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Diabetes & Metabolism 35 (2009) 305–311

Original article

Combination of cardiorespiratory reflex parameters and heart rate variability power spectrum analysis for early diagnosis of diabetic cardiac autonomic neuropathy

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Received 22 September 2008; received in revised form 6 January 2009; accepted 11 January 2009
Available online 30 June 2009

Abstract

Aim. – The study objective was to compare cardiorespiratory reflex (CR-R) parameters and heart rate variability power spectrum (HRV-PS) analysis in the diagnosis of cardiac autonomic neuropathy (CAN) in diabetic patients.

Methods. – Four CR-R tests (Valsalva manoeuvre, deep breathing, and two successive 5-minute periods with the subject supine and standing, respectively) were performed in 399 diabetic patients (58.6% male, median age: 51 years) and 105 healthy controls (40% male, median age: 34 years). Patients with two or more abnormal CR-R parameters were classified as CAN+, while those with only one abnormal CR-R parameter were considered CAN 'borderline'. HRV-PS was performed in all study participants.

Results. – The low-frequency (LF) area with the patient standing was reduced in CAN+ diabetics (median 35.6 normalized units [nu], $n = 31$), in CAN 'borderline' diabetics (median 64.3 nu, $n = 70$) and even in diabetics without CAN (median 89.4 nu, $n = 298$) versus control subjects (median 93.7 nu; $P < 0.001$, $P < 0.001$ and $P < 0.05$, respectively). Adding the abnormal (< 2.5 nu) LF area to the diagnostic criteria in CAN 'borderline' patients caused 11 (15.7%) patients to be considered CAN+.

Conclusion. – Combining abnormal CR-R parameters (I – E and I/E the most specific) with HRV-PS (particularly the LF area with the subject standing) allowed diagnosis of diabetic CAN at an earlier stage.

Keywords: Cardiac autonomic neuropathy; Heart rate variability

Résumé

Combinaison des paramètres des réflexes cardiorespiratoires et de l'analyse spectrale de la variabilité de la fréquence cardiaque pour le diagnostic de la neuropathie autonome cardiaque à un stade plus précoce.

Rationnel. – L'objectif de ce travail était de comparer la valeur des paramètres des réflexes cardiorespiratoires (R-CR) et celle de l'analyse spectrale de la variabilité de la fréquence cardiaque (PS-VFC) pour le diagnostic de la neuropathie autonome cardiaque (NAC) chez des patients diabétiques.

Patients et méthodes. – Quatre tests R-CR (manœuvre de Valsalva, épreuve de respiration profonde et effet de deux périodes successives de cinq minutes en décubitus et en orthostatisme) ont été réalisés chez 399 diabétiques (58,6 % hommes, âge médian : 51 ans) et 105 témoins non diabétiques (40 % hommes, âge médian : 34 ans). Les patients avec deux épreuves anormales ou plus ont été définis comme atteints de neuropathie autonome cardiaque (NAC+) et ceux qui n'avaient qu'une seule épreuve anormale comme NAC « limite ». L'analyse spectrale des variations de la fréquence cardiaque a été effectuée chez tous les participants.

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doi:10.1016/j.diabet.2009.01.006

Résultats. – En orthostatisme, l'aire de basse fréquence (aire LF) était réduite chez les diabétiques NAC+ (moyenne 35,6 nu, $n = 31$), chez les diabétiques NAC « limite » (moyenne 64,3 nu, $n = 70$), ainsi que chez les diabétiques indemnes de NAC (moyenne 89,4 nu, $n = 298$) par rapport à celle des témoins (moyenne 93,7 nu) ($P < 0{,}001$, $P < 0{,}001$ et $P < 0{,}05$, respectivement). Si l'on considérait une aire LF anormale (< 2,5 nu) comme critère diagnostic additionnel chez les patients qui présentaient une seule épreuve anormale, 11 patients avec NAC « limite » étaient reclassés comme NAC+.

