Prevalence of Cardiovascular Autonomic Neuropathy in a Cohort of Patients With Newly Diagnosed Type 2 Diabetes: The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS)

Giacomo Zoppini,[1] Vittorio Cacciatori,[1] Daniele Raimondo,[1] Marialuisa Gemma,[1] Maddalena Trombetta,[1] Marco Dauriz,[1] Corinna Brangani,[1] Isabella Pichiri,[1] Carlo Negri,[1] Vincenzo Stoico,[1] Corinna Bergamini,[2] Giovanni Targher,[1] Lorenza Santi,[1] Karl Thomaseth,[3] Federico Bellavere,[3] Riccardo C. Bonadonna,[1] and Enzo Bonora[1]

Diabetes Care 2015;38:1487–1493 | DOI: 10.2337/dc15-0081

OBJECTIVE

Cardiovascular autonomic diabetic neuropathy (CAN) is a serious complication of diabetes. No reliable data on the prevalence of CAN among patients with newly diagnosed type 2 diabetes are available. Therefore, the aim of this study was to estimate the prevalence of CAN among patients with newly diagnosed type 2 diabetes.

RESEARCH DESIGN AND METHODS

A cohort of 557 patients with newly diagnosed type 2 diabetes with cardiovascular autonomic test results available was selected. Early and confirmed neuropathy were assessed using a standardized methodology and their prevalences determined. A multivariate logistic regression analysis was modeled to study the factors associated with CAN.

RESULTS

In the entire cohort, the prevalence of confirmed CAN was 1.8%, whereas that of early CAN was 15.3%. Prevalence did not differ between men and women. In the multivariate analyses BMI results were independently and significantly associated with CAN after adjusting for age, sex, hemoglobin A_{1c}, pulse pressure, triglyceride-to-HDL cholesterol ratio, kidney function parameters, and antihypertensive treatment.

CONCLUSIONS

CAN could be detected very early in type 2 diabetes. This study may suggest the importance of performing standardized cardiovascular autonomic tests after diagnosis of type 2 diabetes.

Autonomic nervous system dysfunction occurs as a classic complication of diabetes once other causes have been excluded (1). Diabetic autonomic neuropathy is a widespread disorder potentially involving adrenergic, cholinergic, dopaminergic autonomic fibers as well as peptidergic neurons (1). The traditional view of cardiac autonomic neuropathy (CAN) encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics (2). In the natural progression of diabetes, neuropathic complications generally occur after several years of disease. However,

[1]*Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, Azienda Ospedaliera Universitaria Integrata, University of Verona, Verona, Italy*
[2]*Section of Cardiology, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy*
[3]*National Research Council, Institute of Bioengineering, Padua, Italy*

Corresponding author: Giacomo Zoppini, giacomo.zoppini@univr.it.

Received 14 January 2015 and accepted 7 May 2015.

Clinical trial reg. no. NCT01526720, clinicaltrials.gov.

epidemiological evidence showed that a "precocious" involvement in terms of an imbalance of the autonomic system, related principally to an impaired vagal influence and relative sympathetic predominance on the heart, can be present at the time of diagnosis of diabetes mellitus (3). Of particular interest are studies reporting enhanced sympathetic nervous activity as key components associated with poor health outcomes, such as a high risk of cardiac arrhythmia, sometimes culminating in sudden cardiac death (4). The prevalence of confirmed CAN, based on at least two abnormal heart rate test results (5–7) varies from 16.6% to 20% and increases to 65% with age and duration of diabetes (7–9). Despite its prevalence and clinical impact, CAN is still widely underdiagnosed, probably because of the need for training and expertise in the performance of cardiovascular autonomic tests (10). In particular, information on the prevalence of CAN in patients with newly diagnosed type 2 diabetes is scarce and not easy to interpret because of both the different diagnostic approaches in terms of the number and types of tests performed and the differences in the diagnostic cutoff points (11–15). The prevalence of CAN at diagnosis of diabetes is still uncertain (16).

Therefore, the main aim of this study was to estimate the prevalence of CAN by using standardized Ewing tests and validated diagnostic cutoff points in a large cohort of patients with newly diagnosed type 2 diabetes. Moreover, the factors associated with CAN were estimated in multivariate models.

