Cardiovascular Diabetology

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Heart rate variability tests for diagnosing cardiovascular autonomic neuropathy in patients with type 2 diabetes mellitus in advanced stages of kidney disease

João Soares Felício[1*], Maria Antônia Matos Araújo[1], Gabriela Nascimento de Lemos[1], Isabel Jacob Fernandes[1], Licia Oliveira Ruivo[1], Ester da Gama Chambouleyron[1], Lilian de Souza D'Albuquerque Silva[1], Caroline Filgueira Nunes[1], Gisely Mouta de Andrade Paes[1], Franciane Trindade Cunha de Melo[1], Pedro Paulo Freire Piani[1], Ana Regina Bastos Motta[1], Valéria Suênya Galvão Leal[1], Ana Carolina Contente Braga de Souza[1], Natercia Neves Marques de Queiroz[1], Márcia Costa dos Santos[1], Karem Mileo Felício[1] and Priscila Alcântara Barbosa de Figueiredo[1]

Abstract

Cardiovascular Autonomic Neuropathy (CAN) is one of the most devastating complications of Diabetes Mellitus (DM) and presents high morbidity and mortality. Its association with diabetic kidney disease (DKD) worsens the condition even further. CAN diagnosis remains a challenge and is being based on reflex tests which are laborious, risky and difficult to perform. Heart Rate Variability (HRV) tests has been suggested as having high utility in diagnosing CAN, but this issue remains controversial. The aim is to evaluate the sensitivity and specificity of HRV tests to diagnose CAN in patients with type 2 diabetes mellitus (T2DM) and DKD with severely increased albuminuria. This is a cross-sectional study in patients with T2DM and DKD with severely increased albuminuria. A total of 48 subjects were recruited and underwent laboratory and neuropathy assessment. The diagnosis of CAN was first confirmed in 75% (36/48) of patients based on cardiovascular autonomic reflex tests (CARTs). HRV tests (VLF, LF, TP and SDNN) differed between groups with and without CAN (212 vs. 522 ms^2, $p=0.024$; 57 vs. 332 ms^2, $p=0.025$; 359.5 vs. 2733 ms^2, $p=0.007$; 20 vs. 48 ms, $p=0.012$), respectively. The best cut-off points based on ROC curve were $<1{,}117$ ms^2, <152.5 ms^2, $<1{,}891$ ms^2 and <46.5 ms, respectively. VLF and TP reached highest sensitivity values (97% and 92%) and F1 Score of 90%, while LF had best specificity (75%) and TP had best accuracy (85%). Our best model of serial algorithm using VLF as first screening test and TP in sequency obtained a sensitivity of 97% and accuracy of 90%, reducing in 90% the need to perform CARTs. Our findings suggest that it is possible to achieve high sensitivity and accuracy using an algorithm with VLF and TP parameters analyzed in series. It could enable a simpler and early diagnosis, avoiding CARTs complications.

Keywords Type 2 diabetes mellitus, Diabetic kidney disease, Cardiovascular autonomic neuropathy, Diagnostic tests

*Correspondence:
João Soares Felício
felicio.bel@terra.com.br

[1]University Hospital João de Barros Barreto, Endocrinology Division, Federal University of Pará, Mundurucus Street, Guamá, Belém 4487, 66073-000, Pará, Brazil

Background

Cardiovascular autonomic neuropathy (CAN) is one of diabetes neuropathies, which represents the most prevalent, underdiagnosed and undertreated diabetes complication [1]. CAN is a degenerative condition that affects 16.7-34.3% patients with diabetes mellitus (DM), with a multifactorial pathogenesis, related to diabetes duration, poor glycemic control and, more recently, with hypertension [2–4].

CAN early diagnosis is a key factor to manage this condition and slowing its progression is associated with better quality of life and prognosis [5]. The signs and symptoms of autonomic neuropathy should be carefully investigated, as they generally appear in late stages and are usually represented by a non-specific clinic, such as tachycardia, gastroparesis, constipation, diarrhea, erectile dysfunction [6].

The gold standard for diagnosis is cardiovascular autonomic reflex tests (CARTs), which assess autonomic function through provocative maneuvers (lying to standing, deep breathing and Valsalva maneuver) that analyze heart rate and blood pressure [7]. CARTs may expose patients to risks related to increasing of intrathoracic, intraocular, and intracranial pressure and may theoretically be associated with a small risk of intraocular hemorrhage or lens dislocation [8]. These complications could have their frequency increased in more debilitated patients with the presence of other complications associated with diabetes, especially diabetic kidney disease (DKD).

