Feasibility of combining heart rate variability and electrochemical skin conductance as screening and severity evaluation of cardiovascular autonomic neuropathy in type 2 diabetes

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ABSTRACT

Aims/Introduction: Clinical studies show that either heart rate variability (HRV) or electrochemical skin conductance (ESC) alone can serve as a simple and objective method for screening cardiovascular autonomic neuropathy (CAN). We tested the hypothesis that combining these two quantitative approaches can not only reinforce accuracy in CAN screening but also provide a better estimate of CAN severity in patients with type 2 diabetes (T2DM) who had already had CAN in outpatient clinics.

Materials and Methods: Each patient received a complete battery of cardiovascular autonomic reflex tests (CARTs), with ESC measured by SUDOSCAN, time domain of HRV measured by standard deviation of all normal RR intervals (SDNN) and frequency domain of HRV (low frequency [LF], high frequency [HF], and LF/HF ratio), and peripheral blood studies for vascular risk factors. Severity of CAN was measured by CAN score.

Results: The 90 T2DM patients included 50 males and 40 females. Those with more severe CAN had lower values in feet ESC ($P = 0.023$) and SDNN ($P < 0.0001$). Multiple linear regression analysis also showed that feet ESC and SDNN value ($P = 0.003$ and $P < 0.0001$) were significantly associated with CAN score. Combining SDNN and feet ESC also can increase the diagnostic accuracy of CAN with respective to sensitivity and specificity by using receiver operating characteristic analysis.

Conclusions: Combining the results of SDNN and feet ESC can not only assess, but also quantitatively reflect the progress or improvement of autonomic nerve function (including sympathetic and parasympathetic activity) in patients with T2DM.

INTRODUCTION

Diabetic cardiovascular autonomic neuropathy (CAN), the impairment of the autonomic balance of the cardiovascular (CV) system in the setting of diabetes mellitus (DM), strongly influences various CV diseases, causes detrimental effects on the quality of life, and leads to severe morbidity and mortality in patients with type 1 and type 2 diabetes mellitus (T1DM and T2DM)[1,2]. The autonomic nervous system is one of the major homeostatic regulatory systems of the body. Sympathovagal balance failure with a sympathetic dominance is the main trigger of lethal arrhythmias and sudden death.

The prevalence of CAN is variable and depends on the definition and criteria used for diagnosis. A joint consensus statement by the American Diabetes Association (ADA) and

American Academy of Neurology (AAN) has recommended that a battery of cardiovascular autonomic reflex tests (CARTs) should be performed to assess CAN.

Reduced heart rate variability (HRV) is an independent adverse prognostic factor used to detect CAN. HRV represents one of the most promising markers. A joint European Society of Cardiology and North American Society of Pacing and Electrophysiology task force defined and established standards of measurement, physiological interpretation, and clinical use of HRV, and the most widely used methods can be grouped under the time domain and frequency domain methods[3]. Established clinical data based on numerous studies consider HRV have been proven useful in detecting CAN in diabetes patients[4,5]. Recently, portable devices that measure HRV provide cost-benefit along with the simplicity of the measurement and increased compliance for both research and clinical studies[6].

Sudomotor dysfunction, a length-dependent pattern of diseases in thin, non-myelinated sympathetic C-fibers, has been observed in both prediabetic and diabetic patients. The consensus statement of the ADA, AAN, and Latin American Diabetes Association includes assessing the sudomotor function's role in the early diagnosis of CAN in patients with diabetes[7,8]. Sudomotor function can be assessed using SUDOSCAN™ (Impeto Medical, Paris, France), which measures electrochemical skin conductance (ESC), which is a technological advance that calculates sweat function through the quantization of chronoamperometry measures of the hands and feet. It was recently developed to allow the measurement of diabetic small-fiber neuropathy and autonomic dysfunction[9].

Although HRV and ESC are validated and can serve as screening tools for CAN in patients with diabetes[3,10-14], there is paucity of information that focuses on its role in estimating CAN severity in patients with diabetes who was already diagnosed with CAN in outpatient clinics, owing to the possible benefits of exploring the role of the CV autonomic function on subsequent CV events and the consequent development of therapeutic strategies to reduce the prevalence of CV events.

