction. The respiratory frequency was measured to each one patient, and nonsignificant difference was observed between the patients' groups (Table 2). Then, the signals did not have respiratory sinus arrhythmia filtered. Singularities were detected and processed with wavelets, using signal attenuation with a low-pass function. The wavelet used was the first derivative of a Gaussian function, computed in 2^4 scale and centered around 13 Hz, according to the biggest relative powers of the R-wave [40,

41]. The technique established by Clifford and McSharry [42] was followed to detect SBP and diastolic blood pressure values of the PPG signal in conjunction with the ECG signal.

Figure 6: Methodological flowchart of the study.



30 diabetic patients (11 NCAN, 9 SCAN, and 10 ECAN), ECG and PPG signals were acquired over 5 minutes with a sampling frequency of 1000 Hz. Signal features, including R-waves and SBP values, were detected. RR and SBP series were generated, and indices were calculated using the sequence method to evaluate the BRS, considering 0 to 10 lags and HRV analysis. Statistical analysis was carried out using ANOVA, MANOVA, and PCA.

After detecting signal features, including the difference between the consecutive R waves, called the RR series, and consecutive SBP values, called the SBP, BRS was assessed. As described by Porta et al. [43], BRS can be assessed as the slope of the regression line between spontaneous SBP increases or decreases, and linearly related RR interval increases or decreases over at least four consecutive beats (i.e., sequence method). In this work, BRS was measured using the sequence method, however, different lags were assigned to each subject. Sequences were considered if they consisted of three or more intervals (four or more consecutive heartbeats), had lags from 0 to 10, and had a correlation of at least 85% between RR and SBP. For each case, the highest BRS index, the highest number of sequences, the lag of the highest BRS, the lag of the highest number of sequences, the number of sequences of the highest BRS, and the BRS of the highest number of

sequences were obtained.

Initially, univariate analysis of variance (one-way ANOVA) was used to evaluate the significance of the differences between NCAN, SCAN, and ECAN for each variable. Subsequently, two multivariate analysis techniques were used: MANOVA and Principal Component Analysis (PCA). MANOVA was used to verify whether the dependent variables were significantly affected by changes in independent variables, considering the interactions between dependent and independent variables. PCA was used to evaluate the structures and dimensions responsible for the dispersion of the subjects in space. Minitab16 (Minitab Inc®) was used for statistical analysis.

DECLARATIONS

Ethics approval and consent to participate

The institutional research ethics committee approved the study, and all patients provided informed consent.

Consent for publication

"Not applicable".

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

"Not applicable".

Authors' contributions

D.P. and J.M. conceived of the presented idea. D.P. developed the theory and performed the computations. D.P. and J.M. verified the methods. J.M. supervised this work. JM and CM analysed data and

revised this manuscript. All authors discussed the results and contributed to the final manuscript.

