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RESEARCH ARTICLE

Why Not to Use the Handgrip Test in the Assessment of Cardiovascular Autonomic Neuropathy Among Patients with Diabetes Mellitus?

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Abstract: ***Objective:*** Historically, a set of 5 Cardiovascular Autonomic Reflex Tests (CARTs) were considered to be the gold standard in the assessment of Cardiovascular Autonomic Neuropathy (CAN). However, measuring diastolic Blood Pressure (BP) response to sustained handgrip is omitted in recent guidelines. We aimed to assess the association between the handgrip and the other 4 tests as well as to identify determinants of the handgrip test results in diabetic patients.

Patients and Methods: 353 patients with diabetes (DM) were recruited (age: 60.2±7.4 years; female: 57.2%; BMI: 29.3±2.1 kg/m^2; DM duration: 15.6±9.9 years; HbA1c: 7.8±1.4% (66 mmol/mol); with type 1 DM: 18.1%). CAN was assessed by 5 CARTs: the deep breathing test, Valsalva ratio, 30/15 ratio, handgrip and orthostatic hypotension test.

Results: Sensitivity and specificity of the handgrip test in the diagnosis of definite CAN were 24.6% (95%CI 17.7-33.1%) and 79.4% (95%CI 73.3-84.4%), respectively. Results of the handgrip test did not show any association with those of the deep-breathing test (γ=0.004, p=0.563), 30/15 ratio (γ=0.282, p=0.357), Valsalva ratio (γ=-0.058, p=0.436) and orthostatic hypotension (γ=-0.026, p=0.833). Handgrip test abnormality showed an independent association with higher initial diastolic BP (OR 1.05, p=0.0009) and an independent inverse association with the presence of hypertension (OR=0.42, p=0.006).

Conclusion: Our data confirm that the handgrip test should no longer be part of the cardiovascular autonomic testing being highly dependent on hypertensive status and baseline diastolic BP. Exaggerated exercise pressor response is proposed as putative mechanism for the inverse association between abnormal results of the handgrip test and hypertension. Adequate CARTs are important to allow their use in clinical trials and for the prevention of DM-associated complications by initiating early treatment.

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INTRODUCTION

Cardiovascular Autonomic Neuropathy (CAN) is a common complication of diabetes mellitus (DM). Its prevalence varies between 7 and 65% depending on the diagnostic criteria and the studied population increasing with age and DM duration [1]. Moreover, it may be present in newly diagnosed patients with type 1 and type 2 DM and in patients with impaired glucose tolerance [2]. A meta-analysis of 15 longitudinal studies with a follow-up of 1-16 years showed that the definite diagnosis of CAN based on at least 2 abnormal test results is accompanied by a relative risk of mortality of 3.65 [3]. The association between CAN and cardiac death, non-fatal myocardial infarction and silent ischaemia is well-known [4]. CAN was associated with left ventricular dysfunction and hypertrophy in patients with DM type 2 in whom cardiac disease was absent [5]. QT prolongation may lead to severe ventricular arrhythmias and a 2-fold risk of stroke [5]. There is emerging evidence that CAN is an independent predictor of the development and progression of diabetic nephropathy [6, 7].

Therefore, early diagnosis of CAN and thereby identifying patients at high risk of cardiovascular morbidity and mortality is crucial. In clinical practice, assessment of CAN

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is usually based on the Cardiovascular Reflex Tests (CARTs) standardized by Ewing *et al*. [8]. CARTs are safe, non-invasive, clinically relevant, reproducible and easy-to-perform. Hence, they are widely used and considered gold standard measures of CAN [9, 10]. The San Antonio Consensus conference advocated that a battery of quantitative tests including both parasympathetic and sympathetic function tests should be performed and more than one of these tests should be abnormal to verify the diagnosis of CAN [10]. The need to use more tests has been confirmed in recent guidelines [1, 11]. However, measuring diastolic blood pressure (BP) response to sustained handgrip is no longer recommended as an established clinical test [1, 9]. According to the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology, the handgrip test has limited sensitivity and specificity in diagnosing CAN [9]. Furthermore, little attention has been paid to confounding variables influencing the result of handgrip test and evidence is lacking to judge its clinical usefulness.