In this study, we evaluated the feasibility of a time-effective CV autonomic screening service at the outpatient clinic, including the time and frequency domains of HRV as well as the ESC. First, we evaluated which parameters in both HRV and ESC can serve as diagnostic biomarkers in the presence of CAN. Second, we combined the biomarkers in a test battery for CAN severity screening. We tested the hypothesis that CAN score and electrophysiologic parameters (time and frequency domains of HRV and ESC) have a strong association. If our hypothesis is true, when combined as a test battery, these two quantitative approaches can provide a better estimate of CAN severity and improve examinations.

PATIENTS AND METHODS
Study population
A total of 90 patients (≥20 years old) with T2DM who visited the outpatient diabetic clinic at Kaohsiung Chang Gung Memorial Hospital in Taiwan were included. Exclusion criteria included those who (i) suffered from moderate-to-severe heart failure (New York Heart Association class III and IV) and (ii) had any type of arrhythmia that prevents the analysis of HRV, or pacemaker implantation due to any cause. This study was approved by the Ethics Committee of Chang Gung Memorial Hospital Institutional Review Board (201800388B0C501 and 201901363B0).

Baseline clinical and laboratory measurements
All patients underwent complete neurological and physical examinations upon enrollment and at their subsequent follow-ups at the outpatient clinic. A detailed medical history regarding prior use of medications was obtained from the patients and their families through standardized questions. Demographic data, including age, sex, duration of diabetes (years), body mass index (BMI), systolic and diastolic blood pressure (SBP and DSP), waist circumference (WC) during autonomic function testing, underlying disease (hypertension, coronary artery disease [CAD], ischemic stroke, and diabetic retinopathy [DR]), and laboratory parameters, were obtained at baseline.

Biochemical analysis
Blood samples were obtained by antecubital vein puncture in a fasting, nonsedative state between 09:00 and 10:00 AM in the control and study groups to exclude the possible influence of circadian variations. All blood samples were collected in Vacutainer SST tubes (BD, Franklin Lakes, NJ, USA) and centrifuged at 3,000 rpm for 10 min, and serum samples were collected and stored at − 80°C in multiple aliquots prior to biochemical measurement.

Serum levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood sugar, HBA1c, and high-sensitivity C-reactive protein (hs-CRP) were analyzed by the hospital's central laboratory. Estimated glomerular filtration rate (eGFR) in each patient was calculated using an equation for Chinese subjects, as previously described[15]. Urine albumin/creatinine ratio (UACR) is < 30 mg/day; therefore, UACR between 30 and 300 mg/day is called microalbuminuria, and UACR above 300 mg/day is considered macroalbuminuria[16].

Assessment of electrochemical skin conductance and SUDOSCAN risk scores
ESC were assessed with patients placing their hands and feet on electrode plates for 3 min[17]. Measurement was based on an electrochemical reaction between electrodes and chloride ions, after stimulation of small fibers innervating the sweat glands with a low-voltage current (<4 V). An ESC measurement for the hands and feet was generated from the derivative current associated with the applied voltage. Lower ESC values indicated dysfunction of the sweat glands.

SUDOSCAN risk scores were calculated automatically from ESC values, BMI, and age using an algorithm included in the

device software. Scores were presented as percentages. Higher SUDOSCAN risk scores have been related to increased risk of cardiac autonomic abnormalities. Both ESC values and SUDOSCAN risk scores were displayed numerically with graphs on the device monitor[13].

Assessment and scoring of cardiovascular autonomic functions

CARTs are considered as gold-standard measures of autonomic function in patients with diabetes[18]. Parameters that were computed using Ewing's methods, including heart rate responses to deep breathing (E:I ratio), standing (30:15 ratio), and Valsalva maneuver and blood pressure responses to standing[19], were often used by diabetologists. These autonomic parameters were also obtained, and CAN was defined as the presence of at least two abnormal test results[18]. In other words, the patient with CAN score ≥ 2 was defined as having CAN[18]. In this study, we further defined as no CAN, at risk without CAN, mild to moderate CAN, and severe CAN if no, one, two, and more than two abnormal autonomic function testings.

The severity of CAN was quantitated by summation of points obtained from each of the four tests, where each test was given a point of 0 or 1 if it yielded normal or abnormal values, respectively. We termed this severity score as CAN score. Therefore, CAN score provided a score from 0 to 4 points in this study.