DKD is a chronic microvascular complication of DM that affects up to 40% of patients with DM2, and is the leading cause of chronic kidney disease worldwide [9–12]. It has been suggested that DRD contributes significantly to the onset and progression of cardiovascular disease (CVD), through pathophysiological mechanisms such as hypertension, dyslipidemia and endothelial dysfunction, leading to outcomes such as myocardial hypertrophy, acute myocardial infarction and heart failure [13].

In summary, CAN is one of the most devastating complications of DM and presents high morbidity and mortality and could be a useful marker for progression of diabetes complications, especially DKD [7, 14–16]. Its association with DKD worsens the condition even further [2, 15–22]. CAN diagnosis remains a challenge and is being based on reflex tests which are laborious and difficult to perform. Recently, time and frequency domain tests has been suggested as having high utility in diagnosing CAN, but this issue is still controversial [23, 24]. In this context, this study aims to evaluate the sensitivity and specificity of time and frequency domain diagnostic tests, to enable a simpler and early diagnosis, avoiding CARTs complications, which could lead to swifter appropriate therapy and avoid poor outcomes for these patients.

Materials and methods

Study design and pacients

This is a cross-sectional study to evaluate sensitivity and specificity of frequency and time domain tests for CAN diagnostic in patients with diabetes mellitus type 2 (T2DM) and DKD with severely increased albuminuria. A total of 51 subjects with T2DM and DKD were recruited from the Federal University of Pará - Brazil endocrinology department from 2023 to 2024. CAN diagnosis was established according to Toronto consensus (gold standard based on cardiovascular autonomic reflex tests) [7].

It was developed according to the Declaration of Helsinki and the Nuremberg Code and was approved by the University Hospital João de Barros Barreto ethics committee, reference number 88974918.6.0000.0017. Informed Consent Form (ICF) was obtained from all patients.

Inclusion criteria consisted in: (a) providing consent through the ICF obtained prior to any study procedure; (b) patients with type 2 DM aged over 30 years with regular follow-up with an endocrinologist; (c) treatment with oral hypoglycemic agents or insulin at a stable dose for at least three months; (d) patients with DKD and severely elevated albuminuria (> 300 mg/g); (e) patients taking any anti-hypertensive medication must be on a stable dose during 4 weeks prior to study; (f) ability and willingness to perform all study procedures; (g) eGFR > 25 and < 90mL/min/1.73 m^2. Exclusion criteria were (a) patient with type 1 diabetes; (b) hypothyroidism or decompensated hyperthyroidism; (c) pregnant women or women intending to become pregnant or breastfeeding; (d) patients with alcohol abuse or taking recreational drugs that, in the opinion of the researcher, would put the patient's safety and the study procedures at risk; (e) patients with clinically significant arrhythmias that could interfere in CARTs execution.

Data collection

After eligibility criteria evaluation, qualified individuals were started on the study procedures. All patients had a well-established clinical and laboratory diagnosis of T2DM [25]. CAN evaluation was always made in the morning, with fasting capillary glycemia levels between 70 and 250 mg/dl. Subjects were instructed not to use alcohol, caffeine beverages and tobacco for at least 8 h before the test, and not to perform vigorous physical exercises 24 h before examination.

Parameters used to diagnose CAN were deep breathing test, Valsalva coefficient, lying to standing test (30/15 coefficient and systolic blood pressure (SBP) reduction in

orthostasis). Subjects were considered not to have CAN when presenting up to 1 abnormal parameter. The presence of two abnormal parameters was defined as criteria to diagnose definitive CAN [7]. Severe CAN was reported when patients presented orthostatic hypotension.

Patients underwent blood and urine sample collection. HbA1c was analyzed using the HPLC method [26]. Creatinine was analyzed using the kinetic/automated method and the CKD-EPI 2021 equation was used to calculate GFR [27]. The patients underwent albuminuria measurement in an isolated sample by evaluating 3 urine samples isolated on different days, analyzed using the immunoturbidimetry method [24].

Can evaluation

The CARTs used were deep breathing, Valsalva and lying to standing (30:15 coefficient and orthostasis) [28, 29]. Diagnosis was established according to Toronto consensus [7] only based on CARTs results (gold standard). During CAN evaluation, heart rate variability (HRV) and time parameters were also measured with the patient resting in supine position.