The aim of our study was to assess the diagnostic value of the handgrip test in detecting CAN. Furthermore, we evaluated the relationship of handgrip test results with results of the other CARTs and investigated the factors influencing the diastolic BP response to sustained handgrip exercise in patients with DM.

RESEARCH DESIGN AND METHODS

The present cross-sectional study was carried out in patients with DM attending the 1st Department of Medicine, Semmelweis University, Budapest, Hungary. Inclusion criteria were the presence of type 1 or type 2 DM according to the WHO (1999) criteria [12]. Patients hospitalized for acute intercurrent diseases (fever, infection, *etc*.) or for acute metabolic derangements such as diabetic ketoacidosis or hyperglycaemic hyperosmolar state were excluded from our study. Further exclusion criteria included diseases or conditions that may affect autonomic function, such as thyroid and liver diseases, chronic kidney failure, autoimmune or haematological disorders, Parkinson's disease, *etc*. Subjects with a history of arrhythmia, bundle branch block, heart failure, valvular disease, acute coronary syndrome, ischaemic heart disease or pulmonary disorders (COPD) were also excluded. Patients with DM with poor physical status making them unable to perform sustained isometric muscular strain and patients with proliferative retinopathy being at risk of intraocular haemorrhage during Valsalva and handgrip manoeuvre were also not included.

Eligible patients were requested to avoid strenuous physical exercise, caffeine beverages, smoking and alcohol in the 12h preceding cardiovascular autonomic testing. Patients taking antihypertensive agents that might influence the results of the CARTs based on heart rate changes were asked to omit interfering medication, particularly beta-receptor blockers and non-dihydropyridine-type calcium channel blockers in the 24h interval before CARTs were performed.

All participants gave informed consent and the study protocol was approved by the local ethics committee.

Data on age, glycaemic control (HbA1c), DM duration, antidiabetic and antihypertensive medication, insulin treatment, use of diuretics and presence of neuropathic pain requiring specific pain-relieving medication were obtained. Weight and height of eligible subjects were measured and Body Mass Index (BMI) calculated (expressed in kg/m^2). Measurements of office BP and BP values during the manoeuvres were accomplished using an OMRON M2 (Omron Corporation, Kyoto, Japan) automatic upper hand-cuff sphygmomanometer. Office BP values were obtained after a minimal period of 5 min of resting state and the average of three seated BP measurements was determined.

Cardiovascular autonomic function was assessed by the 5 CARTs using Cardiosys 12.1 diagnostic station and Cardiosys-A01 software (MDE Heidelberg GMBH, Heidelberg, Germany). During real-time 12-lead monitoring recorded ECG signals were digitized at 2000 Hz sampling rate with a multichannel data acquisition system connected to a personal computer. Stored data were available for off-line analysis.

CARTs based on heart rate changes mainly reflect parasympathetic function while those based on BP response to manoeuvres explore sympathetic function.

The result of the deep-breathing test was expressed as the difference of the highest heart rate during inspiration and the lowest heart rate during expiration (beat-to-beat variation; beats/min).

Valsalva ratio was assessed as follows: participants were instructed to blow into the mouth piece of Cardiosys connected to the computer and to maintain 40 mmHg airway pressure for a period of 15 sec according to the standardised protocol. The ratio of the longest RR interval after and the shortest RR interval during the manoeuvre was measured.

Result of the Orthostatic Hypotension Test (OHT) was defined as the difference between the systolic BP measured at rest in supine position and the lowest systolic BP after arising.

30:15 ratio was computed as the ratio of the RR intervals of the 30th and 15th (or nearby) cycles following standing up.

Subjects performed sustained handgrip exercise at 30% of maximal voluntary contraction up to 3 min. BP values were measured before the test (initial or baseline diastolic BP) and each minute in the 3 min period during sustained handgrip exercise.

To evaluate severity of cardiovascular autonomic dysfunction, an overall autonomic neuropathy score (CAN score) was obtained by scoring the results of the reflex tests: 0, 1 and 2 for normal, borderline and abnormal test result, according to age-related reference values for heart rate based tests [13].

Similarly, a parasympathetic impairment score was derived from the results of the parasympathetic function tests based on heart rate changes [1]. Normal results of a test were scored with 0, borderline results with 1, and abnormal results with 2.

Confirmed diagnosis of CAN was defined as the presence of ≥ 2 abnormal test results as recommended by recent guidelines [13].