Parameters of heart rate variability (HRV)

To avoid the influence of circadian change on HRV, the recording was done between 9 AM and noon for all patients. The patient was asked to go to the toilet before the test if he/she had desire to urination or defecation. Then 5 min of resting ECG recording was done with the patient lying in supine position with eyes closed. Using the 5-min resting electrocardiogram (ECG) recording, the standard deviation of all normal RR intervals (SDNN) was calculated as the time domain parameter of HRV. In addition, power spectral density analysis of HRV was also done to obtain the frequency domain parameters. Three main spectral components were distinguished in a spectrum calculated from the 5-min recording, i.e., high frequency (HF, 0.15–0.4 Hz), low frequency (LF, 0.04–0.15 Hz), and very low frequency (VLF, 0–0.04 Hz). The components of LF and HF were computed both in absolute values of power (ms^2) and in normalized unit (n.u.). The LF/HF ratio, regarded as an index of sympathovagal balance, was also calculated. The aforementioned computing process was done by Kubios HRV Standard version 3.2 (Kubios Oy, Finland).

Statistical analysis

Data are expressed as means ± standard deviations (SDs) or medians (interquartile ranges (IQR)). Categorical variables were compared using chi-square or Fisher's exact tests. Continuous variables that were not normally distributed by Kolmogorov–Smirnov test were logarithmically transformed to improve normality and compared. Five separate statistical analyses were performed. First, patients were stratified into four groups according to the severity of CAN. Second, Spearman non-parametric correlation analysis was used to evaluate the relationship between the CAN score and the variables that included age, diabetes duration, BMI, and parameters of ESC and time and frequency of HRV. Third, two stepwise models of multiple linear regression analysis were performed to evaluate the influence of independent variables on the mean CAN score (severity of CAN). Factors of HRV or ESC that were significantly correlated with the mean CAN score were assessed using the model 1 multiple linear regression analysis. Subsequently, results from model 1 were further analyzed using the model 2 multiple linear regression analysis. Fourth, receiver operating characteristic (ROC) curves were generated for those significant parameters in linear regression model as well as their combinations, in predicting the presence of CAN. The area under the ROC curve (AUC) for the presence of CAN in addition to the sensitivity, specificity, and Youden's index of each parameter was also calculated. The statistical analyses were conducted using the SAS software package, version 9.1 (2002, SAS Statistical Institute, Cary, NC, USA). All probabilities were two-tailed and $P < 0.05$ were regarded as significant.

RESULTS

General characteristics of patients with diabetes stratified by severity of cardiovascular autonomic neuropathy

The 90 patients with diabetes included 50 males and 40 females. Patient characteristics and baseline underlying diseases at assessment are presented in Table 1.

Laboratory and autonomic function testings of patients with type 2 diabetes stratified by the severity of cardiovascular autonomic neuropathy

Those with more severe CAN had higher UACR ($P = 0.012$). Those with more severe CAN had lower values in parasympathetic parameters, including the Valsalva ratio (VR), E:I ratio, and 30:15 ratio ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively), and lower values in feet ESC (µS) ($P = 0.023$), BP change related to standing ($P < 0.0001$), SDNN (<0.0001) and coefficient variation of R-R intervals (CVR-R) (%) (<0.0001). Those with more severe CAN had a higher prevalence of orthostatic hypotension ($P < 0.0001$) (Table 2).

Effect of autonomic function parameters on cardiovascular autonomic neuropathy scores

Correlation analysis parameters used to evaluate the effects of autonomic function parameters on CAN score are listed in Table 3. The significant statistical results (correlation coefficient, P-value) on CAN score were as follows: age (year) ($r = -0.332$, $P = 0.002$), diabetes duration (year) ($r = 0.240$, $P = 0.025$), SDNN (ms) ($r = -0.462$, $P < 0.0001$) (Figure 1a), LF (power