The VNS-MICRO software (Neurosoft, Ivanovo, Russia) was used to analyze data [30, 31]. The test begins with patients in supine positions and an electrocardiographic record for 300 s was performed. R waves are highlighted by the software and each regular RR interval is analyzed by a math algorithm and then expressed through an amplitude diagram of heart rate oscillation (HR fluctuations per second) versus heart rate (HR) in hertz. Total amplitude of HRV spectrum is distributed in three bands: (1) Very Low Frequency (VLF) component (0.01 to 0.04 Hz), which is related to vasomotor tonus (sympathetic control); (2) Low Frequency (LF) component (0.4 to 0.15 Hz), associated with baroreceptor reflex; and (3) High Frequency (HF) component (0.15 to 0.5 Hz), related to parasympathetic control (vagus nerve). These represent frequency domain parameters, which also include Total Power (TP), a set of three combined spectral bands and LF/HF ratio, which reflects balance between sympathetic and parasympathetic systems.

Besides those items, software provided time domain parameters. RRmin (minimum RR interval), RRmax (maximum RR interval); RRNN (mean length of regular RR intervals); and SDNN (standard deviation of all NN intervals).

As no extended analysis of all HRV and time parameters in large normal populations has been performed, reference values described by Angelink et al. 2001[30] were based on studies involving few patients. Therefore, values should have been considered as approximate, and no definite clinical decisions should be based on them.

Statistical analysis

Data collected was organized and analyzed using SigmaStat 3.5® (Jandel Scientific Corporation, Chicago, Illinois) and Statistical Package for Social Sciences (SPSS 22®). The analysis was carried out according to the distribution values (considering normality by the Shapiro-Wilk test $p > 0.05$) and for the null hypothesis rejected a $p < 0.05$ in all tests. For variables with a normal distribution, the Student's t-test for two independent samples and ANOVA repeated measures were used. For variables with a non-normal distribution, the Mann-Whitney U test was used for independent samples and the Kruskal Wallis test for more than two samples. For categorical variables, the chi-square or Fisher test was used, with data reported as absolute values and frequency (%). For variables with a normal distribution, data are reported as mean ± standard deviation; for non-normal distribution, data are reported as median (quartile 25–75). For sensitivity, specificity, accuracy, prevalence, positive predictive value (PPV) and negative predictive value (NPV), the standard formulas were used, and cross-analysis of the paired tests was carried out to obtain the joint sensitivity and specificity. The best cut-off point was defined based on Youden Index (J) and, additionally, a ROC (Receiver Operating Characteristic) curve was constructed. The cut-off with maximum sensitivity and specificity in the ROC curve was defined as the minimum value in the equation $\sqrt{}$ [(1– sensitivity)2 + (1– specificity)2] and the accuracy was estimated based on the area under the ROC curve. Predictive values and likelihood ratios were also calculated from the values of sensitivity and specificity. Sensitivity, specificity and diagnostic accuracy were expressed as percentage.

Our sample size was calculated based on an expected power of 0.8 to detect the difference between the most used HRV parameter (VLF) in patients with and without CAN. The number of patients necessary was 45 cases, with an expected difference in median equals to 600 ms^2, an expected standard deviation of 1000 and an alpha of 0.05. Since this group was composed by 48 patients, the power of our study to identify difference between those variables was > 0.8 and considered satisfactory.

Results

A total of 51 patients with DM2 and DRD were recruited, but three patients were excluded because they were unable to undergo reflex tests. A total of 48 subjects underwent diagnostic tests for CAN, and 36 (75%) had a confirmed diagnosis of CAN. As expected, those patients presented long-term diabetes, associated to a higher frequency of complications (Table 1).

The comparison of autonomic neuropathy tests (CARTs, HRV and time domain parameters) in patients with and without CAN are shown in Table 2. The parameters (HRV and time domains) that showed significant

Table 1 Clinical and laboratorial parameters of patients with DM2 and DRD with severely increased albuminuria

Clinical features	*N* = 48
Age (Years)	66 ± 6.7
Sex (M/F)	27/21
BMI (kg/m^2)	29.5 ± 3.9
Time of T2DM diagnosis (years)	16.8 ± 8.4
Dyslipidemia (yes %)	47 (91.6)
Hypertension (yes %)	45 (93.7)
Systolic blood pressure (mmHg)	138.6 ± 17.2
Diastolic blood pressure (mmHg)	70.4 ± 8.3
Cardiovascular event (yes %)	5 (10.4)
Peripheral neuropathy (yes %)	37 (77.1)
Ankle brachial index	0.81 ± 0.08
Retinopathy (yes %)	8 (16.6)
Smoking (yes %)	16 (33.3)
Glycemia (mg/dl)	161 ± 61.1
Glycated hemoglobin (%)	8.3 ± 1.3
Creatinine (mg/dL)	1.44 ± 0.41
Clearance (CKD-EPI) (mL/min/1.73 m^2)	51.23 ± 13.7
Mean albuminuria (mg/g)	614.00 ± 584.6
Mean albuminuria (log_{10})	2.56 ± 0.5
Autonomic neuropathy (yes %)	36 (75)
Beta-blockers (yes %)	16 (33%)
ACEI/ARB (yes %)	47 (98%)

Source: Author data.

