Original Article

Diagnosis, Staging, and Associated Conditions of Cardiovascular Autonomic Neuropathy in Libyan Patients with Diabetes

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ABSTRACT

Background: Cardiovascular autonomic neuropathy (CAN) can affect daily activities and patients' quality of life and evoke potentially life-threatening outcomes in diabetes mellitus (DM). **Objectives:** We aimed to identify and characterize CAN and associated disorders in Libyan patients with DM at National Diabetes Hospital. **Patients and Methods:** Ninety-nine patients with DM seen in the outpatient clinics from October 2017 to April 2018 at National Diabetes Hospital were prospectively evaluated. Assessments for CAN were made by clinical symptoms and signs, cardiovascular autonomic reflex tests, and echocardiogram. Patients with potentially confounding concomitant medical conditions were excluded. CAN is defined as possible (one abnormal cardiovagal test), confirmed (two abnormal such tests), and severe (with concomitant orthostatic hypotension and heart rate abnormality). **Results:** Sixty-two percent of the studied patients (mean age: 52 ± 1.5 years, 53% – female) with DM had CAN. CAN diagnosis was possible in 18% of these patients, confirmed in 6%, and severe in 38%. The presence of severe CAN was associated with hypoglycemic unawareness ($P = 0.01$), dyslipidemia ($P = 0.012$), and microvascular diabetic complications ($P = 0.04$). **Conclusions:** In this cohort of relatively old and high-risk cardiovascular disease, patients with diabetes, uncontrolled blood pressure, associated dyslipidemia, presence of microvascular complication of diabetes, and history of hypoglycemic unawareness were strongly associated with a severe form of cardiac autonomic neuropathy with potentially serious clinical consequences. Larger and more detailed studies are needed to elucidate further the complex association between hypoglycemia and cardiac autonomic dysfunction.

Keywords: Cardiovascular autonomic neuropathy, diabetes mellitus, heart rate variability, orthostatic hypotension, resting tachycardia

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INTRODUCTION

Diabetes mellitus (DM) is a growing health challenge, and its burden is increasing on the health-care systems worldwide. This problem is worsened by the association of DM with several serious and fatal complications, including diabetic autonomic neuropathies. Cardiac autonomic neuropathy (CAN)[1,2] is one of the most severely debilitating forms of these autonomic neuropathies in DM patients. The autonomic nervous system modulates complex physiological mechanisms to help preserve blood pressure (BP) and heart rate (HR) within a normal range.

The Toronto Consensus Panel defined CAN as the impairment of cardiovascular autonomic control in patients with DM after the exclusion of other medical causes.[3,4] The diagnosis of CAN involves an evaluation of signs and symptoms for abnormal cardiovascular autonomic control. Standardized noninvasive cardiovascular autonomic reflex tests (CARTs) are safe and feasible to administer and have good sensitivity, specificity, and reproducibility for diagnosing CAN in patients with diabetes.[4-7]

Screening for CAN is recommended in patients with type 2 diabetes (T2D) and type 1 diabetes (T1D) at diagnosis or after 5 years of disease, respectively. CAN is particularly evident in patients with poor glycemic control (hemoglobin A1c [HbA1c]>7%), at least one major cardiovascular disease risk factor, or other chronic complications associated with DM (level B). In asymptomatic patients, screening for CAN may also be required for preoperative risk assessment for major surgical procedures (level C).[4] A classification of CAN is based on "early involvement" (two borderline test results or one abnormal result on HR test), "definite involvement" (two or more abnormal results on HR tests), and "severe involvement" (development of orthostatic hypotension).[8]

Diabetic CAN is further classified into phases: the subclinical phase is associated with decreased HR variability (HRV), the early phase with resting tachycardia, and the advanced phase with exercise intolerance, cardiomyopathy with left ventricular (LV) systolic dysfunction,[9,10] orthostatic hypotension, and silent myocardial ischemia (MI). A meta-analysis of 12 studies identified a higher rate of silent MI in patients with versus without CAN (20% vs. 10%).[11] CAN can independently predict the progression of diabetic nephropathy.[12] CAN is also an independent risk factor for all-cause mortality in patients with T1DM (EURODIAB study) and T2D (ACCORD study).[4,13,14] A meta-analysis of 15 longitudinal studies reported an association between CAN and higher mortality.[15]

CAN is also associated with a five-fold increased risk of cardiovascular mortality[16] and can be used for cardiovascular risk stratification, including a marker for a greater risk of intraoperative cardiovascular liability. There are limited data on CAN in different populations. The present study aims to identify and characterize CAN and its associated disorders in a sample of Libyan patients with diabetes.