Conclusion. – La combinaison d'un paramètre des réflexes cardiorespiratoires anormal (principalement I-E et I/E) et de l'analyse spectrale des variations de la fréquence cardiaque (particulièrement l'aire LF chez le sujet en orthostatisme) pourrait permettre de diagnostiquer une NAC diabétique à un stade plus précoce.

Mots clés : Neuropathie autonome cardiaque ; Variabilité de la fréquence cardiaque ; Diabète sucré

1. Introduction

Among chronic diabetic complications, cardiac autonomic neuropathy (CAN) occupies a singular position due to its high prevalence and the extent of its clinical manifestations [1–4]. In the absence of specific and effective treatment, all efforts in the past few decades have been directed at defining CAN with higher accuracy, and at developing and standardizing diagnostic methods with greater reliability to obtain earlier and tighter control of hyperglycaemia [5,6], hypertension, dyslipidaemia and other factors [7,8] to prevent their progression. This is important as there is now general consensus that CAN is a risk factor for cardiovascular events and mortality [9,10].

Measurement of cardiorespiratory reflexes (CR-R) is the closest method there is to a gold standard in the diagnosis of CAN [1–4], despite the lack of agreement on the cutoff values for age- and gender-related abnormalities, and on the number of abnormal values required for a positive diagnosis [11–14]. CR-R measurements are time-consuming as three steps are necessary—instructing the patients, practicing and performing—and even then, there is a high level of failure when performing the heart rate (HR) deep-breathing (6DB) test, the Valsalva manoeuvre (VM) and the standing test. Thus, it is of paramount importance to find a sensitive, inexpensive diagnostic method that is easy to perform in an outpatients setting and, if possible, does not require the patient's active cooperation. Such a method might be HR variability power spectrum (HRV-PS) analysis to estimate the frequency of the component values, given that several studies involving relatively small numbers of diabetic patients [15–19] have shown good sensitivity and accuracy as well as an additional advantage: the values were independent of the intrinsic HR [19], and of the functional and structural state of the heart [20], thus offering greater specificity.

In the present study, both methods were used to evaluate autonomic cardiac dysfunction in a large sample of diabetic patients to test the hypothesis that combining both types of parameters can result in diagnosis of diabetic CAN at an earlier stage.

2. Study subjects

Our study's target population was from the autonomous communities of Madrid, Aragón, País Vasco and Galicia. The diabetic patients were outpatients at the University Hospital San Carlos, University Hospital Lozano Blesa, University Hospital Cruces and University Hospital of Santiago, respectively. The public healthcare systems in the geographical areas covered by these four university hospitals are representative of the Spanish Caucasian population. The study's main centre was the University Hospital of Santiago (Service of Endocrinology and Nutrition).

Altogether, 522 diabetic patients, including both types (451 type 2) and genders, were recruited consecutively in a systematic way: every day, one out of every two diabetics attending outpatients was recruited. Excluded were patients who: had hypertension (defined as BP ≥ 140/90 mmHg), as some CR-R parameters are abnormal in non-treated, non-obese, non-diabetic, complication-free hypertensives [21]; had any type of arrhythmia or heart block; cardiac, pulmonary or more-than-moderate renal insufficiency (creatinine > 124 μmol/L); had a body mass index (BMI) > 35 (obesity) to facilitate performance of the CR-R tests; consumed more or equal to 20 units per week of alcohol (in Spain, 10 g of ethanol per unit); smoked more or equal to 15 cigarettes per day; or had any other known cause of cardiovascular autonomic dysfunction. In addition, 115 non-diabetic control subjects (who also did not meet any other exclusion criteria) were recruited from among hospital colleagues, nurses and employees, and a small number of relatives of diabetic patients who had a normal glucose tolerance test.