Table 2 Test parameters for patients with and without a diagnosis of CAN

Variables	Without CAN	With CAN	*p*
	N = 12	*n* = 36	
Frequency domain parameters			
TP (ms^2)	2733 (536-3178.5)	359.5 (111–1278)	0.007
VLF (ms^2)	522 (212–1925)	212 (75.5-499.5)	0.024
LF (ms^2)	332 (96-1091)	57 (22.5–182)	0.025
HF (ms^2)	415.5 (72-1298)	72(15-451.5)	0.086
LF/HF (ms^2)	1.12 (0.37–2.35)	0.63 (0.36–1.3)	0.617
Time domain parameters			
RRmin (ms)	685 ± 272	708 ± 185	0.909
RRmax (ms)	1125 (906–1224)	935 (758–1070)	0.287
RRNN (ms)	935 ± 226	832 ± 244	0.122
SDNN (ms)	48 (24–60)	20 (11–34)	0.012
Autonomic cardiac reactivity tests			
Deep breathing	1.13 (1.09–1.20)	1.06 (1.03–1.06)	0.027
Orthostatism test (30:15)	1.08 (1.05–1.75)	1.04 (1.02–1.06)	0.003
Valsalva Maneuver	1.43 (1.22–1.65)	1.18 (1.14–1.28)	0.006

CAN = Cardiovascular autonomic neuropathy. TP = Total power. VLF = Very low frequency. LF = Low frequency. HF = High frequency. RRmin = Shorter RR Interval. RRmax = Longest RR. Interval. RRNN = mean value of normal RR intervals. SDNN = Standard deviation of normal RR intervals.

Source: Author data.

differences between groups (TP, VLF, LF and SDNN) were subsequently evaluated as tests for the diagnosis of CAN.

Figure 1 shows data from ROC curves analysis with HRV and time domain parameters. Based on Youden Index (J), optimal cut-off points to diagnosing CAN for TP, LF, VLF and SDNN were < 1891 ms^2, < 152,5 ms^2, < 1117 ms^2 and < 46,5 ms, respectively

A summary of HRV and time parameters individual diagnostic performance is shown on Table 3. In addition, matching these tests in parallel has not improved the results.

In addition, we have tested a few models of serial and sequential algorithms using HRV and time domain parameter to diagnose CAN and trying to avoid performing CARTs. Our best model used VLF as first screening test and TP in sequence. This algorithm presented a sensitivity of 97%, specificity of 67%, accuracy of 90%, with positive and negative likelihood ratios of 2.93 and 0.04. We achieved these results using CARTs in only 10% of the sample, enabling a fast-screening procedure and avoiding CARTs adverse events in 90% of patients.

At the end of the model, 39/48 (81%) patients had abnormal tests results, 35 True Positives (33 identified by abnormal VLF + TP and 2 by CARTs) and 4 False Positives. Among 9/48 (19%) normal results, there was 1 False Negative and 8 healthy individuals, 5 of these identified at the screening by normal VLF and 3 by CARTs (Fig. 2).

Discussion

Our study was the first to evaluate the diagnostic performance of HRV and time parameters for CAN in patients with long-standing T2DM and advanced DKD, with severely increased albuminuria. Our findings suggest that it is possible to achieve high sensitivity and accuracy using an algorithm with the VLF and TP parameters analyzed in series. This algorithm achieved a sensitivity of 97%, specificity of 67%, accuracy of 90%, with positive and negative likelihood ratios of 2.93 and 0.04. In our serial model, the need to perform CARTs, initially used in all patients (*N* = 48), would be reduced to 10% of individuals. These procedures would enable a fast-screening process and avoid CARTs adverse events in 90% of patients. This would be an important advance, considering a fragile population with several comorbidities and risks associated to its realization. Additionally, when evaluated individually the HRV and time parameters (VLF, LF, TP and SDNN), showed also to be useful to diagnose CAN, presenting sensitivity of 97%, 75%, 92% and 89%, and specificity of 42%, 75%, 67% and 67% respectively, in this population.