RESEARCH DESIGN AND METHODS

Subjects

The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) is an ongoing study of patients with newly diagnosed type 2 diabetes. The study has been registered as a clinical trial (ClinicalTrials.gov identifier NCT01526720). As of 1 January 2002, all patients referred to the Division of Endocrinology and Metabolic Diseases of the University of Verona School of Medicine whose diabetes was diagnosed in the past 6 months were asked to participate in this research. The clinical evidence on which the diagnosis of type 2 diabetes was made is reviewed and the diagnosis confirmed. Patients are drug-naïve or, if already treated with antidiabetic drugs, undergo a treatment washout of at least 1 week before tests are performed. Among the exclusion criteria are age >75 years, non-Italian ancestry, insulin treatment, and presence of anti-GAD antibodies, malignancies, and any condition severely impairing liver and/or kidney function. In this observational cross-sectional analysis we report the data collected from 557 patients, all of whom have cardiovascular autonomic tests available. The cohort comprised 68% of all subjects evaluated (n = 813). Seventy-three subjects did not have complications evaluated and 183 refused to be tested for CAN. Patients included in the study have similar characteristics as those not included in the analyses, as shown in Table 1.

Subjects under study consumed a weight-maintaining diet containing 200–250 g carbohydrate/day for at least 3 days before studies. Body weight of all subjects was stable for at least 1 month before studies. No subject participated in any heavy exercise. Each subject gave informed written consent before participating in the research, which was approved by the Human Investigation Committee of the Verona City Hospital. Standard clinical phenotypes were measured in all patients.

Clinical and Laboratory Data

BMI was calculated by dividing weight in kilograms by the square of height in meters. Blood pressure was measured with a standard mercury manometer. Venous blood was drawn in the morning after an overnight fast in all patients. Serum creatinine (measured using a Jaffé rate-blanked and compensated assay), lipids, calcium, and other biochemical blood measurements were determined by standard laboratory procedures (DAX 96; Bayer Diagnostics, Milan, Italy). Hemoglobin A_{1c} (HbA_{1c}) was measured according to the standard operating procedure of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Reference using an automated high-performance liquid chromatography analyzer (Bio-Rad Diamat, Milan, Italy); the upper limit of normal for our laboratory was 5.8% (40 mmol/mol). Patients were considered to have arterial hypertension if their blood pressure values were >140/90 mmHg or they were taking any antihypertensive drugs. Glomerular filtration rate (GFR) was estimated from the four-variable Modification of Diet in Renal Disease (MDRD) study equation (17). The urinary albumin excretion rate was measured from a 24-h urine sample by an immunonephelometric method. The presence of albuminuria (defined as urinary excretion of >30 mg albumin/day) was confirmed by at least two of three consecutive samples. Nephropathy was defined as albuminuria and/or estimated GFR_{MDRD} <60 mL/min/1.73 m^2. Presence of diabetic retinopathy was diagnosed with indirect ophthalmoscopy after pupillary dilation by a single ophthalmologist (S. Casati, Azienda Ospedaliera Universitaria Integrata Verona).

Cardiovascular Autonomic Tests

Cardiovascular autonomic function was assessed by a computerized system, as previously described (18), following the criteria presented by Ewing and Clarke (5). Quantitative studies of autonomic function are also well described elsewhere (10). Patients should be requested to avoid strenuous physical exercise in the 24 h preceding the cardiovascular tests. We recommended not consuming beverages containing caffeine, as well as not smoking or drinking alcohol, at least 2 h before the tests. The three heart rate tests were performed while the patient was fasting or at least 2 h after a light meal. A personal computer collected, stored, and processed R-R intervals and blood pressure (systolic, diastolic, and mean) and analyzed heart rate and blood pressure variations during lying to standing (LS), deep breathing (DB), and Valsalva maneuver (VM). The diagnostic definition of CAN based on this battery of tests allows an indication of the severity of the neuropathy, showing independent parasympathetic and sympathetic functions and reducing the probability of false positives.

No agreement exists on the number of abnormal cardiovascular tests required to reach a diagnosis of CAN (7). In the light of the evidence available, experts have proposed that at least two abnormal heart rate tests (below the 5th percentile) are required for the diagnosis of CAN (confirmed autonomic neuropathy). Only one abnormal test or two borderline tests (between the 5th and 10th percentiles) identifies the condition of "early or uncertain" CAN. The severe form of autonomic neuropathy is diagnosed when confirmed CAN is associated with orthostatic hypotension. DB, LS, and VM are considered indexes of mainly "cardiovagal" function, with similar high sensitivity and specificity, whereas the

Table 1—Clinical characteristics of the 557 subjects with newly diagnosed type 2 diabetes compared with subjects with no cardiovascular autonomic tests (CATs), stratified by the absence or presence of early and confirmed CAN