2.1. Material and methods

A combination of CR-R and HRV-PS tests was carried out in a single session on each study participant between 0930 and 1330 h in a quiet room with a temperature maintained at 22 to 24° C. A VM test (standardized to 40 mmHg over 15 seconds, using the mercury column of a modified sphygmomanometer) generated two parameters: Valsalva ratio (the highest HR during VM divided by the lowest HR post-VM); and VR-10, (the HR at 10 seconds of the VM divided by the HR at 10 seconds post-VM). Also, a 6DB test (standardized to six deep breaths over 1 minute, guided by a breathing cue device [WR Medical Electronics Co., Stillwater, MN, USA]), generated another two parameters: I – E (mean inspiration HR minus mean expiration HR; and I/E, mean inspiration HR divided by mean expiration HR). These tests were performed twice, and the higher value used in the analyses. In the standing test, the highest HR after standing was divided by the lowest HR. An additional parameter was the root mean square of successive differences (RMSS)—the mean of both supine and standing HR).

Other individual factors were controlled for as well. Subjects undergoing testing had either fasted or taken breakfast at least 3 hours before, with no coffee or tea; they had emptied their bladder, and had had a 15-minute relaxation period prior to recording the RR intervals, with a 3-minute period of relaxation between each test. Subjects were also advised to abstain from alcohol and tobacco during the previous 24 hours.

The patient's diagnosis of peripheral polyneuropathy was taken from the last annual screening, according to the hospital's criteria and based on the neuropathy impairment score (NIS) at the big toe (light touch pressure, pinprick, vibration, joint position and ankle reflex), and the attributes of neural conduction (peroneal, tibial and sural). Retinopathy was assessed (by the hospital ophthalmology department) using bilateral biomicroscopy and indirect ophthalmoscopy with the eyes in mydriasis.

A package including the RR-interval recording along with a database of the anthropometrics, blood pressure, smoking and drinking status, and analytical data was sent to the reference centre in Santiago.

The RR intervals were obtained directly from a computerized measurement system comprising a PSION-II lZ64 pocket organizer equipped with an RS-232 data-acquisition card, using a 200-Hz sampling acquisition frequency (Medimatica, Martinsicuro, Italy). The card is connected to three electrodes through a potentiometer for adjusting signal volume [22]. The system features a specially designed potential-difference meter that measures electrical heart activity. The RR intervals are measured through a high- and low-wave filtering system that identifies QRS complexes. The margin of error in identifying these complexes is greatly reduced by the simultaneous operation of a frequency control, which identifies as abnormal all QRS complexes that appear after time intervals 40% longer or shorter than the previous one. The RR intervals were obtained using software developed ad hoc for the present study. After data transfer to a personal computer, any 'artifacts' in the RR time series were detected visually and corrected after repeat sampling at 4 Hz using cubic splines. Tachograms with a high percentage of artifacts (greater than 10%) were rejected.

For the HRV-PS analysis, autoregressive modelling was used, as it is statistically more robust in comparison to fast Fourier transform estimates [23]. This was applied to a recording of 257–512 RR intervals obtained over 5 minutes with the subject supine, after a 10-minute relaxation period prior to recording the RR intervals, followed by 5 minutes in the standing position. The analyses were performed using a spontaneous breathing rate, as comparisons of spontaneous and metronome-guided breathing in the assessment of vagal modulation by Bloomfield et al. [24], among others, could find no differences between the two types of breathing.

The frequency-band range—low frequency (LF), 0.04–0.15 Hz, and high frequency (HF), 0.15–0.4 Hz—was selected according to those proposed by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology [23]. The normalized PLF(%) and PHF(%) indices were estimated as PLF(%) = (PLF/[PLF + PHF]) × 100 and PHF(%) = (PHF/[PLF + PHF]) × 100. Power spectra of the resampled RR time series were estimated after linear detrending by fitting an autoregressive model from the Yule–Walker method using the IDENT toolbox of the MATLAB 6.5 software (MathWorks, Natick, MA, USA). The order of the model was adjusted for each case using Akaike's information criterion. The power of the LF and HF bands were computed by individual spectral components using the residuals theorem, and the LF/HF index was obtained as recommended [23]. Home-developed software was used for the analysis and quantification of the CR-R parameters [11]. A CR-R parameter was considered abnormal when it was lower than the 2.5th percentile in the controls (Table 1). CAN + was defined as two or more abnormal parameters out of six. When only one of these parameters was abnormal, the subject was considered CAN 'borderline'.