Ewing's cardiovascular autonomic reflex testing (CART), the actual gold standard for diagnosing CAN [7], is a fully established tool and a powerful marker of

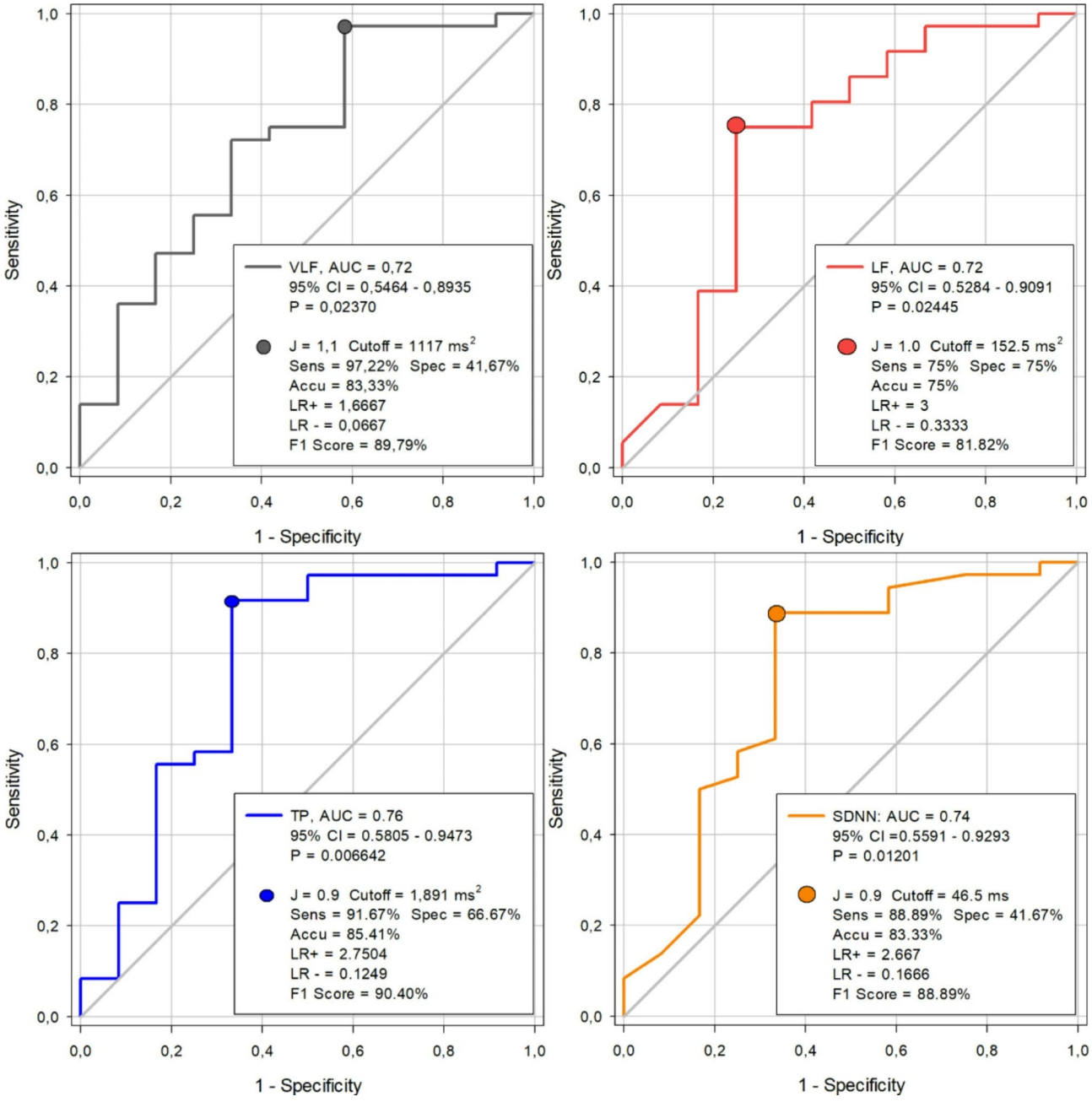

Fig. 1 Cut-off points for HRV parameters on the diagnosis of can based on ROC curve. AUC: Area under the ROC curve; 95% IC: confidence interval; J: Youden Index; Sens: Sensitivity; Spec: Specificity; Accu: Accuracy; LR+: Positive Likelihood Ratio; LR -: Negative likelihood ratio; TP = Total power. VLF = Very low frequency; LF = Low frequency; SDNN = Standard deviation from normal RR intervals.