PATIENTS AND METHODS

Study cohort

Adult patients (18 years and older) diagnosed with DM were seen at the National Diabetes Hospital outpatient clinic in Tripoli, Libya, between October 2017 and April 2018. They were prospectively enrolled for diagnostic evaluation after informed consent for CAN and associated conditions [Tables 1 and 2], highlighting the CARTs. Patients who had any systemic illness (e.g., congestive heart failure, coronary artery disease, arrhythmia, and thyroid dysfunction) or were on adrenergic antagonists that could confound the results of the autonomic function tests were excluded from the final analysis.

Assessment and data acquisition

Baseline demographic data (e.g., age, sex, body mass index (BMI), and history of cardiovascular risk factors) were taken. Echocardiographic findings and details related to type, duration, and associated complications of DM were evaluated. Transthoracic echocardiography (Vivid 7 GE) was used to assess LV systolic function, wall thickness, and ischemic changes based on the American Society of Echocardiography recommendations.[17]

Patients were also evaluated for the diagnosis and staging of CAN and associated conditions based

Table 1: Distribution of the patients' sociodemographic characteristics and degree of cardiovascular autonomic neuropathy severity

Characteristic	Total, n (%)	Status of CAN				P
		None, n (%)	Possible, n (%)	Definite, n (%)	Advanced, n (%)	
Sex						
Female	53 (53)	23 (60)	6 (33.3)	3 (50)	21 (55)	0.247
Male	46 (46)	14 (36.8)	12 (66.3)	3 (50)	17 (44.7)	
Age						
<30	9 (9)	2 (5.4)	3 (16.6)	0	3 (7.8)	0.72
31-50	37 (37)	12 (32.4)	6 (33.3)	3 (50)	16 (42)	
51-65	40 (40)	15 (40.5)	7 (38.8)	3 (50)	15 (39.4)	
>66	14 (14)	8 (21.6)	2 (11)	0	4 (10.5)	
Smoker						
None	70 (70)	29 (78.3)	10 (55.5)	5 (83.3)	26 (68.4)	0.807
Active	19 (19)	6 (16.2)	5 (27.7)	1 (16.6)	6 ((15.7)	
Passive	4 (4)	1 (2.7)	1 (5.5)	0	2 (5.2)	
Ex-smoker	7 (7)	1 (2.7)	2 (11.1)	0	4 (10.5)	
BMI						
Normal	34 (34)	11 (29.7)	7 (38.8)	0	16 (42.1)	0.419
Overweight	43 (43)	15 (40.5)	8 (44.4)	4 (66.6)	16 (42.1)	
Obese	17 (17)	8 (21.6)	2 (11.1)	2 (33.3)	5 (13.1)	
Morbid obesity	5 (4)	3 (8.1)	1 (5.5)	0	1 (2.6)	

CAN: Cardiovascular autonomic neuropathy, BMI: Body mass index

Table 2: Distribution of the patients' clinical characteristics and degree of cardiovascular autonomic neuropathy severity