Table 1 | Baseline characteristics of patients with Type 2 diabetes

Characteristics	Patients (%) (n = 90)
Age (year)	67.5 ± 8.1
Sex (male/female)	50/40
Diabetes duration (year)	11.5 ± 8.6
Body mass index	27.1 ± 4.6
Waist circumference (cm)	90.6 ± 11.7
SBP (mmHg)	137.8 ± 17.9
DBP (mmHg)	76.5 ± 12.4
Baseline underlying disease	
Hypertension (%)	67 (74.4)
Coronary heart disease (%)	11 (12.2)
Cerebrovascular events (%)	32 (35.6)
Hyperlipidemia (%)	70 (77.8)
Chronic kidney diseases (%)	47 (52.2)
Retinopathy, n (%)	50 (55.6)
Proteinuria, n (%)	37 (41.1)
Other concomitant medications	
ACE inhibitor or ARB (%)	64 (71.1)
Beta-blocker (%)	27 (30)
Calcium channel blocker (%)	45 (50)
Diuretics (%)	18 (20)
Antiplatelet medications (%)	26 (28.8)
Statins (%)	69 (76.7)

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; DBP, diastolic blood pressure; IQR, interquartile range; *n*, number of cases; OHA, oral hypoglycemic agent; SBP, systolic blood pressure.

density, ms^2) ($r = -0.455$, $P < 0.0001$), HF (power density, ms^2) ($r = -0.227$, $P = 0.033$), and feet ESC (μS) ($r = -0.313$, $P = 0.0003$) (Figure 1b).

Effects of the variables of heart rate variability and electrochemical skin conductance on cardiovascular autonomic neuropathy score

The effects of the variables on CAN score in patients with T2DM according to correlation analysis are listed in Table 4. Based on the correlation analysis, only significant variables were enrolled into stepwise linear regression models. Results from the model 1 analysis (Table 3) revealed that only SDNN, HF, LF, feet ESC value, age and diabetes duration were significantly associated with CAN score and were enrolled into stepwise linear regression models. Results from the model 1 analysis (Table 4) revealed that SDNN, feet ESC and age were significantly associated with mean CAN score while there was no association with HF, LF and diabetes duration. Therefore, we included SDNN, feet ESC and age in the Model 2 regression analysis and found that SDNN, feet ESC and age were independently associated with mean CAN score in the patients with T2DM.

Diagnostic accuracy for standard deviation of normal RR interval, feet electrochemical skin conductance, as well as their combinations in predicting cardiovascular autonomic neuropathy, using receiver operating characteristic curve analysis

Statistical analyses for SDNN, feet ESC, and their combinations in predicting CAN using ROC curve analysis were as follows: SDNN ($P < 0.0001$), feet ESC (μS) ($P = 0.009$), and a combination of SDNN and feet ESC ($P < 0.0001$). Further, the sensitivity, specificity, and AUC by using ROC curve analysis, are listed in Table 5.

DISCUSSION

Clinical data show that clinical manifestations of CAN appear in T2DM, and the estimated 5-year mortality is approximately 50%[20]. Thus, evaluating the severity of CAN regression or progression is important for risk stratification and subsequent management. To our knowledge, efforts on those patients who had already suffered from CAN remain unsatisfactory.

Major findings of our study

Our study produced three major findings. First, the parameters of HRV and ESC including SDNN, total power of HF and LF, and feet ESC had been shown to bear a significant negative correlation with CAN score, indicating that they are surrogate markers of CAN severity. Second, integrating SDNN and feet ESC as a test battery can reinforce the accuracy in estimating the severity of CAN according to multiple linear regression analysis. Third, combining SDNN and feet ESC also can increase the diagnostic accuracy of CAN with respective to sensitivity, specificity by using ROC analysis[21].

Combining feet ESC and SDNN reinforce the accuracy in CAN screening service

Our study showed 36.3 % (33/90) had one abnormal autonomic function testings but did not reach the diagnostic criteria of CAN (equal or more than two abnormal autonomic function testings). The false negative rate of the diagnosis of CAN, according to cut-off value of each test in ROC analysis, were highest in feet ESC, followed by SDNN, and its combination. One may argue that only SDNN or feet ESC alone can serve as a simple and objective method for screening CAN. In clinical practice, HRV and feet ESC reflect patient's physiological conditions during the examinations and there are several clinical confounding factors that could influence the results, including age, gender, average heart rate and respiratory rate, co-morbidities, and drugs. To our knowledge, there are several methods that could increase the sensitivity and specificity of these diagnostic tools, including repeating the same methods (HRV or ESC) for several times during the follow-up period, recording HRV for a longer time period, or combining different diagnostic methods. Therefore, the combination of these two quantitative methods (sudomotor [sympathetic cholinergic] and cardiovagal [parasympathetic] system function) cannot only