Table 3 Sensitivity, specificity, accuracy, and positive and negative predictive values for cut-offs of CAN parameters

Test (Cutoff)	Sens	Spec	Accu	PPV	NPV	F1 score
VLF (1,117 ms^2)	97%	42%	83%	83%	83%	90%
LF (152.5 ms^2)	75%	75%	75%	90%	50%	82%
TP (1,891 ms^2)	92%	67%	85%	89%	73%	90%
SDNN (46.5 ms)	89%	67%	83%	89%	67%	89%

Note: Sens: Sensitivity; Spec: Specificity; Accu: Accuracy; PPV: Positive Predictive Value; NPV: Negative Predictive Value; VLF = Very low Frequency. LF = Low Frequency. TP = Total Power. SDNN = Standard deviation from normal RR intervals

Source: Author data

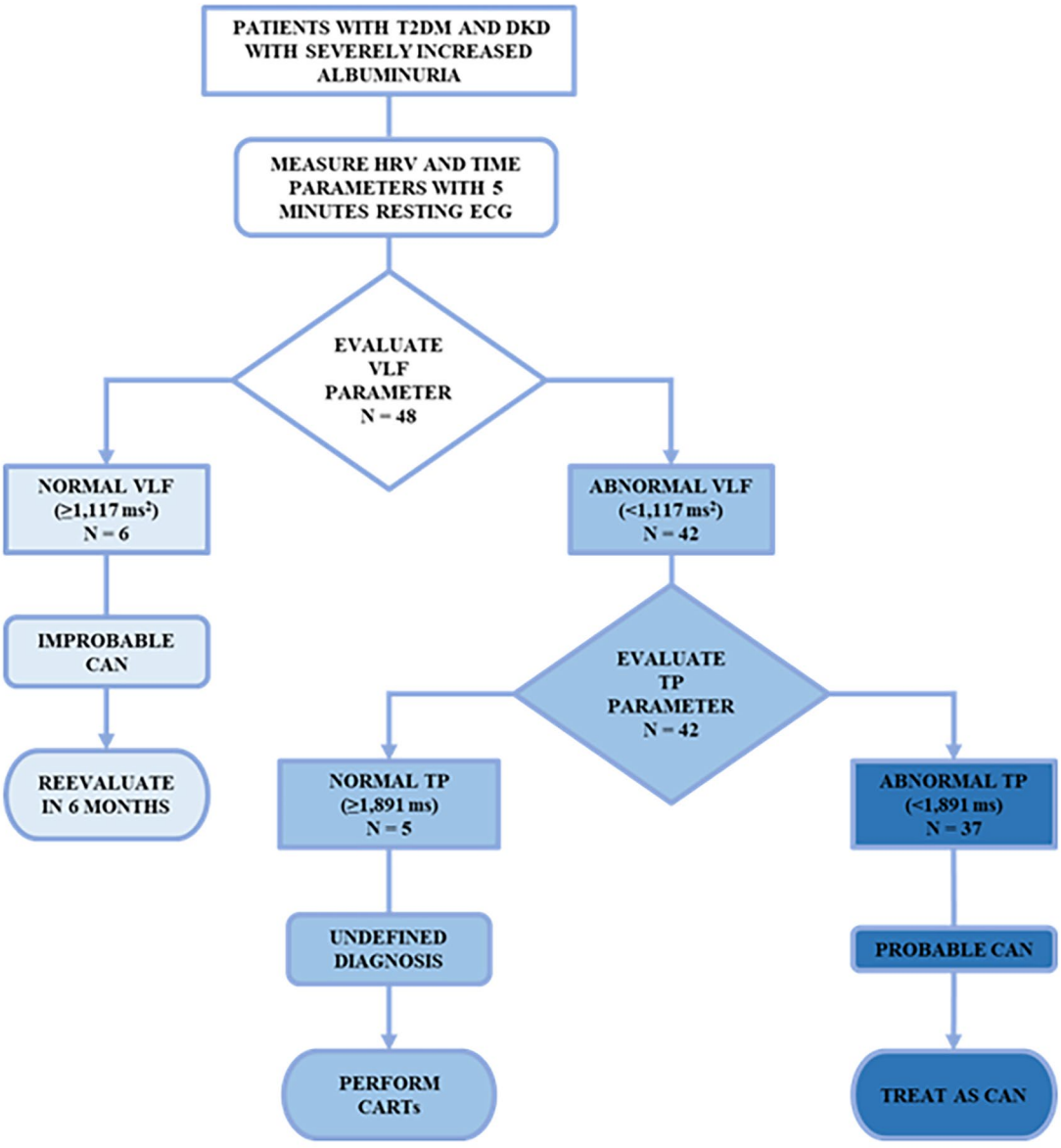

Fig. 2 Can diagnostic algorithm in patients with T2DM and advanced stages DKD. T2DM: Type 2 diabetes mellitus; DKD: Diabetic kidney disease; EKG: electrocardiogram; VLF = Very low Frequency; TP = Total Power; CAN: Cardiovascular autonomic neuropathy.

cardiovascular prognosis, with good sensitivity (93% to deep-breathing and lying-to-standing, and 98% to Valsalva), specificity (100% if three abnormal tests and 98% if two abnormal tests) [32] and reproducibility (coefficients of variation: 9.2, 12.6, and 6.4% for the Valsalva, deep-breathing, and lying-to-standing tests, respectively) [7, 33–35]. However it is widely used, these tests are difficult to perform, due to its laborious procedures, and have some limitations in clinical practice, especially in fragile populations. For example, mobility challenges or the presence of Charcot osteoarthropathy are cited as difficulties to perform lying-to-standing test [36]; evaluating postural hypotension is not reliable for patients with fluid retention, something to be considered in a DKD population [37], and severe arrythmias figure as a contraindication [2]. As consequence, they are not routinely realized, leading to CAN underdiagnosis. The Valsalva Maneuver increases intrathoracic, intraocular and intracranial pressures and may be associated with an increased risk of retinal hemorrhages, which is why it is recommended to avoid it in patients with proliferative retinopathy [7]. In addition, in individuals with T2DM and CAN, exercise tolerance is significantly reduced because of poor physical conditions that compromise their aerobic capacity

[38], and when associated with DKD, this condition is worsened [39]. As a result, it is important to find alternatives to simplify screening process and avoid clinical complications of CARTs, reducing exposure to tests that patients may not be able to perform.