2.2. Statistical analysis

The data are presented as medians and interquartile (IQ) ranges (25th–75th percentiles) or as the absolute values and percentages for numerical and categorical variables, respectively. Mann–Whitney and Khi^2 tests (the latter using the Yates correction where appropriate) were used for comparisons of numerical and categorical variables, respectively. To assess statistical differences across groups, and tendencies towards increases in these differences according to the increase in independent variables, we used the Jonckheere–Terpstra and Khi^2 tests for linear tendencies of numerical and categorical variables, respectively. Two-tailed P values < 0.05 were considered statistically significant. Data on the reproducibility of the HRV-PS were not included in the design of this study, as previous data from the Hoorn Research Group [20] showed that both tests—CR-R and HRV-PS—have moderate reproducibility (v.g. CV 40% for I – E and LF power). Data were processed using Statistical Package for Social Sciences software (SPSS 13.0, Chicago, IL, USA).

2.3. Ethics

The present study was carried out according with the World Medical Association's fifth revision of the Declaration of Helsinki (1964) for research in humans, and was approved by the respective institutional ethics review boards. Informed consent was obtained from all patients and controls participating in the study.

3. Results

Of the original package sent to the reference centre in Santiago, 399 diabetic patients (58.6% male, median age: 51 years, IQ range: 35–62 years) and 105 controls (40% male, median age: 34 years, IQ range: 21–55 years) were evaluated. A large number of patients were not evaluated because of failure in the 6DB and standing tests and, in some cases, due to a tachogram showing a greater than 10% rate of 'artifacts'. As the median age of the evaluated control group did not exactly match that of the diabetic patients (21–65 years), and given that CR-R parameters

Table 1
Values of CR-R in diabetic patients and controls according to age-based grouping.

Parameters	Diabetic patients		Controls	
CR-R test	Age < 55 years ($n = 227$)	Age ≥ 55 years ($n = 172$)	Age < 55 years ($n = 79$)	Age ≥ 55 years ($n = 26$)
VR	1.83 (1.56–2.10)	1.45 (1.32–1.67)	1.95 (1.66–2.23) 2.5th, 1.39	1.55 (1.29–1.73) 2.5th, 1.08
VR-10	1 (0.93–1.07)	1 (0.95–1.05)	1.02 (0.95–1.09) 2.5th, 0.73	1.02 (0.98–1.08) 2.5th, 0.9
I – E	22.1 (15.1–29.3)	10.7 (6.3–17.2)	27.7 (21.8–34.9) 2.5th, 6.91	13.2 (8.8–19.9) 2.5th, 3.04
I/E	1.33 (1.22–1.47)	1.15 (1.09–1.24)	1.41 (1.31–1.53) 2.5th, 1.10	1.20 (1.12–1.30) 2.5th, 1.03
Max/min	0.90 (0.85–1.04)	0.79 (0.95–1.05)	1.10 (0.96–1.15) 2.5th, 1.06	1.05 (0.98–1.10) 2.5th, 0.86
RMSSD	2.17 (1.52–3.20)	1.27 (0.93–1.80)	2.86 (2.07–3.73) 2.5th, 1.40	1.51 (1.10–1.87) 2.5th, 0.69