Characteristic	Total, n (%)	Status of CAN				P
		None, n (%)	Possible, n (%)	Definite, n (%)	Advanced, n (%)	
Type of diabetes						
Type 1	14 (14)	2 (5.4)	5 (27.7)	1 (16.6)	6 (15.7)	0.154
Type 2	85 (85)	35 (94.5)	13 (72.2)	5 (83.3)	32 (84.2)	
Duration of diabetes (years)						
<1	15 (15)	9 (24.3)	3 (16.6)	1 (16.6)	2 (5.2)	0.143
1-9	43 (43)	18 (48.6)	7 (38.8)	1 (16.6)	17 (44.7)	
>10	41 (41)	10 (27)	8 (44.4)	4 (66.6)	19 (50)	
Diabetes therapy						
OAD	29 (29)	15 (40.5)	4 (22.2)	2 (33.3)	8 (21)	0.296
Combined	27 (27)	8 (21.6)	7 (38.8)	0	12 (31.5)	
Insulin	43 (43)	14 (37.8)	7 (38.8)	4 (66.6)	18 (47.3)	
Hypertension control						
Controlled	31 (31)	18 (48.6)	5 (27.7)	1 (16.6)	7 (18.4)	0.006
Uncontrolled	68 (68)	19 (51.3)	13 (72.2)	5 (83.3)	31 (81.5)	
Microvascular complications						
Yes	57 (57)	15 (40.5)	11 (61.1)	4 (66.6)	27 (71)	0.007
No	42 (42)	22 (59.4)	7 (38.8)	2 (33.3)	11 (28.9)	
CAN symptoms						
Overt	16 (16)	7 (18.9)	2 (11.1)	1 (16.6)	6 (15.7)	0.907
Silent	83 (83)	30 (81)	16 (88.8)	5 (83.3)	32 (84.2)	
Hypoglycemic unawareness						
Yes	76 (76)	27 (72.9)	11 (61.1)	4 (66.6)	34 (89.4)	0.02
No	23 (23)	10 (27)	7 (38.8)	2 (33.3)	4 (10.5)	

CAN: Cardiac autonomic neuropathy, OAD: Oral antidiabetic drugs

on Ewing's methodology for autonomic function tests [Supplementary Materials 1 and 2]. The existence of one abnormal cardiovagal test result identifies the condition as possible or early CAN. The presence of at least two abnormal results is necessary for a definite or confirmed diagnosis

of CAN. If orthostatic hypotension is present in addition to HR test abnormalities, this identifies severe or advanced CAN.

Statistical analysis

Analysis was performed using the Statistical Package for the Social Sciences program version 16 (SPSS Inc. Released 2007. SPSS for Windows, version 16.0. Chicago, SPSS Inc.). The data are presented as frequency and percentages. Descriptive analysis using cross-tabs, with the application of Chi-square tests, resulted in Asymp. Sig. (two-sided); $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Ninety-nine patients with DM (mean age: 52 ± 1.5 years) were evaluated for CAN. Table 1 highlights the demographics. There were marginally more females (53%), and most were nonsmokers (70%). Type 2 DM was present in 85% of the cases (based on clinical classification), 29% were on oral hypoglycemic agents, 27% of the patients were receiving combined insulin and oral antidiabetic drugs, and 43% were on insulin only. There were 15% newly diagnosed (<1 year), 43% of their DM duration were <9 years, and about 41% were more than 10 years. The duration of diabetes was 12.7 ± 9.5 years. BMI was normal (18.5%–24.5%) in 34% of the cases, 43% were overweight (BMI = 25%–29.5%), 17% were obese (BMI = 30%–39.5%), and 5% with morbid obesity (BMI ≥40%). BMI with mean ± standard deviation was 32.29 ± 7.5. BP was measured for every patient after 15-min rest, uncontrolled (≥140/90) in 68% of the studied cases. Diabetic microvascular complications were present in 54% of the cases [Table 1].

Cardiac autonomic neuropathy – symptoms and stages

Symptom of CAN (orthostatic symptoms, such as light-headedness, dizziness, blurred vision, or fainting) was overt in only 16 cases. Six of them had a severe degree of CAN. Eighty-three percent of the patients were asymptomatic (silent), and 32 of them had an advanced stage of CAN.

CAN could be detected in 62% of the studied cases. They were classified as (1) subclinical phase:

decreased HR variability and early involvement (two borderline test results or one abnormal result on HR test) were possible in 18%, (2) early phase: resting tachycardia and substantial involvement (two or more abnormal results on HR tests) were in 6% (resting HR: 80.58 ± 1.02), and (3) advanced stage: severe involvement (development of orthostatic hypotension) was in 38%.

History of hypoglycemic unawareness was present in 76% of the studied cases, with 34% having an advanced CAN stage. A significant association was evident with hypoglycemic unawareness ($P = 0.02$), uncontrolled BP ($P = 0.006$), dyslipidemia ($P = 0.012$), and microvascular diabetic complications ($P = 0.007$) [Table 2].