Statistical Analysis

The Kolmogorov-Smirnov test for normality was performed on all variables. Normally distributed data are expressed as mean ± SD while non-normally distributed variables are described as median/geometric mean and interquartile range.

To evaluate the diagnostic accuracy of the handgrip test *vs.* diagnosis of confirmed CAN (presence of ≥2 different abnormal test results), sensitivity and specificity were determined using 2x2 contingency tables and χ^2 test. Categorical variables are reported as n (%).

Since most results of CARTs were not normally distributed, association between the results of the handgrip test and clinical parameters were analysed using Spearman's rank correlation (rho). Kruskal's monotony coefficient gamma (γ), also known as the Goodman-Kruskal test, was used to evaluate the association between abnormality of the handgrip test and abnormality of the other tests. For comparison between groups with normal and abnormal handgrip test result, the Mann-Whitney U-test or paired t-test for continuous variables and χ_2 test or Fisher's exact test for categorical data were performed as required.

In order to adjust for the confounding effect of antihypertensive medication on the association between handgrip test results and hypertension as well as the association between handgrip test results and initial diastolic BP, additional multiple logistic regression analysis was performed. Variables associated ($p < 0.1$) with abnormal handgrip test result were included in this analysis.

All analyses were performed using STATISTICA 12.5 software (Statsoft Inc, Tulsa, USA). Statistical significance was defined as $p < 0.05$ (two-sided value).

RESULTS

A total of 353 diabetic patients were included: 64 (18%) with type 1 and 289 (82%) with type 2 DM; 225 (63.7%) patients had coexistent hypertension (30 with type 1 and 195 with type 2 DM). Hypertensive subjects had known hypertension and were taking antihypertensive medication. The main characteristics of the study population are shown in Table **1**.

Diagnosis of CAN was present in 36.8% of the patients. Abnormal results of the deep-breathing test, the Valsalva ratio, 30/15 ratio, handgrip and orthostatic hypotension test were proven in 59.5, 19.5, 4.5, 22 and 32.3%, respectively.

Compared with the definition of CAN based on the presence of at least 2 abnormal reflex tests, sensitivity of the handgrip test for detecting CAN was 24.6% (95% CI 17.7 - 33.1%) and specificity was 79.4% (95% CI 73.3 - 84.4%) ($p = 0.384$). Abnormal results of the handgrip test did not show any association with abnormality of the deep-breathing test ($\gamma = 0.004$, $p = 0.563$), Valsalva ratio ($\gamma = -0.058$, $p = 0.436$), 30/15 ratio ($\gamma = 0.282$, $p = 0.357$) and the parasympathetic impairment score ($\gamma = 0.059$, $p = 0.465$), respectively. An association between results (scores) of the handgrip and the OHT failed to be proven ($\gamma = -0.026$, $p = 0.833$) (Table **2**). For comparison, associations of the three parasympathetic tests, as well as parasympathetic score with OHT are also shown in Table **2**.

Table 1. Main clinical characteristics of the study population.

Parameters (n=353)	
Mean age (years)	60.2±7.4*
Gender (male/female)	151/202
HbA1c	7.8±1.4% (61.2±15.8 mmol/mol)*
Diabetes duration (years)	15.6±9.9*
Type 1 / type 2 diabetes	64 (18%) / 289 (82%)
BMI (kg/m^2)	28.7 (25; 32)#
Hypertension (yes/no)	225 (63.7%) / 128 (36.3%)
Antihypertensive medication (n=225) ACEi or ARB/CCB/Diuretics/AG	174/87/151/40
Statin use	160/353 (45%)
Aspirin use	147/353 (42%)

* mean ± SD
# geometric mean (interquartile range).
ACEi: ACE Inhibitors; ARB: Angiotensin Receptor Blockers,; CCB: Calcium Channel Blocker. diuretics included hydrochlorothiazide and indapamide. AG: Alpha1-Receptor antagonists.

In order to identify factors influencing handgrip test results, patients with and without handgrip test abnormality were compared in terms of demographic and clinical characteristics. Patients with abnormal handgrip test result had significantly higher mean office systolic BP (140 *vs.* 130 mmHg, $p = 0.007$) and higher mean baseline diastolic BP (83 *vs.* 76 mmHg, $p = 0.0004$) than those with normal handgrip test result. In contrast, diagnosis of hypertension was more frequent in patients without handgrip test abnormality [186 (67.7%) *vs.* 39 (50%), $p = 0.0076$] (Table **3**).