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ABBREVIATIONS

BRS: Baroreflex Sensitivity

CAN: Cardiovascular Autonomic Neuropathy

DM: Diabetes Mellitus

DM1: Diabetes Mellitus Type 1

DM2: Diabetes Mellitus Type 2

ECAN: Established Cardiovascular Autonomic Neuropathy

HRV: Heart Rate Variability

NCAN: No Cardiovascular Autonomic Neuropathy

PCA: Principal Components Analyze

QT: Interval between Electrocardiogram Q and T waves

RR: Interval between Electrocardiogram R waves

SBP: Systolic Blood Pressure

SCAN: Subclinical Cardiovascular Autonomic Neuropathy

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Figures

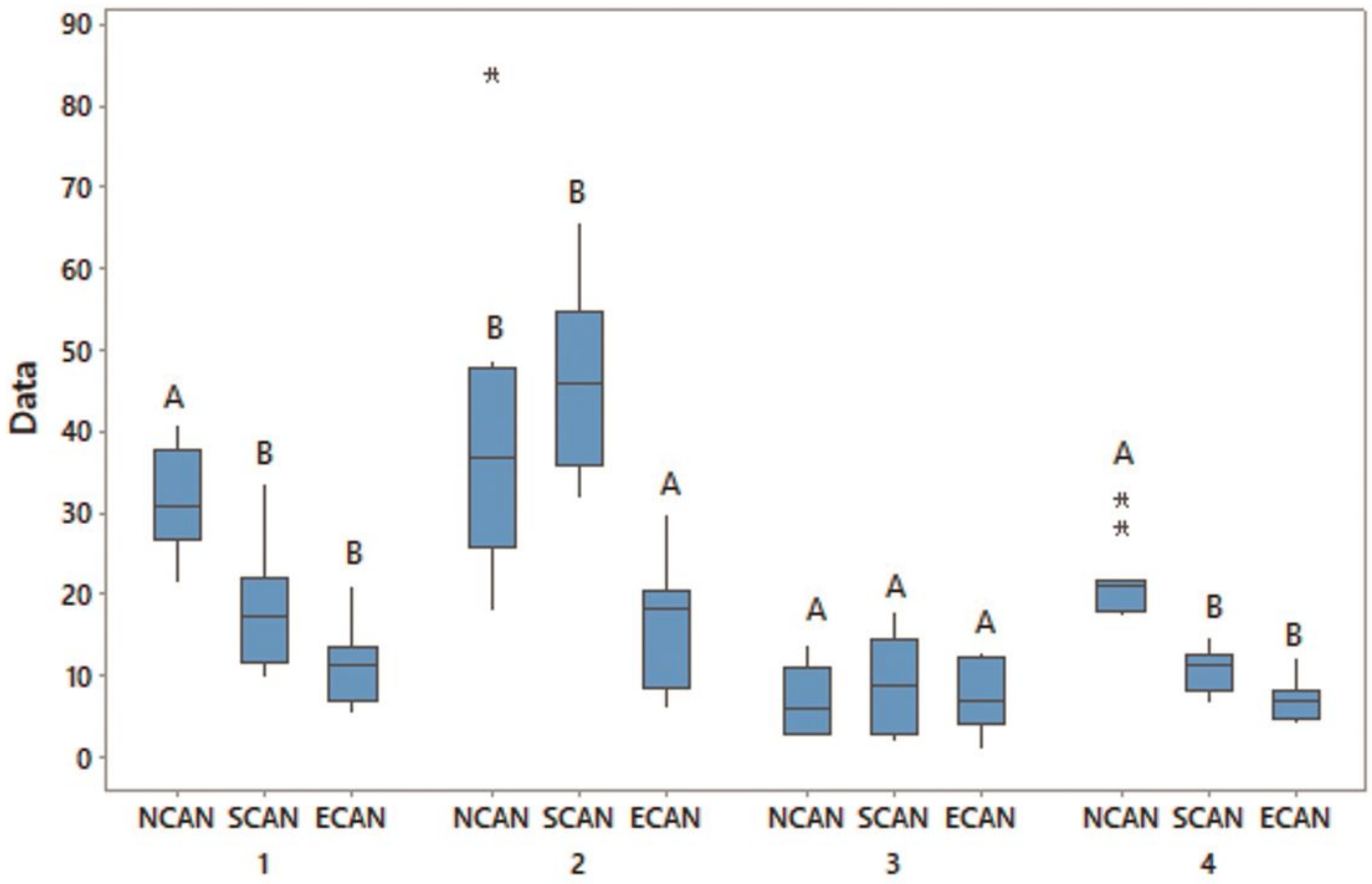

Figure 1

Boxplot of HRV values, defined as RR intervals, for different patient groups.