Biochemical associations of cardiac autonomic neuropathy

We meant that out of the total number of the patients who had advanced stage of CAN (38 patients) 19 cases (50%) of the advanced CAN patients had an average HbA1c control , whereas 16 cases (42,1%) of the advanced CAN patients had a poor HbA1c control. Fasting lipid profiles were abnormal even with described treatment (no compliance) in 54 cases. There were 22 cases with advanced stage of CAN with abnormal lipid profile, about 45 cases with an average level under treatment, and 16 cases with advanced CAN [Table 3].

DISCUSSION

This pilot study aimed to evaluate cardiovascular autonomic neuropathy (CAN) by assessing associated conditions in diabetic patients attending diabetes and cardiac clinics.

CAN was associated with an increase in sudden death in the ACCORD study in which intensive glycemic control was contemplated.[14] In this study, the coexistence of numbness of the feet indicative of exacerbation of diabetic peripheral neuropathy and fixed HR indicative of CAN provoked the susceptibility to an incident.[15] Similar to the present study, there was a significant association between the presence of both severe CAN and other microvascular complications ($P = 0.04$).

Table 3: Distribution of patients' biochemical laboratory test and degree of cardiac autonomic neuropathy severity according to Ewing's tests

Characteristic	Total, n (%)	Status of CAN				P
		None, n (%)	Possible, n (%)	Definite, n (%)	Advanced, n (%)	
HbA1c level						
Targeted ≤7%	10 (10)	5 (13.5)	2 (11.1)	0	3 (7.8)	0.679
Average 8-9%	49 (49)	20 (54)	7 (38.8)	3 (50)	19 (50)	
Poor >9%	38 (38)	12 (32.4)	7 (38.8)	3 (50)	16 (42.1)	
Missed	2 (2)	0	2 (11.1)	0	0	
Fasting lipids						
Normal	45 (45)	18 (48.6)	5 (27.7)	0	16 (42.1)	0.012
Abnormal on treatment	54 (54)	13 (35.1)	13 (72.2)	6 (100)	22 (57.8)	
Missed	0	6 (16.2)	0	0	0	

CAN: Cardiac autonomic neuropathy, HbA1c: Hemoglobin A1c

Seventy-one percent of the patients have severe CAN and microvascular complications.

Similar results were also found in the EURODIAB study, a large cohort study of T2DM patients with CAN.[17] The presence of retinopathy and albuminuria was associated with severe CAN. EURODIABE demonstrated that over a 7.5-year follow-up, diabetic retinopathy and higher levels of microalbuminuria predicted CAN progression.[17]

Cardiac autonomic dysfunction leads to many clinical complications, such as orthostatic tachycardia, orthostatic bradycardia, and hypotension, and can cause arrhythmias and sudden death.[6] The predictive significance of resting HR is a valuable tool for cardiovascular risk stratification and as a therapeutic target in high-risk patients.[6]

Whereas the loss of HRV is the early finding of CAN, resting tachycardia and fixed HR are typical late features in patients with autonomic dysfunction. Resting HRs of 90–100 b. p. m. and rarely up to 130 b. p. m. arise. The highest resting HRs have been discovered in patients with vagal nerve dysfunction before sympathetic nerve injury; in those with a sign for both vagal and sympathetic participation, the rate resumes near normal but still raised. A fixed HR that is insensitive to moderate exercise, stress, or sleep signifies nearly complete cardiac denervation. A reduced HR reaction to adenosine receptor agonists was shown in metabolic syndrome and diabetes, recognized as earlier stages of CAN.[18]

We meant based on large cohort study there are a strong correlation between elevated resting heart rate

and was associated with an increased risk of dying from IHD and from all causes of death.[19]

We found a weak correlation between symptoms and the degree of CAN ($P = 0.907$) in agreement with a recent study.[20] Patients with orthostatic hypotension classically have light-headedness and presyncopal symptoms. However, many patients stay asymptomatic even with substantial reductions in BP and advanced stage of CAN. Orthostatic symptoms can furthermore be misinterpreted as hypoglycemia and can be provoked by drugs.[16] In a small cohort of patients with T2D, there were comparable associations between loss HRV resulting from 48-h Holter electrocardiography and spontaneous hypoglycemic events detected by continuous monitoring CGM.[6]

On the other hand, the existence of CAN may raise the risk of hypoglycemia through hypoglycemia unawareness and consequent compromise ability to return euglycemia through sympathoadrenal dysfunction or delayed gastric emptying.[18]