Diastolic BP changes during handgrip test correlated negatively to baseline diastolic BP values ($rho = -0.286$, $p < 0.01$), while office systolic BP had no effect ($rho = -0.0169$, NS) on the scale of BP increase during handgrip test. In multiple logistic regression analysis, handgrip test abnormality was related to the initial diastolic BP values (OR: 1.05, 95% CI: 1.02 - 1.08; $p = 0.0009$) and inversely related to the presence of hypertension (OR: 0.42, 95% CI: 0.23 - 0.78, $p = 0.006$), with these relationships being independent of antihypertensive medication (Table **4**).

Abnormal results of the OHT test (defined as a systolic BP fall of at least 20 mmHg) were influenced neither by clinical characteristics such as age ($rho = 0.059$, NS), BMI ($rho = 0.122$, NS), DM duration ($rho = -0.102$, NS), HbA1c ($rho = -0.139$, NS), baseline systolic BP ($rho = -0.109$, NS) nor by the presence of hypertension ($\chi^2 = 0.042$, NS).

DISCUSSION

The objectives of our study were to assess the relationship of handgrip with the other CARTs in the diagnosis of CAN as well as to identify the factors having influence on the diastolic BP response to sustained handgrip.

Table 2. Associations between abnormality (*i.e.* score) of the handgrip test and the abnormalities of the other cardiovascular reflex tests (CARTs; for comparison, associations of orthostatic hypotension test (OHT) are also indicated).

CARTs Scores	Handgrip Score	p
Deep Breathing	$\gamma = 0.04$	0.563
Valsalva manoeuvre	$\gamma = 0.058$	0.436
30/15 ratio	$\gamma = 0.282$	0.357
Orthostatic hypotension	$\gamma = -0.026$	0.833
Parasympathetic score	$\gamma = 0.059$	0.465
	OHT score	**p**
Deep Breathing	$\gamma = 0.219$	0.046
Valsalva manoeuvre	$\gamma = 0.311$	0.006
30/15 ratio	$\gamma = 0.470$	0.059
Parasympathetic score	$\gamma = 0.301$	0.003

γ = Monotony coefficient gamma

Table 3. Comparison of demographic and clinical characteristics of diabetic subjects with or without handgrip test abnormality (univariate analysis).

	Abnormal Handgrip Test Result (n=78)	Normal Handgrip Test Result (n=275)	*p*
Age (years)	58.8±12.6	60.6±12.5	0.224*
BMI (kg/m^2)	29.0±6.3	29.4±5.8	0.517*
Diabetes duration (years)	10.9 (8; 20)	10.0 (6; 20)	0.573
HbA1c (%)	7.8 ±1.4	7.7±1.5	0.340*
HbA1c (mmol/mol)	61.2±15.8	60.9±15.8	0.338*
Confirmed diagnosis of CAN	32 (41.0%)	98 (36.6%)	0.384 #
Deep Breathing (1/min)	8.94 (7; 13)	8.54 (6; 12)	0.510
Valsalva ratio	1.27 (1.18; 1.32)	1.24 (1.17; 1.32)	0.507
30/15 ratio	1.15 (1.09; 1.22)	1.17 (1.1; 1.22)	0.542
Orthostatic hypotension (mmHg)	6 (0; 12)	7 (2; 14)	0.988
Parasympathetic score	2.94 (2; 4)	2.8 (2; 4)	0.527
CAN score	3.33 (3; 4)	3.21 (2; 4)	0.468
Diagnosis of hypertension	39 (50.0%)	186 (67.7%)	0.0076 #
Office systolic blood pressure (mmHg)	140 (126; 152)	130 (120; 144)	0.007
Baseline diastolic blood pressure (mmHg)	83 (76; 92)	78 (72; 86)	0.0004
ACEi/ARB	43 (55.0%)	183 (66.5%)	0.087 #
CCB	22 (28.2%)	65 (23.6%)	0.836 #
Alfa receptor antagonist	7 (8.97%)	37 (13.5%)	0.023**
Diuretics	25 (32.1%)	104 (37.8%)	0.046 #
Insulin therapy	37 (47.4%)	134 (48.7%)	0.951 #
Neuropathic pain	11 (14.0%)	35 (12.7%)	0.690 #