Table 2 | Laboratory and autonomic function testings in patients with Type 2 diabetes stratified by the severity of cardiovascular autonomic neuropathy

	Without CAN		With CAN		P-value
	No CAN (n = 21)	At risk without CAN (n = 33)	Mild to moderate CAN (n = 25)	Severe CAN (n = 11)	
Laboratory test findings					
Total cholesterol(mmol/L)	158.0 ± 35.5	152.8 ± 36.5	159.2 ± 32.2	159.5 ± 34.0	0.895
Triglyceride(mmol/L)	109.8 ± 51.5	145.5 ± 82.6	148.4 ± 87.9	169.2 ± 55.7	0.145
HDL-C (mmol/L)	47.1 ± 9.2	45.4 ± 12.1	45.6 ± 13.0	42.1 ± 6.8	0.65
LDL-C (mmol L)	88.9 ± 31.0	82.9 ± 22.3	86.5 ± 25.6	83.1 ± 24.4	0.882
HbA1c (%)	6.8 ± 0.8	6.8 ± 0.9	7.0 ± 0.7	7.4 ± 1.0	0.102
eGFR	62.3 ± 22.1	60.0 ± 28.1	57.0 ± 22.3	40.9 ± 26.0	0.122
UACR	14.2 (8.8,47.3)	10.5 (7.7,76.6)	19.4 (8.5, 90.2)	102.7(44.9, 1529.1)	0.012*
Hs-CRP	1.6 ± 0.9	1.8 ± 1.4	1.7 ± 0.8	1.7 ± 0.7	
Cardiac autonomic reflex tests					
CAN score	0	1.0 ± 0	2.0 ± 0	3.2 ± 0.4	<0.0001**
E:I ratio	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.05	1.1 ± 0.04	<0.0001**
30/15 ratio	1.2 ± 0.1	1.1 ± 0.04	1.0 ± 0.03	1.0 ± 0.02	<0.0001**
Valsalva ratio	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.05	1.2 ± 0.08	<0.0001**
Orthostatic Hypotension	0	0	0	5	<0.0001**
BP change related to standing (mmHg)	-3 (−11, 3)	-3 (−8.5,3.5)	-5.5(−15.0, −1.0)	-14 (−23, −10)	<0.0001**
Heart rate variability					
SDNN (ms)	34.7 ± 26.2	22.9 ± 10.6	17.2 ± 7.7	14.2 ± 6.8	<0.0001**
CVR-R (%)	3.93 ± 2.84	2.64 ± 1.09	2.02 ± 0.83	1.74 ± 0.70	<0.0001**
Frequency domain					
LF (power density, ms^2)	63.8 (36.1, 203.3)	98.1(31.9,139.5)	23.2(14.7, 78.8)	16.5(5.1, 29.0)	0.028*
LF (n. u.)	57.0 ± 20.8	45.8 ± 22.8	49.4 ± 25.8	46.2 ± 14.3	0.351
HF (power density, ms^2)	50.9 (6.1, 174.2)	101.1(35.9, 198.0)	31.9 (15.5, 51.2)	12.2 (8.9, 38.1)	0.066
HF (n . u.)	43.0 ± 20.8	54.2 ± 22.8	50.6 ± 25.8	53.8 ± 14.3	0.351
LF/HF ratio	1.7 (0.7, 2.7)	0.8 (0.4, 2.5)	1.0 (0.3, 2.9)	0.9 (0.6, 1.3)	0.29
Sudoscan					
Hands ESC, µS	50.6 ± 18.1	48.8 ± 23.0	42.1 ± 17.2	46.4 ± 14.3	0.472
Feet ESC, µS	59.4 ± 16.1	55.7 ± 17.8	44.5 ± 16.6	45.2 ± 19.7	0.023*
Sudoscan-risk scores (%)	34.3 ± 6.5	36.7 ± 8.9	38.0 ± 6.1	38.6 ± 6.0	0.305

Data are presented as means ± standard deviations or median (IQR) n(%). *P < 0.05; **P < 0.01. CAN, cardiac autonomic neuropathy; CVR-R, coefficient variation of R-R intervals; ESC, electrochemical skin conductance; HF, high frequency; IQR, interquartile range; LF, low frequency; n, number of cases; n.u., normalized unit; SDNN, standard deviation of normal RR interval.

reinforce the accuracy of CAN screening, but also is a time-saving method in the outpatient clinic.