It has been suggested that frequent occurrence of hypoglycemia may diminish the counter-regulatory hormones and reduce autonomic nervous system responses to succeeding hypoglycemic episodes.[19]. Further studies described that controlled hypoglycemia caused a progressive decrease in HRV in patients with T1D.[19] A current report in adults with T1D showed that a greater rate of spontaneous nocturnal hypoglycemia was related to a decline in the LF power.[18] Our data similarly propose that CAN may increase the risk of hypoglycemic stress in patients

with DM, even though this cannot be established from the present study due to the cross-sectional nature of this study. We noticed that these relations are independent of glucose control (HbA1c values) based on an insignificant P = (0.679). At the same time, we found that uncontrolled hypertension, dyslipidemia, presence of retinopathy, and history of hypoglycemic unawareness were strongly associated with a severe form of CAN, suggesting an impaired autonomic function, which was independent of glycemic control as assessed by the HbA1c. This study found no significant P value between progressions to the advanced stage of CAN with sex, age, BMI, duration of DM, orthostatic symptoms, smoke stat, and mode of diabetes treatment [Table 1].

This study has some limitations. It is a small and single-center study. Patients were not followed for the long term to deduce outcome data and link with baseline findings. Thus, the prognostic implications of our findings remain to be delineated. Nonetheless, reports on CAN using novel measures for diagnosis and staging are limited, particularly from different parts of the world.

CONCLUSIONS

In this relatively high-risk cardiovascular disease cohort, patients with diabetes, uncontrolled BP, dyslipidemia, microvascular diabetic complication, and hypoglycemic unawareness are at increased risk of severe cardiac autonomic neuropathy. Large prospective studies are warranted to elucidate the complex interplay between hypoglycemia and cardiac autonomic dysfunction.

Patients with DM who are expected to have CAN should be examined for cardiac autonomic neuropathy before an exercise program. Patients with CAN must be depending on their apparent effort, not HR, to avoid dangerous levels of exercise intensity. Silent infarction can postpone suitable therapy. Thus, patients with CAN necessitate extra caution, and cardiovascular autonomic function testing might be an essential element in the risk evaluation of patients with DM and coronary artery disease. Finally, every patient with DM (T1D after 5 years of diagnosis and T2D at presentation) must be examined for possible CAN by a cardiologist.

Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

The Bioethics Committee approved the study at the Biotechnology Center, Tripoli, Libya (Ref Number 25-2021). Verbal consent was obtained from all participants.

REFERENCES

1. Dyck PJ, Albers JW, Andersen H, Arezzo JC, Biessels GJ, Bril V, *et al.* Diabetic polyneuropathies: Update on research definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev 2011;27:620-8.
2. Albers JW, Pop-Busui R. Diabetic neuropathy: Mechanisms, emerging treatments, and subtypes. Curr Neurol Neurosci Rep 2014;14:473.
3. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, *et al.* Diabetic neuropathy: A position statement by the American Diabetes Association. Diabetes Care 2017;40:136-54.
4. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, *et al.* Cardiovascular autonomic neuropathy in diabetes: Clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011;27:639-53.
5. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care. 1985;8:491-8
6. von Känel R, Nelsen RA, Mills PJ, Ziegler MG, Dimsdale JE. Relationship between heart rate variability, interleukin-6, and soluble tissue factor in healthy subjects. Brain Behav Immun 2008;22:461-8.
7. Vinik AI. The conductor of the autonomic orchestra. Front Endocrinol (Lausanne) 2012;3:71.
8. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985;8:491-8.
9. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007;115:387-97.
10. Dinh W, Füth R, Lankisch M, Bansemir L, Nickl W, Scheffold T, *et al.* Cardiovascular autonomic neuropathy contributes to left ventricular diastolic dysfunction in subjects with type 2 diabetes and impaired glucose tolerance undergoing coronary angiography. Diabet Med 2011;28:311-8.
11. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553-79.
12. Astrup AS, Tarnow L, Rossing P, Hansen BV, Hilsted J, Parving HH. Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care 2006;29:334-9.
13. Soedamah-Muthu SS, Chaturvedi N, Witte DR, Stevens LK, Porta M,

Fuller JH, *et al.* Relationship between risk factors and mortality in type 1 diabetic patients in Europe: The EURODIAB Prospective Complications Study (PCS). Diabetes Care 2008;31:1360-6.