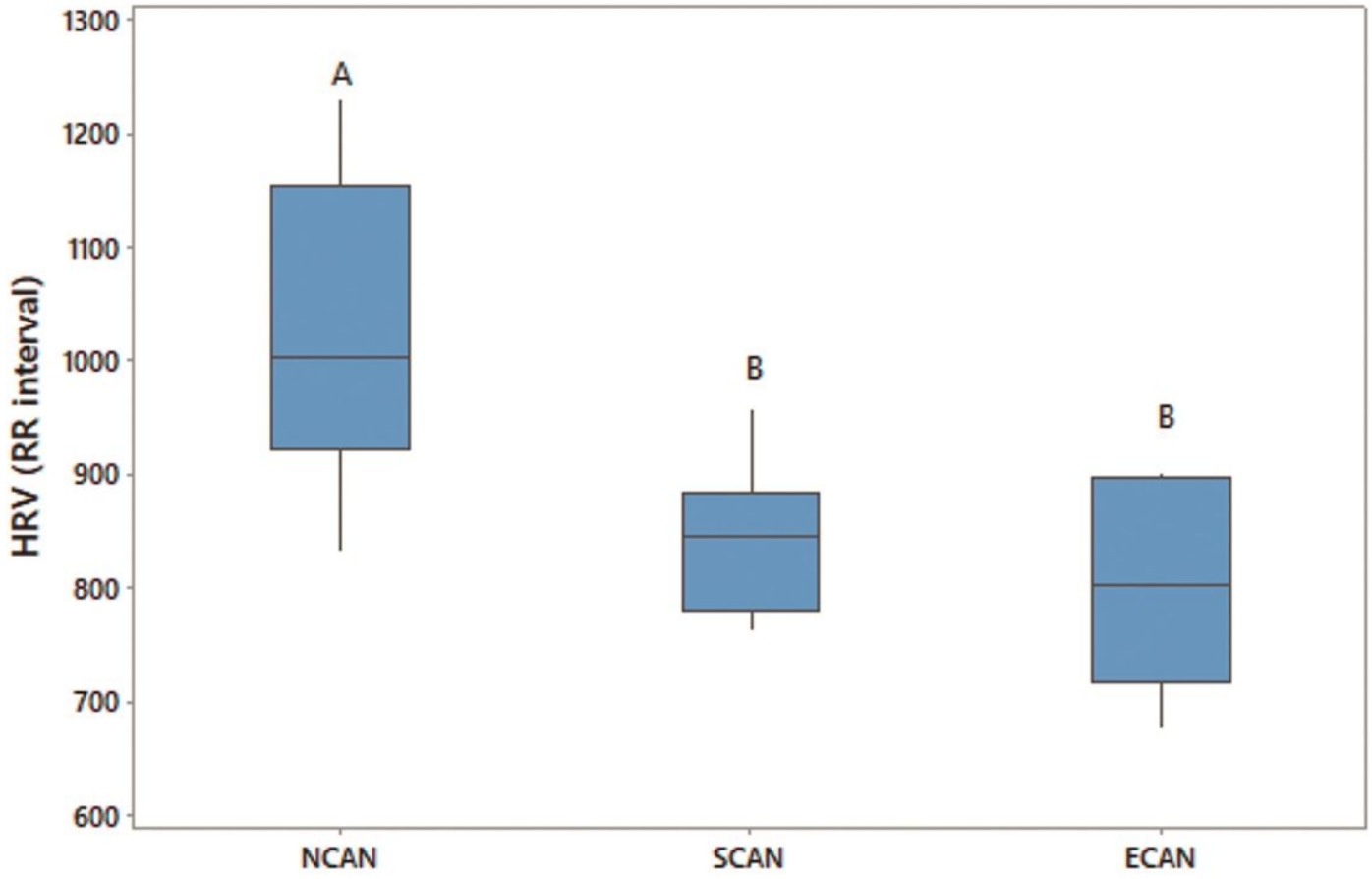

Figure 2

Boxplot depicting values of the variables 1, 2, 3 and 4 for different patient groups.