Risk factors associated with the severity of CAN

The pathophysiological mechanism of CAN development is multifactorial, and several studies reported the important role of CV risk factors, such as diabetes duration, degree of glycemic control, SBP, triglyceride levels, and BMI, in the development of CAN[22-25]. Further, there is enough evidence that CAN may precede DM[23]. A framework for screening and early multifactorial interventions (hyperglycemia, hypertension, dyslipidemia, and microalbuminuria) is the best prospect for preventing or halting CAN and its devastating sequelae and is the gold standard of diabetic care in T2DM[26] and T1DM[27]. Diabetes duration is an important risk factor for CAN. CAN is a length-

dependent pattern of disease, and parasympathetic activity can be damaged in the early phase of CAN with sympathetic predominance. As the diseases progress, sympathetic denervation occurs in the late stage of CAN[28]. Our study also showed that all parasympathetic parameters (e.g., VR, E:I ratio, 30:15 ratio, and SDNN) were significantly lower in proportion to the severity of CAN.

Feet ESC and SDNN as electrophysiologic markers for evaluation of CAN severity

CARTs are the gold standard for the diagnosis of CAN. Nevertheless, there are some limitations to perform the whole test battery in clinical practice. It takes around 30 min to complete all the tests, and thus screening a large number of patients with diabetes may be impossible in some busy clinics. In addition,

Table 3 | Correlation analysis of autonomic function parameters on cardiac autonomic neuropathy score in patients with type 2 diabetes

Variables	CAN score	
	r	*P*-value
Age (year)	−0.332	0.002**
Diabetes duration (year)	0.240	0.025*
Body mass index	0.05	0.642
SDNN (ms)	−0.462	<0.0001**
LF (n. u.)	−0.115	0.291
HF (n. u.)	0.115	0.291
LF (power density, ms^2)	−0.455	<0.0001**
HF (power density, ms^2)	−0.227	0.033*
LF/HF ratio	−0.124	0.253
Hands ESC, μS	−0.132	0.226
Feet ESC, μS	−0.313	0.0003**

*P-value < 0.05. **P-value < 0.01. CAN, Cardiac autonomic neuropathy; ESC, electrochemical skin conductance; HF, high frequency; LF, low frequency; *n*, number of cases; n.u., normalized unit; *r*, correlation coefficient; SDNN standard deviation of normal RR interval.

the tests sometimes may not be done due to patients' limitation, such as patients' underlying conditions (e.g., dementia or parkinsonism) and those who are unable to do deep breathing or Valsalva maneuver, or patients who are unable to stand. Therefore, a simplified effective method may be needed in busy outpatient clinics so that most patients can be screened or frequently followed up.

A clinical study showed that ESC reduction is proportional to the skin nerve fiber density[29] and can be used as a noninvasive measurement and is correlated with the gold standard measurement for the severity of small fiber neuropathy (e.g., skin biopsy with quantization of intra-epidermal nerve fiber density). Furthermore, ESC can be a clinically meaningful tool to measure severity and follow-up for progression and regression[30]. Although several studies have demonstrated that SUDOSCAN risk score is a good screening test for CAN[13,31], the diagnostic accuracy of CAN in our study is not established. The available information was calculated automatically from ESC values, BMI, and age using an algorithm included in the device software as we did not know the detailed algorithm of SUDOSCAN risk scores. However, several CV risk factors, such as diabetes duration, degree of glycemic control, SBP, triglyceride levels, and diseases of central or peripheral autonomic dysfunctions, could contribute to the severity of CAN. Further, different ethnic groups also can affect the normal values of ESC that seemed to lower in Chinese populations[32]. However, other studies and our study showed that feet ESC is significantly correlated with the severity of CAN[13], which is compatible with the length-dependent pattern of diseases.