14. Pop-Busui R, Evans GW, Gerstein HC, Fonseca V, Fleg JL, Hoogwerf BJ, *et al.* Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. Diabetes Care 2010;33:1578-84.

15. Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: A meta-analysis. Diabetes Care 2003;26:1895-901.

16. Kuehl M, Stevens MJ. Cardiovascular autonomic neuropathies as complications of diabetes mellitus. Nat Rev Endocrinol 2012;8:405-16.

17. Menon AS, Dixit A, Garg MK, Girish R. Cardiac autonomic neuropathy in patients with type 2 diabetes mellitus at high risk for foot ulcers. Indian J Endocrinol Metab 2017;21:282-5.

18. Wakabayashi S, Aso Y. Adiponectin concentrations in sera from patients with type 2 diabetes are negatively associated with sympathovagal balance as evaluated by power spectral analysis of heart rate variation. Diabetes Care 2004;27:2392-7.

19. Nauman J, Janszky I, Vatten LJ, Wisløff U. Temporal changes in resting heart rate and deaths from ischemic heart disease. JAMA 2011;306:2579-87.

20. Low PA, Benrud-Larson LM, Sletten DM, Opfer-Gehrking TL, Weigand SD, O'Brien PC, *et al.* Autonomic symptoms and diabetic neuropathy: A population-based study. Diabetes Care 2004;27:2942-7.

SUPPLEMENTARY MATERIAL

Supplementary Material Appendix 1: Description and definitions of normality and abnormality in the cardiovascular autonomic reflex tests*

Test	Technique	Normal response and values
Beat-to-beat HRV	With the patient at rest and supine, HR is monitored by ECG while the patient breathes in and out at six breaths per minute, paced by a metronome or similar device	A difference in HR of >15 beats per minute is normal, and <10 beats per minute is abnormal. The lowest normal value for the expiration-to inspiration ratio of the R-R interval decreases with age: 20-24 years, 1.17; 25-29, 1.15; 30-34, 1.13; 35-39, 1.12; 40-44, 1.10; 45-49, 1.08; 50-54, 1.07; 55-59, 1.06; 60-64, 1.04; 65-69, 1.03; and 70-75, 1.02
HR response to standing	During continuous ECG monitoring, the R-R interval is measured at beats 15 and 30 after standing	Normally, tachycardia is followed by reflex bradycardia. The 30:15 ratio should be >1.03, borderline 1.01-1.03
HR response to the Valsalva maneuver	The subject forcibly exhales into the mouthpiece of a manometer to 40 mmHg for 15 s during ECG monitoring	Healthy subjects develop tachycardia and peripheral vasoconstriction during strain, intrathoracic overshoot bradycardia, and rise in BP with the release. The normal ratio of longest R-R to shortest R-R is >1.2, borderline 1.11-1.2
Systolic BP response to standing	Systolic BP is measured in the supine subject. The patient stands, and the systolic BP is measured after 2 min	The normal response is a fall of <10 mmHg, borderline fall is a fall of 10-29 mmHg, and abnormal fall is a decrease of >30 mmHg
Diastolic BP response to isometric exercise	The subject squeezes a handgrip dynamometer to establish a maximum. The grip is then squeezed at 30% maximum for 5 min	The normal response for diastolic BP is a rise of >16 mmHg in the other arm, borderline 11-15 mmHg

*Ewing DJ *et al.*[5] HR: Heart rate, HRV: HR variation, ECG: Electrocardiography, BP: Blood pressure

Supplementary Material Appendix 2: Normal, borderline, and abnormal values in tests of cardiovascular autonomic function*

Tests reflecting mainly parasympathetic function	Normal	Borderline	Abnormal
HR response to Valsalva maneuver (Valsalva ratio)	≥1.21	1.11-1.20	≤1.10
HR (R-R interval) variation (beats/min)	≥15	11-14	≤10
During deep breathing (maximum-minimum HR), immediate HR response to standing (30:15 ratio)	≥1.04	1.01-1.03	≤1.00
Tests reflecting mainly sympathetic function			
BP response to standing (fall in systolic BP mmHg)	≤10	11-29	≥30
BP response to sustained handgrip (increase in diastolic BP mmHg)	≥16	11-15	≤10

*Ewing DJ *et al.*[5] BP: Blood pressure, HR: Heart rate