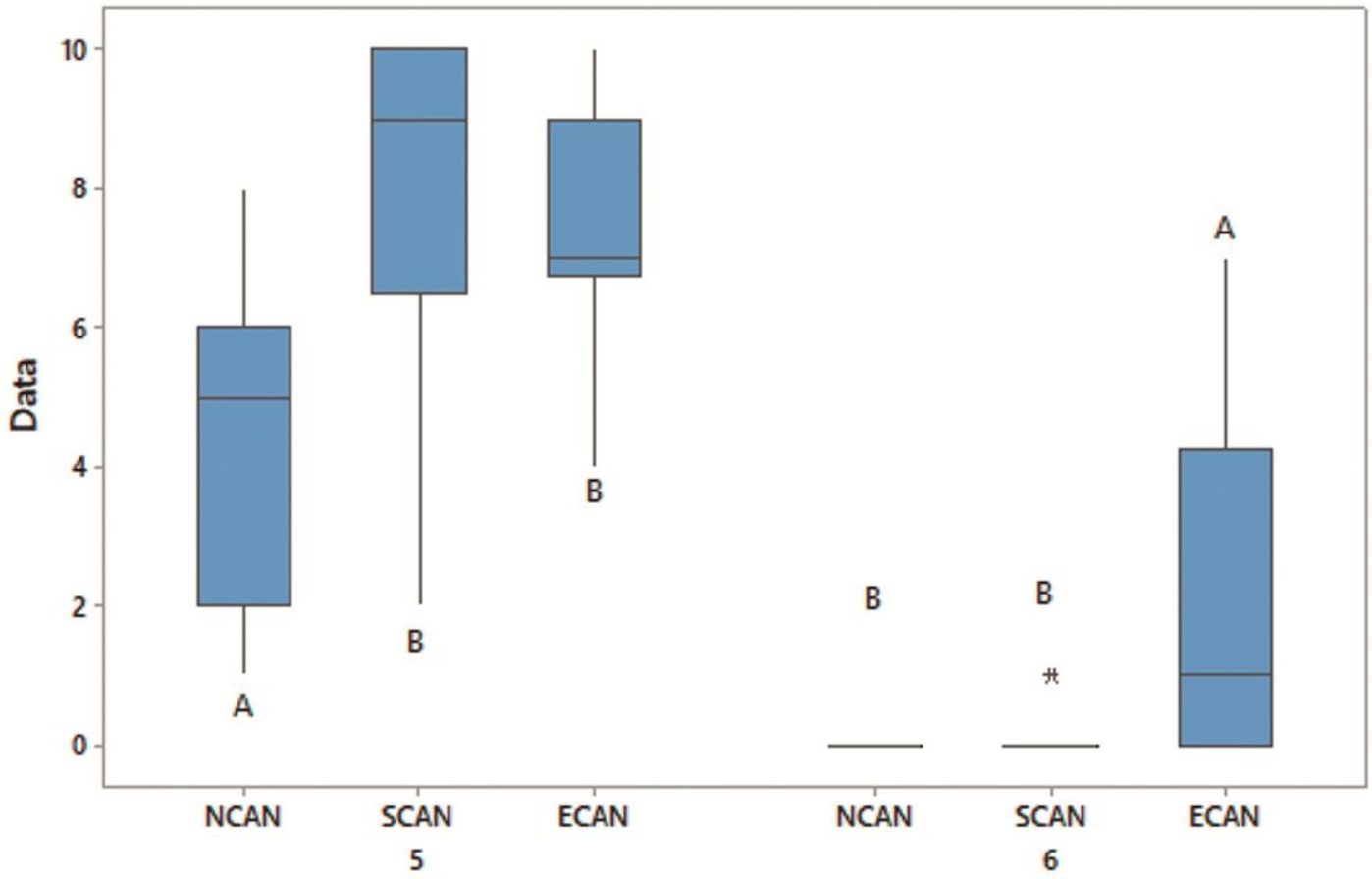

Figure 3

Boxplot depicting values of the variables 5 and 6 for different patient groups.