Data are expressed as medians and interquartile ranges (25th–75th percentiles); the 2.5th percentile is the cutoff point of abnormality of CR-R parameters.
nu: normalized units; VR: Valsalva ratio; VR-10: Valsalva ratio at 10 seconds; I – E, I/E: inspiration minus expiration, inspiration divided by expiration; Max/min (standing): highest heart rate/lowest heart rate; RMSSD: root mean square of successive differences (mean of supine and standing positions).

are age-dependent [11], we analyzed the histogram of values for CR-R parameters and noted that differences were particularly evident from age 55 onwards; for this reason, this was established as the dividing age. CR-R values from the controls and diabetic patients according to whether they were less than or more or equal to 55 years are shown in Table 1. According to the criteria used (≥ two abnormal CR-R parameters), 31 out of 399 diabetic patients (7.7%) were classified as CAN+, while the proportion of diabetics with one abnormal parameter ('borderline') was 70 out of 399 (17.5%).

Values of HRV-PS parameters of diabetes patients (according to CAN−, CAN 'borderline' or CAN+) and controls, with all subjects in supine and standing positions, are shown in Table 2. Alterations in the HRV-PS parameters correlated with the stage of diabetic CAN, as defined by CR-R criteria (Table 2). In absolute units, both HF and LF areas tended to decrease in healthy

Table 2
Spectral component values (normalized units [nu]) with study participants in supine and standing positions.

Power spectral components (nu)	Controls ($n = 105$)	Diabetic patients ($n = 399$)		
		CAN− ($n = 298$)	CAN 'borderline'[a] ($n = 70$)	CAN+[b] ($n = 31$)
Supine				
LF (nu)	74.2 (53.7–89.7)	75.8 (48.1–75.8)	73.0 (20.2–85.8)	33.3 (2.1–69.3)***
LF (area)	271.2 (99.2–745.7)	156.9 (43.1–426.0)**	55.5 (7.7–159.9)***	7.3 (0.0–20.1)***
HF (nu)	25.7 (10.3–46.2)	24.1 (9.8–51.8)	26.9 (14.1–79.7)	66.7 (29.5–100)***
HF (area)	100.3 (27.1–266.6)	35.2 (11.7–151.1)	16.4 (6.6–60.5)***	8.3 (3.2–20.3)***
LF/HF	2.8 (1.1–8.7)	3.1 (0.9–9.1)	2.7 (0.2–6.1)	0.5(0.02–2.2)***
Standing				
LF (nu)	93.7 (74.7–99.7)	89.4 (58.2–98.9)*	64.3 (25.6–95.5)***	35.6 (13.8–78.0)***
LF (area)	281.9 (101.6–661.1)	131.9 (34.4–389.0)***	82.4 (16.2–219.9)***	7.4 (0.5–19.6)***
HF (nu)	6.2 (0.2–25.2)	10.5 (1.0–41.7)	64.4 (4.4–74.3)***	64.3 (21.9–86.9)***
HF (area)	20.1 (0.69–52.9)	10.3 (1.4–40.7)	3.5 (8.8–37.1)	5.3 (3.6–10.6)
LF/HF	15.0 (2.9–421.1)	8.4 (1.3–99.4)*	3.8 (0.3–21.3)***	0.5 (0.1–3.5)***

Data are expressed as medians and interquartile ranges (25th–75th percentiles).
$P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ for differences between each subgroup of diabetic patients and controls.
LF: low frequency; HF: high frequency.
[a] CAN 'borderline': one abnormal CR-R parameter.
[b] CAN+: two or more abnormal CR-R parameters.

Table 3
Demographic and clinical characteristics of study controls and diabetics, and risk factors previously related to diabetic CAN.