Data are reported as mean + SD or geometric mean/median and interquartile range. Between-group comparisons were carried out by Mann-Whitney U-test or two-sample t-test (*) where appropriate.
χ^2-test or **Fisher's exact test for categorical variables were used as indicated. Categorical data are reported as n (%).
Abbreviations:
BMI: Body Mass Index
HbA1c: Haemoglobin A1c
CAN: Cardiovascular Autonomic Neuropathy
ACEi/ARB: Angiotensin-Convertase Enzyme Inhibitor/Angiotensin Receptor Blocker
CCB: Calcium Channel Blocker

Table 4. The relationship between handgrip test abnormality and hypertension as well as initial diastolic blood pressure values adjusted for the confounding antihypertensive medication (multiple logistic regression analysis).

Variables p <0.1 from table 4 are included in the analysis.

	OR	95% CI	p
Hypertension	0.42	0.23-0.78	0.006
ACEi/ARB	0.96	0.49-1.84	0.909
Alpha-receptor antagonists	1.04	0.42-2.59	0.931
Diuretics	1.14	0.61-2.13	0.696
Initial diastolic blood pressure	1.05	1.02-1.08	0.0009

Abbreviations: ACEi/ARB: angiotensin-convertase enzyme inhibitor/angiotensin receptor blocker.

According to our data, very low sensitivity and moderate specificity of the handgrip test *vs.* definite diagnosis of CAN could be demonstrated. Results of the handgrip test did not show any association with results of the deep breathing test, 30/15 ratio and the partially sympathetically controlled Valsalva ratio. An association between results of the handgrip test and the OHT could not be proven. Potential confounding factors having influence on handgrip test outcomes were the presence of hypertension and baseline diastolic BP.

CAN is an early and frequent complication and is accompanied with poor prognosis among patients with DM. In epidemiological studies performed on patient populations with both type 1 and type 2 DM, the prevalence rates of confirmed CAN ranged from 16.6 to 20% [14, 15]. The prevalence of CAN increases with age and DM duration reaching as high as 65% in patients with long-standing type 2 DM [16]. In our study, the prevalence rate of CAN based on the presence of ≥2 abnormal cardiovascular reflex tests was 37%. Considering age, DM duration and the proportion of type 2 DM patients in our study population, this prevalence is in accordance with the literature [1, 3, 6, 11, 13-14].

As CAN imposes an enormous increase in cardiovascular morbidity and mortality among patients with DM, early diagnosis and timely intervention with risk reduction strategies are crucial. Safe, non-invasive tests with high sensitivity and reasonable specificity are warranted. Cardiovascular reflex tests are widely used for identifying patients with CAN. In a study by Ewing *et al*. [17], a weak but significant correlation between the rise in diastolic BP in response to handgrip and Valsalva ratio was demonstrated. Patients with handgrip test abnormality had lower Valsalva ratio compared with those with normal handgrip test results [17]. When compared with spectral analysis of heart rate variability as a reference test, BP tests added on to heart rate tests did not improve diagnostic performance of CARTs [18]. Sustained handgrip test had significant weaker associations with $LnLF_{DAY}$ – a measure of prevalent sympathetic function – than OHT did [18]. However, particular reasons why the handgrip test has limited diagnostic performance remained elusive.

In the present study, we confirmed that handgrip test has poor sensitivity and moderate specificity for diagnosing CAN. No associations between handgrip test results and other CART outcomes could be established. Furthermore, a negative association between handgrip test abnormality and hypertension was demonstrated showing that patients with both DM and hypertension are unlikely to exhibit reduced diastolic BP response to handgrip exercise. The putative mechanism behind this phenomenon could be an exaggerated exercise pressor reflex related to increased sympathetic activation in patients with DM and hypertension.