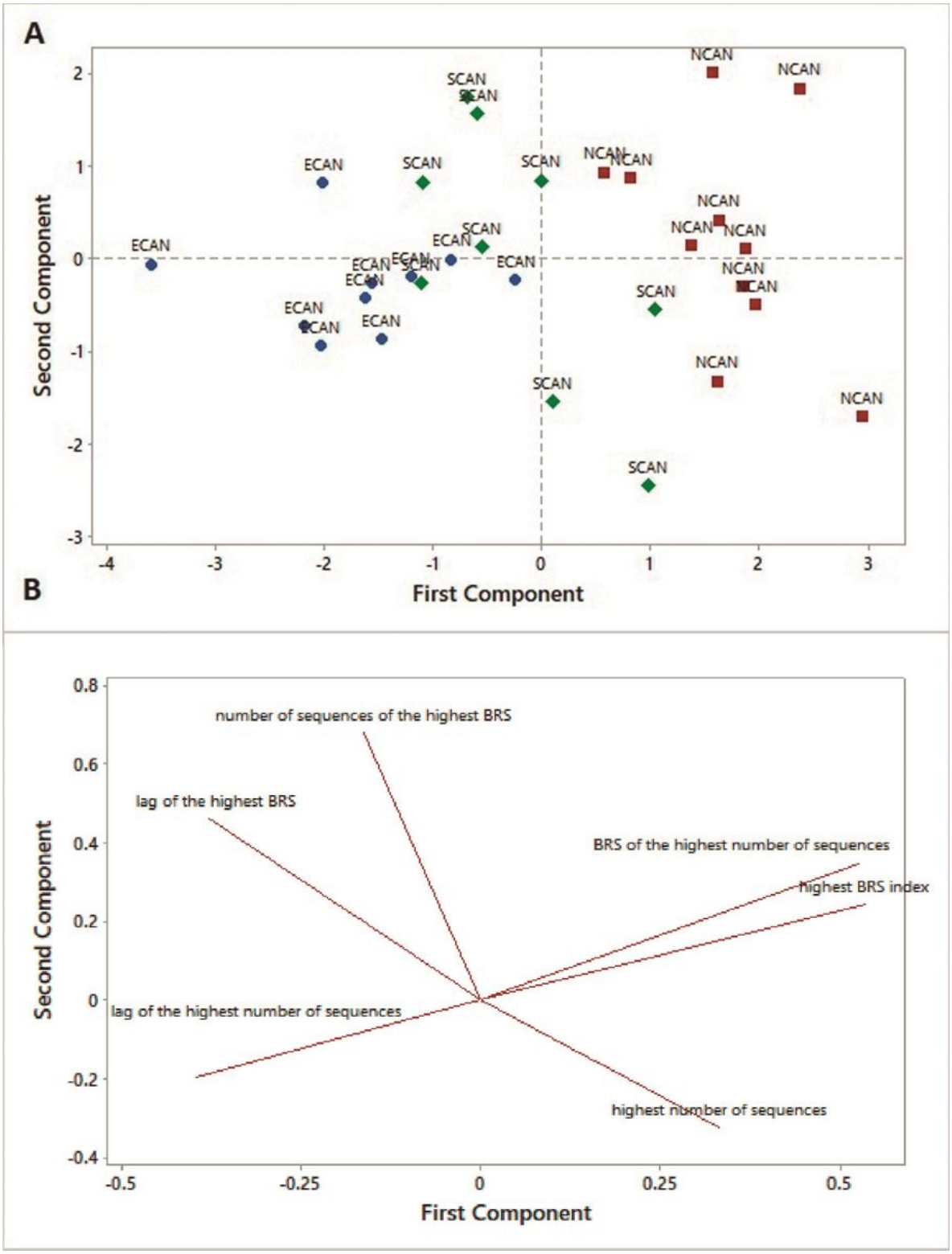

Figure 4

Score and loading plots of the Principal Component Analysis. (A) Two-dimensional dispersion of patients regarding the first two principal component scores, explaining approximately 70% of the data variance. (B) Loading graph identifying variables with the greatest effect on each component or the variables responsible for data clusters. NCAN patients shown by red squares are positioned in the second and

fourth quadrants and ECAN patients shown by blue circles are positioned in the 10 first and third quadrants. SCAN patients shown by green diamonds are positioned between NCAN and ECAN patients