HRV and time parameters analysis, based on electrocardiographic short-term measurements (5, 10–20 min) [40, 41], is an easy-to-use, non-invasive technique with good reproducibility [42, 43], as well as efficiently assessing sympathetic and parasympathetic activity [42–44]. As no comprehensive investigations of all HRV indices in large normal populations have yet been performed, normalized values were obtained from studies involving small number of subjects [45]. The values should therefore have been considered as approximate, and no definite clinical conclusions should be based on them. Therefore, studies in many subgroups of patients need to be performed to establish reliable thresholds [45].

Several studies have demonstrated the applicability of HRV and time parameters [46–51]. Tang et al. used Bayesian models to determine normality values for time and frequency parameters, which showed sensitivity and specificity of over 80%, and the results were comparable to Ewing's diagnostic battery [23]. A Spanish study that compared CARTs and the heart rate variability power spectrum (HRV-PS) found that the LF parameter was the best for diagnosing NAC [52]. In our study, TP was the well-balanced parameter to be used alone to diagnose CAN in this population. The Atherosclerosis Risk in Communities (ARIC) Study, which followed more than 13,000 patients over 16 years, concluded that low heart rate variability was associated with the development of severe renal outcomes, such as hospitalizations for renal failure, dialysis and death from acute renal failure in patients with previous chronic disease [53]. Recently, the study by Min et al. tried to identify the behavior of HRV in patients with end stage renal disease, a population in which CAN is quite prevalent, and all the time and frequency parameters were altered, in the groups with or without DM [54]. Both articles show that the association of CAN and ESRD (end-stage renal disease) leads to poor outcomes, and that the assessment of time and frequency domain parameters is useful to screening CAN earlier and mitigate complications. In addition, some studies have demonstrated that patients with Non Albuminuric DKD also have increased cardiovascular risk. Therefore, albuminuria should always be evaluated in parallel with e-GFR in those patients [15]. Finally, HRV parameters have been linked to a higher cardiovascular risk, especially with lipid and glucose markers [55] and it has been associated to several cardiovascular biomarkers, such as genetic, inflammatory and cardiac performance [56]. Nevertheless, in this study, our main objective was to identify a screening method that could avoid or even replace cardiovascular autonomic neuropathy reflex tests to diagnose CAN. Our serial model could diagnose CAN and avoid performing CARTs in 90% of this population. It could be meaningful for managing this relevant diabetes complications which remain underdiagnosed. We did not evaluate the correlation between HRV parameters and other cardiovascular risk biomarkers.