The exercise pressor reflex aims the adaptation of autonomic nerve control of the cardiovascular system during exercise to meet the perfusion and metabolic demand of working skeletal muscle. Static (isometric) exercise causes heart rate-dependent increases in cardiac output accompanied by increased or unchanged peripheral vascular resistance resulting in substantially elevated BP [19, 20]. There is emerging evidence that hypertension is associated with excessive pressor responses during muscle contraction; both mechanically (mechanoreflex) and metabolically (metaboreflex) driven components of the pressor reflex are suggested being involved [21, 22]. Patients with hypertension were shown to produce exaggerated BP elevation and enhanced muscle sympathetic nerve activation during isometric handgrip exercise compared with age-related healthy controls [23, 24]. Increased muscle metaboreflex activation leading to augmented pressor responses was demonstrated in older moderately hypertensive adults [25] and in pre-hypertensive state [26]. The exercise pressor reflex might occur promptly: BP elevation and heart rate increased within 30-60 sec during static contraction in an experimental study [27]. More than two-thirds of our patients with DM had hypertension; this exaggerated exercise pressor reflex might explain why abnormal handgrip test results were not commonly found in our patients, even if they may have been already affected by CAN. The inverse association between abnormality of the handgrip test and hypertension indicates that low sensitivity of the handgrip test might be the result of a 'masking effect' of hypertension.

Previously, both hypertension and DM were found to be associated with impaired autonomic function: they negatively influence most CARTs [28], baroreflex sensitivity and heart rate variability [29-31] with their effects being additive [32, 33]. Nevertheless, the handgrip test seems to be the only measure of CAN being conversely affected by DM and hypertension. Hence, handgrip test outcomes might reflect the counteraction between increased sympathetic activity being a

feature of essential hypertension and sympathetic impairment attributable to diabetic autonomic neuropathy.

Hypertension is a frequent comorbidity of DM. Using ambulatory BP monitoring (ABPM), hypertension could be diagnosed in 54 and 29% of type 2 DM patients with/without CAN in spite of normal office BP and no history of hypertension [30]. In this context, hypertension may remain unrecognized in up to half of the patients with CAN [30]. Therefore, the effects of hypertension on handgrip test results cannot be overestimated in routine clinical practice. The known or unknown hypertension involving sympathetic overactivity prevents detecting CAN in the majority of our patients when sympathetic dysfunction is assessed by the handgrip test.

Autonomic dysregulation characterized by sympathetic overactivity and parasympathetic impairment has been proposed as a mechanism of developing hypertension [34]. The role of baroreflex in attenuating the circulatory response to exercise has been recently described [35]. Consequently, one could speculate that augmented BP response to sustained muscular strain and hypertension itself may be at least partially compelled by parasympathetic impairment leading to 'pseudo-normal' handgrip test results in patients with diabetic neuropathy.

In our study, diastolic BP changes in response to handgrip test were inversely correlated with baseline diastolic BP. The higher the baseline values were, the less was the extent of diastolic BP elevation and the greater was the chance of handgrip test abnormality. It would be conceivable that baseline diastolic BP is a limiting factor of diastolic BP increase during the test. Noteworthy, reduced diastolic BP as a consequence of increased central artery stiffness is a common feature of hypertension in the elderly. Hence, the association between lower diastolic BP and normal handgrip test results may be also attributed to atherosclerosis and hypertension in our population. Still, the independent association between higher baseline diastolic BP values and handgrip test abnormality needs further investigation.

The strength of the present study is the well-defined patient population. All drugs, especially antihypertensive agents being of potential influence on the outcomes of the cardiovascular reflex tests were included in the statistical analysis. Therefore, an independent inverse association between abnormality of the handgrip test and the presence of hypertension could be revealed.

A limitation of this study could be the absence of information on physical activity. Muscle function might influence the pressor response during isometric exercise. However, the exclusion of patients with relevant comorbidities and poor physical status should have excluded those with significantly impaired muscle function. Moreover, overweight status of our patients was only assessed by calculating BMI that does not provide any information on the type of obesity. Visceral adipose tissue accumulation may contribute to sympathetic nervous system activation [36, 37]. Although no association between handgrip test results and BMI was found, the influence of visceral obesity on our results cannot be entirely excluded.