Sympathetic and parasympathetic stimuli directly influence heart rate and are responsible for the physiologic variation in HRV. HRV can be evaluated in the time and frequency
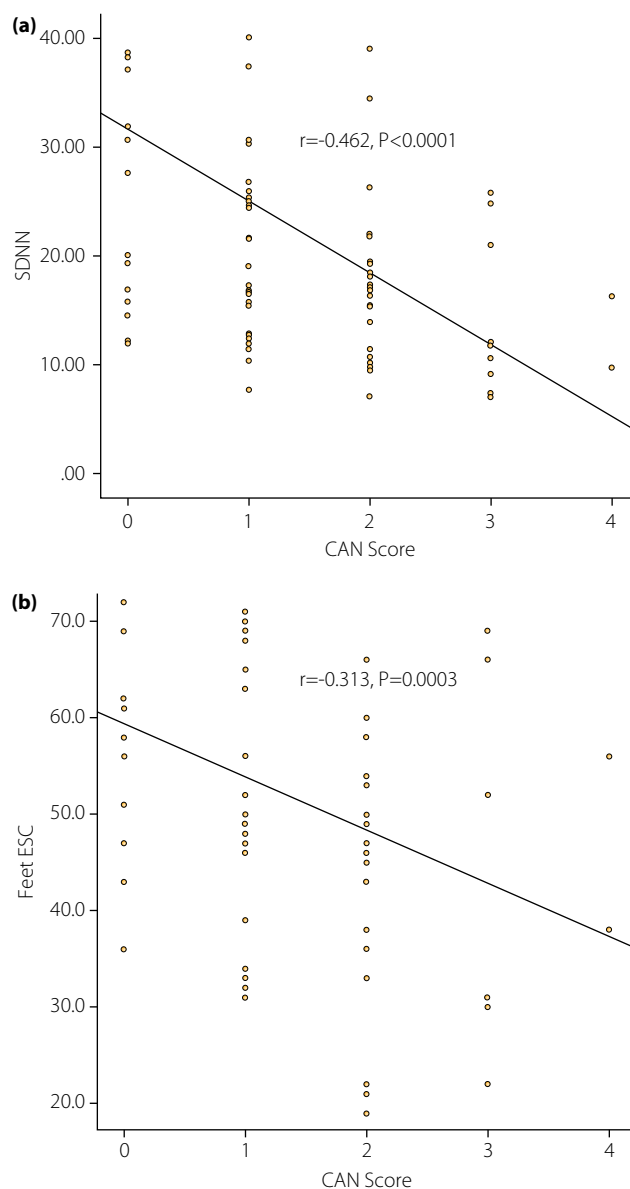
Figure 1 | Relationship between SDNN value and CAN score (a) and relationship between Feet ESC value and CAN score (b) in patients with type 2 diabetes.

domains[33]. Short-term HRV may be another time-saving tool that can be used in busy clinics. Only a 5-min resting recording of ECG is needed for computation and the requirement of patient's cooperation is decreased.

There are several different approaches of HRV in the time and frequency domains. Among these methods, the time domain parameter, SDNN, is the most intuitional with the simplest computation. Furthermore, the parameter is essential for almost all portable HRV devices. Since variance is mathematically equal to the total power of spectral analysis, SDNN reflects all the cyclic components responsible for the variability

Table 4 | Effects of the variables of heart rate variability and electrochemical skin conductance on cardiac autonomic neuropathy score according to correlation analysis

Variables	CAN score					
	Model 1[α]			Model 2[β]		
	Regression coefficient	Standard error	P-value	Regression coefficient	Standard error	P-value
Constant	5.523	0.915	<0.0001**	5.79	0.844	<0.0001**
SDNN (ms)	-0.025	0.006	<0.0001**	-0.025	0.006	<0.0001**
Feet ESC, μS	-0.014	0.005	0.014*	-0.015	0.005	0.003*
Age (year)	-0.046	0.012	<0.0001**	-0.046	0.011	<0.0001**
Diabetes duration (year)	0.009	0.012	0.453			
HF (power density, ms^2)	0.002	0.004	0.718			

A, Model summary: r^2, 0.34; β, model summary: r^2, 0.353. Formula: CAN score, 5.79-0.025 x (SDNN)-0.046x (Age) −0.015x (Feet ESC). Regression coefficient for each individual variable. *P-value < 0.05. **P-value < 0.01. CAN, Cardiac autonomic neuropathy; ESC, electrochemical skin conductance; HF, high frequency; SDNN standard deviation of normal RR interval.