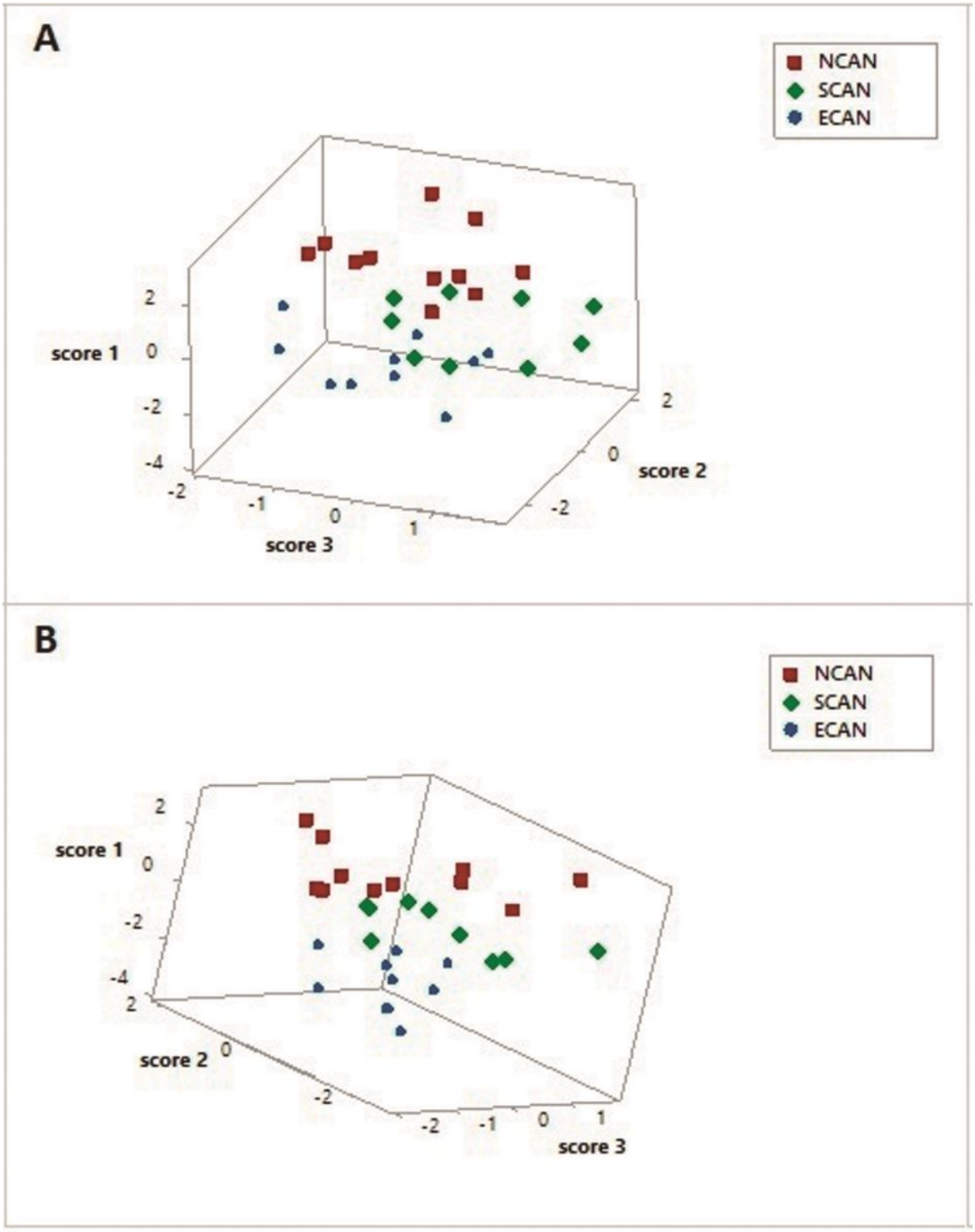

Figure 5

Three-dimensional dispersion of patients concerning the scores of the first three principal components.Explaining approximately 80% of the data variance. Part B shows the same graph of part A, rotated for better visualization.