	Controls ($n = 105$)	Diabetic patients ($n = 399$)			
		CAN– ($n = 298$)	CAN 'borderline'[a] ($n = 70$)	CAN+[b] ($n = 31$)	P*
Age (years)	34 [32–67]	51 [32–62]	52 [43–62]	50 [40–54]	0.01
Men (n)	42 (40%)	176 (59.1%)	40 (57.1%)	18 (54.5%)	NS
Weight (kg)	66 [58–77]	73 [64–82]	74 [67–83]	76 [70–82]	NS
Height (m)	1.65 [1.59–1.72]	1.65 [1.58–1.72]	1.63 [1.58–1.70]	1.64 [1.56–1.75]	NS
Body mass index (kg/m^2)	23.7 [20.9–27.1]	26.6 [23.5–29.7]	27.1 [24.1–31.0]	26.7 [24.9–32.4]	NS
HbA_{1C} (%)	–	7.6 [6.6–8.5]	7.5 [6.5–8.3]	7.9 [7.0–9.3]	NS
Diabetes duration (years)	–	11 [6–18]	14 [7–22]	14 [9–23]	0.01
Serum creatinine (> 124 μmol/L)	–	7 (2.3%)	1 (1.4%)	1 (3.2%)	NS
Microalbuminuria (30–299 mg/24 h)	< 30 mg/24 h	41 (13.8%)	12 (17.1%)	10 (32.3%)	0.01
Diabetes duration (years)	–	11 [6–18]	14 [7–22]	14 [9–23]	0.01
Retinopathy	–	66 (22.1%)	17 (24.3%)	13 (41.9%)	0.03
Polyneuropathy	–	52 (17.4%)	17 (24.3%)	13 (41.9%)	0.001

Data are expressed as medians and interquartile ranges [25th–75th percentiles] or as the absolute number of diabetic patients and respective percentage.
NS: not significant.
Trend test in diabetic patients.
[a] CAN 'borderline': one abnormal CR-R parameter.
[b] CAN+: two or more abnormal CR-R parameters.

controls more than in CAN– diabetics, CAN 'borderline' diabetics and CAN+ diabetics.

However, in normalized units (nu) as recommended by the Task Force [23], the HF area tended to increase in parallel with the stage of CAN (Table 2). The most prominent and consistent alteration was found in the LF area, particularly when standing. The LF area (nu) in the standing position was reduced in CAN+ diabetics, CAN 'borderline' diabetics and even in diabetics without CAN compared with controls (Table 2). By adding the LF area in the standing position (< 2.5 nu) to the diagnostic criteria for CAN, 11 out of 70 (15.7%) patients with 'borderline' CAN became CAN+.

The presence of CAN was associated with duration of diabetes, microalbuminuria, retinopathy and peripheral neuropathy (Table 3). CAN was not associated with age, gender, BMI, glycated haemoglobin or renal dysfunction, as estimated by serum creatinine.

4. Discussion

In addition to the reasons already outlined in this report, the early diagnosis of diabetic CAN is now more necessary than ever, as an autonomic cardiac dysfunction has recently been reported in a substantial number of individuals who are glucose-intolerant [25], who have impaired fasting glucose or who are the glucose-tolerant, but insulin-resistant, offspring of type 2 diabetic patients [26]. These emerging findings serve to highlight the connection between metabolic factors and diabetic neuropathy.

Previous studies [15–19] have had limitations such as a small population sample, poorly described inclusion and/or exclusion criteria, restriction of control subjects, inclusion of type 1 diabetic patients with known autonomic dysfunction, or data from CR-R and HRV-PS tests performed at different times. In the present study, all of these factors were taken into account by the study design and controlled for. In particular, we used a large population sample size, strict inclusion and exclusion criteria, and tight temporal and environmental conditions during the acquisition of the RR intervals.

The decision to use the less than 2.5th percentile as an abnormality threshold for parameters derived from the CR-R, and to consider having two or more of these parameters as the diagnostic criteria for CAN, had the consequence that none of the controls was ascribed to the CAN+ group. It is possible that, as an unwanted effect, the number of CAN+ diabetic patients was, therefore, underestimated in the present study compared with other studies [14]. This possibility is supported by the fact that we have detected alterations in HRV-PS frequency bands in

patients with only one CR-R abnormal parameter and who were, therefore, defined as CAN 'borderline', and even in diabetic patients diagnosed as CAN– by CR-R tests.