Table 5 | Sensitivity, specificity, and area under the curve using receiver operating characteristic curve analysis for SDNN, feet electrochemical skin conductance, as well as their combinations in predicting cardiovascular autonomic neuropathy

Significant parameters	Cut-off value[†]	AUC (95% CI)	Sensitivity (%)	Specificity (%)	P-value
SDNN (ms)	16.45	0.737 (0.63–0.85)	76.1	56.2	<0.0001**
Feet ESC, μS	44.5	0.674 (0.56 − 0.93)	82.6	40.6	0.009**
Combined SDNN and Feet ESC	0.372	0.779 (0.68 − 0.88)	79.4	70	<0.0001**

*P < 0.05; **P < 0.01. [†]The positive or negative test results of feet ESC, SDNN, and its combination were according to cut-off value of each test in ROC analysis. The false negative rate of the diagnosis of CAN by feet ESC, SDNN, and its combination were 40.7% (22/54), 27.8% (15/54) and 18.5% (10/54), respectively. ESC, electrochemical skin conductance; ROC, receiver operating characteristic; SDNN standard deviation of normal RR interval.

at the time of recording. Although parameters in the frequency domain may provide information about parasympathetic and sympathetic modulations or balance in addition to cardiovagal function[34], it bears some pitfalls in the interpretation of results. Application of the such technique is critically dependent on the understanding of the underlying physiology, the mathematical analyses used, and the many confounders and possible technical artifacts. The measurement of VLF, LF, and HF power components is usually made in absolute values of power (power density, ms^2). LF and HF may also be measured in normalized units (n.u.), which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF (n.u.) and HF (n.u.) emphasizes the control and balance between the sympathetic and parasympathetic systems and tends to minimize the effect of the changes on the values ond HF total compo Nevertheless, normalized units should always be quoted wsolute values of the LFF power in order to describe tely the distribution or in spectral component study showed that the of LF and HF total

powreased in proportion to the severity to CA bothd HF power negativerelated with the CAN sct implies that both parasympathetic and sympathetic impairments were more severe as the severity of CAN progressed.

Although the LF components of frequency-domain measures of heart rate variability was generally accepted to reflect the activity of the sympathetic nervous system. Goldstein and colleagues have demonstrated that the LF power was mixed sympathetic and parasympathetic influences by baroreflex modulation of autonomic outflows in central origin[35]. This may explain the complex nature of LF power and exceedingly poor relationship to sympathetic nerve activation. In addition, the ratio of LF to HF could be used to quantify the changing relationship between sympathetic and parasympathetic nerve activities but it provides only a single degree of analysis. The ratio of LF to HF cannot accurately quantitatively reflected the severity of CAN. Therefore, only SDNN alone may neglect the sympathetic component and the combination is a better model in predicting the severity of CAN.

The combination of electrophysiologic biomarkers (SDNN and feet ESC) as a simplified test battery is a feasible time-effective CV autonomic screening service for outpatient clinics and can be completed in less than 10 min. It also reduces clinic visits and provides objective and quantitative measures of CAN in those patients who already had CAN. Similar to the idea of a one-stop service for microvascular screening[36], our test battery can serve as a one-stop CAN screening service. If the test battery shows CAN progression, they will resort to the more sophisticated and specific, but ultimately more time-consuming complete autonomic function testings (e.g., CARTs).

Study limitations

This study is a cross-sectional prospective study. Although we observed close relationship between SDNN and feet ESC and the severity of CAN in this observational study, the role of the test battery together with the combination of the two electrophysiologic biomarkers (SDNN and feet ESC) in a longitudinal study to evaluate the regression or progression of CAN is mandatory. Second, the case numbers are not large, and our study only included a Chinese population with T2DM. We did not include those patients who had pre-diabetes, non-diabetes or normal control. It may cause potential bias in statistical analysis or casual statistical finding. Finally, patients on medications known to cause orthostatic hypotension or otherwise affect autonomic testing were asked to stop the drug for five half-lives, if this was not harmful to the patient's well-being. The medications of beta-blocker and calcium channel blockers are likely to influence the autonomic test results. However, due to ethical considerations, these drugs were not stopped for five half-lives. These drugs were omitted on the day of the study and resumed after the test. Although the lingering effects from medication cannot be entirely excluded, we have done our best to reduce the influence from medication to an acceptable range.

Conclusion

Combining the results of SDNN and Feet ESC can not only assess, but also quantitatively reflect the progress or improvement of autonomic nerve function (including sympathetic and parasympathetic activity) in patients with T2DM.

ACKNOWLEDGMENTS

DISCLOSURE

The authors declare no conflict of interest.

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