There are many current softwares for HRV analysis, each with its strengths and limitations, and choosing one of these tools may depend mostly on specific research needs and the user's familiarity with the software environment. Specially for diagnosing CAN, most studies rely on diagnostic accuracy of HRV parameters using softwares with linear algorithms [57, 58], such as time (SDNN, SDANN, pNN50, ecc) and frequency domains (VLF, LF, HF, HF/LF ratio). The first is based on statistical calculations of consecutive RR intervals and the last on indexes based on spectral analysis [59]. In our study, VNS Micro by Neurosoft was used for HRV data acquisition, an easy to carry and read tool, offering rhythmograms, spectrograms, measurement tables, and automatic interpretation according to the International standard [23]. Kubios HRV is known for its comprehensive analysis capabilities relying not only on linear analysis, providing also nonlinear HRV parameters, not affected by nonstationarity, as it happens for linear indexes [59] and shows the ability to correct artifacts and remove trends enhances the accuracy of HRV measurements [57, 58]. HRVanalysis provides advanced time-frequency analysis using wavelet transform, more nuanced understanding of HRV changes over short periods, which is essential for early detection of CAN [60]. Recently, The SCN4ALL Pulse Rate Variability Analysis Module has been validated against Kubios HRV, which provides validated PRV indices similar to HRV, figuring as an alternative for HRV analysis for remote monitoring of autonomic function, extremely useful in telemedicine scenario [61].

The main limitations of our study were a small sample and few patients without CAN (control group). We are studying individuals with long standing T2DM and severely increased albuminuria, which leads to a high CAN prevalence [62] and increased difficulties to recruit patients in a control group [63]. Our most sensitive parameter (VLF) also showed low specificity. But we compensated this issue proposing a serial algorithm model that reached higher values. Additionally, HRV and time parameters have some confounder factors, for example DKD patients are known to have increased sympathetic nerve activity [64, 65], therefore we cannot determinate whether HRV and time parameters alterations are due to renal impairment or to CAN itself.

As we are aware, this is the first study to propose a diagnosis algorithm in patients with T2DM and severely

increased albuminuria, reaching high sensitivity in a risky group for developing CAN.

Conclusion

This study proposes the possibility of using HRV parameters to diagnose CAN in patients with T2DM and severely increased albuminuria. A serial algorithm was created using VLF and TP parameters, obtaining high sensitivity and accuracy, and reducing 90% the need to perform CARTs. Further studies with a larger population are needed to confirm our findings.

Abbreviations

ACEI	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blocker
ARIC	Atherosclerosis risk in communities
CAN	Cardiovascular autonomic neuropathy
CARTS	Cardiovascular reflex tests
CKD-EPI	Chronic kidney disease epidemiology collaboration
CVD	Cardiovascular disease
DKD	Diabetes kidney disease
DM	Diabetes mellitus
eGFR	Glomerular filtration rate
ESRD	End Stage Renal Disease
HbA1c	Glycated hemoglobin
HF	High Frequency
HPLC	High-performance liquid chromatography
HR	Heart rate
HRV	Heart rate variability
HRV-PS	Heart rate variability power spectrum
ICF	Informed consent form
LF	Low frequency
NPV	Negative predictive value
PPV	Positive predictive value
ROC	Receiver operating characteristic
RRmax	Maximum RR interval
RRmin	Minimum RR interval
RRNN	Mean length of regular RR intervals
SBP	Systolic blood pressure
SDNN	standard deviation of all NN intervals
SPSS	Statistical Package for Social Sciences
T2DM	Type 2 diabetes mellitus
TP	Total power
VLF	Very low frequency

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Author contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. J.S.F, M.A.M.A., G.N.L., P.A.B.F., L.S.D.S., took part in conception and design of study. I.J.F., E.G.C., A.R.B.M., C.F.N., G.M.A.P. and V.S.G.L. were responsible for acquisition of data, while G.N.L., M.A.M.A., and L.O.R. have done the analysis and interpretation of data. N.N.M.Q., F.T.M., K.M.F., A.C.C.B.S., P.P.F.P., M.C.S., J.S.F. have drafted the manuscript together. All authors have revised the manuscript critically and approved the version to be published.

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Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by University Hospital João de Barros Barreto ethics committee, in accordance with the national legislation, resolution 466/12 (National Health Council). A written informed consent form to participate in this study was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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