The OHT test results were associated with measures of all three parasympathetic function tests, including the Valsalva ratio, confirming previous observations [38]. The association between OHT and abnormal Valsalva ratio is plausible, as OHT is an established test of sympathetic function as well, and circulatory changes during the Valsalva manoeuvre are also partially controlled by sympathetic pathways. The former association may be attributable to the fact that the possibility and extent of sympathetic impairment is expected to increase with severity of parasympathetic dysfunction. OHT was independent of anthropometric data and was neither influenced by the presence of hypertension nor by initial supine systolic BP suggesting that it is a reliable test. This latter finding is in contrast with previous observations that the magnitude of the orthostatic BP fall is related to the baseline supine BP with 25% of the variance of the systolic BP fall being due to the supine BP [38, 39]. This discrepancy might be explained by the fact that systolic BP values in our patients were not very high and they were treated with antihypertensive medication.

This topic has several important therapeutic implications. Alpha-lipoic acid, the most powerful pathogenetic therapeutic option of diabetic peripheral neuropathy [40-42] is also considered as the best choice for the treatment of CAN [43]. Alpha-lipoic acid enhances endoneurial blood flow by preventing the inhibition of nitric oxide synthetase and this way precludes ischaemic damage to nerve tissues [41]. Another pathogenetic option is the transketolase activator benfotiamine, inhibiting harmful alternative metabolic pathways, such as advanced glycation end product formation, the hexosamin and the protein-kinase-C pathways [44], as well as the polyol pathway [45].

Our data indicate that the presence of hypertension was identified as the main confounder for the presence of a false negative handgrip test. The majority of patients with DM, especially those with autonomic neuropathy, have hypertension as well [30, 32, 36, 46, 47]. Most patients with DM and hypertension are treated with ACE inhibitors, while these drugs might have an effect on autonomic function as well. Didangelos *et al*. [48] assessed the effect of ACE inhibition or angiotensin receptor blockade, and their combination on CAN in patients with DM. After 12 months, improvement of CAN was observed in patients treated both with quinapril or losartan, while their combination was slightly even more effective [48]. Beneficial effect of quinapril on CAN has also been observed in another study [49], while no significant changes in CAN was detected after 12 months treatment with trandolapril [50].

In the context of parasympathetic-sympathetic imbalance, relative sympathetic overactivity influences the action of beta-blocking agents; cardioselective beta-blockers have a beneficial effect on cardiac autonomic dysfunction [51]. Our results confirm that assessment of orthostatic BP changes should be considered as the only reliable measure of sympathetic nerve function. Some drugs, like hydrochlorothiazide, alpha- and non-selective beta-blocking agents might aggravate OHT, while beta-blockers with intrinsic sympathomimetic properties (partial agonists) have a role in the therapy of OHT.

In summary, the handgrip test has low sensitivity for CAN and shows no association with the other reflex tests established for assessing CAN in DM. Our data provide evidence that the handgrip test should no longer be part of the cardiovascular autonomic testing being highly dependent on both the hypertensive status and baseline diastolic BP. Among these factors, the presence of hypertension seems to be decisive. Instead of measuring diastolic BP response to handgrip exercise, OHT test should be used for the evaluation of sympathetic dysfunction as recommended in the latest guidelines [1].

Adequate CARTs are important to allow their use in clinical trials and for the prevention of DM-associated complications by initiating early treatment.

LIST OFABBREVIATIONS

ABPM	=	Ambulatory Blood Pressure Monitoring
ACE inhibitor	=	Angiotensin-Convertase Enzyme Inhibitor
BP	=	Blood Pressure
CAN	=	Cardiovascular Autonomic Neuropathy
CART	=	Cardiovascular Reflex Test
CI	=	Confidence Interval
COPD	=	Chronic Obstructive Pulmonary Disease
DM	=	Diabetes Mellitus
ECG	=	Electrocardiogram
LnLF day	=	Daytime Low-Frequency Power
NS	=	Not Significant
OHT	=	Orthostatic Hypotension Test
OR	=	Odds Ratio

AUTHORSHIP

AEK researched data, wrote, reviewed and edited manuscript; **MK** researched data, co-wrote and edited manuscript. OEV helped researching data and edited manuscript. **CL**, **ZP**, **II**, **VJH** and **KK** contributed to the revision of the manuscript. AGT reviewed and edited manuscript and reviewed statistical analysis. **VS** reviewed, edited manuscript and contributed to discussion. **PK** helped with the concept, supervised the study and revised manuscript. **PK** is the guarantor of this work and, as such, had full access to the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. All the authors approved the text.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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