As the frequency ranking and autoregressive model were chosen according to the guidelines of the Task Force [23], which are different from those used in other cited studies, any comparison of results is limited—although all of the studies, including the present one, support the validity of HRV-PS analysis as a useful method for assessing autonomic cardiac dysfunction in diabetes.

The main findings of our study are the low LF band with the subject supine and, in particular, failure to increase the LF band on standing.

However, we acknowledge certain limitations of the present study, some of which are technical and some of which derive from its multicentre design. One technical limitation is that the interface we used had a sampling rate of 200 Hz, which is lower than the 250–500 Hz recommended by the Task Force [23], and may have created a 'jitter' in identification of the fiducial point, thus introducing an error in RR-interval measurements that could have altered the spectrum in some recordings. However, we are confident that, as the 'artifacts' on the RR time series were visually detected and corrected after resampling at 4 Hz using cubic splines, this possible error was most likely overcome.

The multicentre nature of the study has intrinsic disadvantages when comparing two or more diagnostic methods—specifically, the introduction of multiple test providers with the inevitable concomitant increase in operator-related data scatter. Nevertheless, we estimate that our centralized data processing and analyses had a buffering effect on all of our limitations, thus increasing the validity and allowing for generalization of the study results and conclusions.

As the ages of the controls did not precisely match those of the diabetic patients and because CR-R parameters are age-dependent [1–4,11,13], we decided to analyze the histogram of values for CR-R parameters, and found that differences were particularly noticeable from age 55 onwards, leading us to establish this as the dividing age.

However, the present study has a further limitation that it shares with every other study of diabetic autonomic cardiac dysfunction that attempts to evaluate a new diagnostic method: the lack of a specific 'gold-standard' method as a comparator. Another common limitation of this and other such studies is inherent to HRV spectral analysis and concerns the physiological role of the main spectral components. The LF band is considered by some to be a marker of sympathetic modulation (especially when expressed in normalized units); however, at present, the predominating opinion [23] is that it includes both sympathetic (mostly) and parasympathetic influences. In any case, LF and HF reflect neither the efferent sympathetic nor parasympathetic tone (the quantity of neural traffic to the heart), but only efferent sympathetic and parasympathetic HRV modulation [27].

In our opinion, once complete automation of the technique is achieved, analysis of the HRV in the frequency domain should be included as a diagnostic method for early-stage diabetic autonomic cardiovascular dysfunction. Indeed, using the largest sample of diabetic patients published so far, we have found that LF bands (nu) are already significantly reduced (in both supine and standing positions) in diabetic patients who have only one CR-R abnormal parameter—defined as CAN 'borderline'—and, on standing, in patients defined as CAN–.

In addition, on using the less than 2.5th percentile as the cutoff point for abnormality of CR-R parameters and adding the abnormal (< 2.5 nu) LF area in the standing position to the CAN diagnostic criteria of one abnormal CR-R parameter, 15.7% of diabetic patients considered CAN 'borderline' became CAN+.

So, although more prospective studies are required, the combination of one abnormal CR-R parameter (mainly I – E or I/E during the 6DB test and VR) and the LF area (nu) with the subject standing could allow diagnosis of diabetic CAN at an earlier stage. Furthermore, whether or not the dysfunction detected in our study by HRV-PS analysis reflects functional or structural neural alterations as well, and whether or not such alterations are reversible, are questions that remain to be answered by prospective studies.

Conflicts of interests

None.

Acknowledgements

The authors are grateful to Professor Jesús R. Requena (USC, Department of Medicine) for revision of the manuscript and to Professor Miguel A. García-Gonzalez (UPC, Centre of Research in Biomedical Engineering) for his many technical suggestions. This study was supported by the Spanish Diabetes Society (SDS) and by an educational grant from Novo Nordisk (Spain).

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