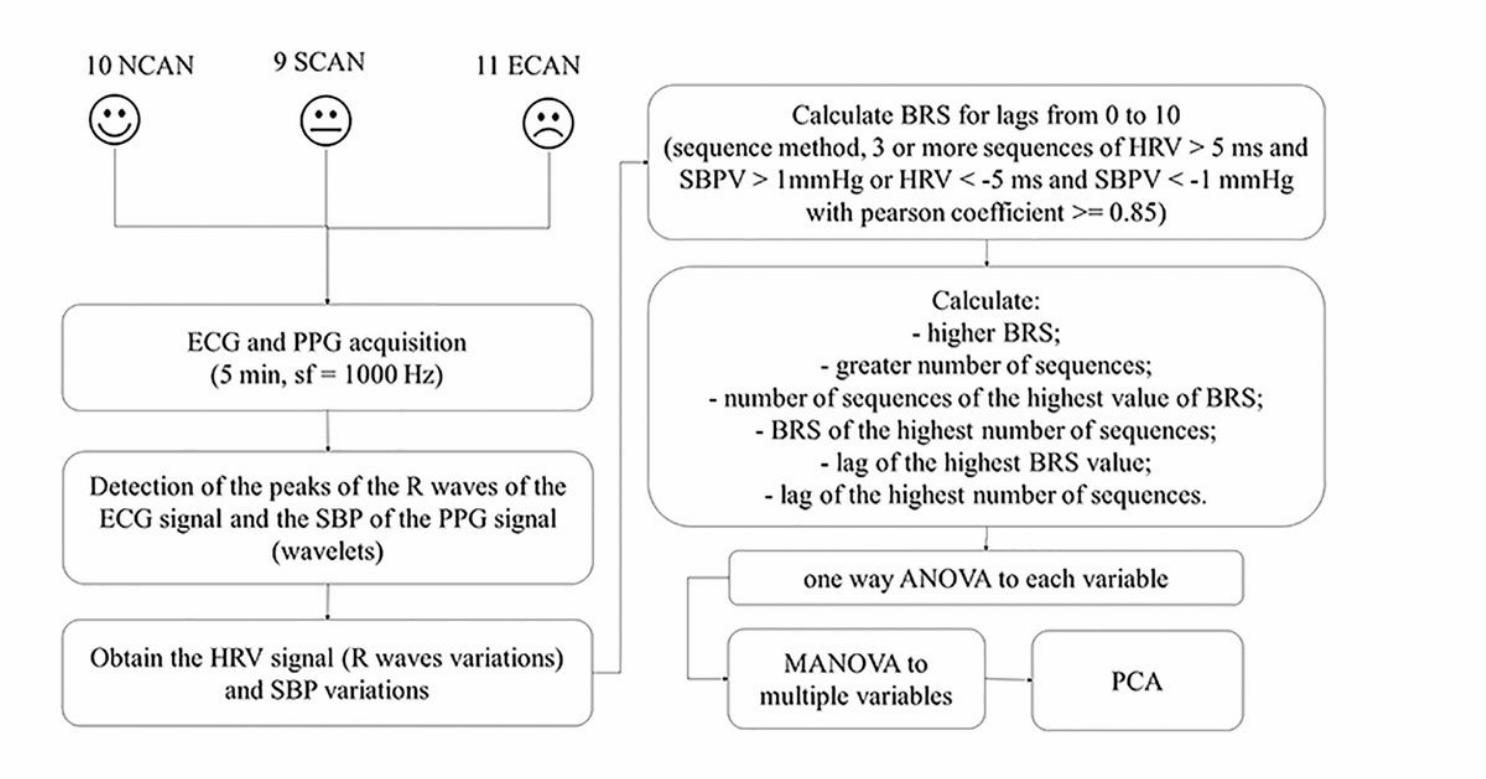

Figure 6

Methodological flowchart of the study. 30 diabetic patients (11 NCAN, 9 SCAN, and 10 ECAN), ECG and PPG signals were acquired over 5 minutes with a sampling frequency of 1000 Hz. Signal features, including R-waves and SBP values, were detected. RR and SBP series were generated, and indices were calculated using the sequence method to evaluate the BRS, considering 0 to 10 lags and HRV analysis. Statistical analysis was carried out using ANOVA, MANOVA, and PCA.