				Subjects by CAN category			
	Subjects with no CAT (n = 256)	Subjects with CAT (n = 557)	P value*	Absent (n = 461)	Early (n = 86)	Confirmed (n = 10)	P value*
Age (years)	58.1 ± 10.3	58.3 ± 10	0.78	57.8 ± 9.8	58 ± 9.6	56.7 ± 6.6	0.92
Weight (kg)	84.4 ± 18	82.7 ± 16	0.17	81.9 ± 15.3	87.2 ± 17.8	97.25 ± 29.1	0.001
BMI (kg/m^2)	30.3 ± 5.8	29.8 ± 5.2	0.31	29.54 ± 5.0	31.4 ± 5.2	33.7 ± 7.9	0.001
Systolic blood pressure (mmHg)	135.3 ± 18.6	135.8 ± 16.7	0.73	136 ± 16.5	136.9 ± 17.3	134.2 ± 13.7	0.85
Diastolic blood pressure (mmHg)	83.2 ± 10.1	83.7 ± 9	0.52	83.8 ± 8.5	84.2 ± 10.6	84 ± 11.6	0.93
Pulse pressure (mmHg)	52.1 ± 13.7	52.1 ± 13.6	0.99	52.1 ± 15.5	52.7 ± 13.7	48.8 ± 17.2	0.70
Fasting glycemia (mmol/L)	7.4 ± 1.8	7.2 ± 1.7	0.25	7.15 ± 1.6	7.58 ± 2.1	6.91 ± 1.0	0.87
HbA_{1c} (%)	7.0 ± 1.5	6.9 ± 1.2	0.06	6.8 ± 1.1	7 ± 1.1	6.9 ± 1.3	0.39
HbA_{1c} (mmol/mol)	53.0 ± 16.2	51.9 ± 12.3	0.06	50.8 ± 12.2	53.0 ± 12.3	51.9 ± 14.6	0.39
LDL cholesterol (mmol/L)	3.0 ± 0.9	3.0 ± 0.9	0.89	3.02 ± 0.9	3.1 ± 0.9	2.92 ± 0.6	0.19
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.2 ± 0.3	0.68	1.18 ± 0.3	1.14 ± 0.3	1.32 ± 0.7	0.20
TGs (mmol/L)	1.7 ± 1.1	1.6 ± 0.9	0.12	1.62 ± 0.9	1.6 ± 1.0	1.32 ± 0.6	0.93
TG-to-HDL cholesterol ratio	3.8 ± 3.3	3.5 ± 2.7	0.19	3.5 ± 2.6	3.7 ± 3.3	2.6 ± 1.6	0.501
Estimated GFR_{MDRD} (mL/min/1.73 m^2)	83.6 ± 21.2	84.9 ± 21.3	0.42	84.4 ± 21.3	86.9 ± 22.0	88.9 ± 18.5	0.52
Statins	21.1	19.6	0.627	18.8	22.4	30.0	0.528
Antihypertensive drugs	57.2	53.9	0.09	50.4	68.2	90.0	0.001
Central sympatholytics				6.7	9.4	10.0	0.63
Diuretics				10.6	18.8	0.0	0.05
β-Blockers				21.9	38.8	40.0	0.002
Calcium antagonists				17.3	24.7	10.0	0.21
ACE inhibitors				29.9	24.7	40.0	0.47
Angiotensin II antagonists				8.4	24.7	10.0	0.001
Antiplatelets drugs	21.5	14.8	0.02	13.3	22.4	20.0	0.080
Background retinopathy	3.2	4.2	0.76	3.9	5.3	10.0	0.249
Preproliferative retinopathy	0.9	0.6	0.76	0.2	2.6	0.0	0.249
Microalbuminuria	15.1	11.0	0.14	10.0	17.6	0.0	0.260
Macroalbuminuria	1.8	0.8	0.14	0.7	1.4	0.0	0.260
Cardiac frequency (bpm)				66.3 ± 10.4	65.9 ± 10.1	67.6 ± 10.5	0.889
LS				1.19 ± 0.14	1.12 ± 0.12	1.01 ± 0.17	<0.001
DB				20.8 ± 8.4	12.2 ± 7.1	11.2 ± 4.8	<0.001
VM				1.56 ± 0.49	1.27 ± 0.19	1.20 ± 0.08	<0.001
Postural hypotension (mmHg)				−5.4 ± 12.7	−5.5 ± 13.4	−2.3 ± 20.3	0.767

Data are mean ± SD or percentages, unless otherwise specified. *Significance was tested using a t test or χ^2 test. TG, triglyceride.

orthostatic hypotension test is considered a test of sympathetic function (19). None of the three heart rate tests is clearly superior to the other two in terms of diagnostic characteristics. However, although the three heart rate tests explore mainly parasympathetic function, the nervous pathways and reflex mechanisms involved are not identical; the sympathetic activity and baroreflex have a contributory role in the orthostatic test and VM (10,18,20). Hence, experts generally consider a hierarchy of levels of sensitivity in these measures. For example, an abnormality in the heart rate variability alone, as shown in DB, may be the earliest stage; an abnormality in the Valsalva response may define an intermediate stage; and the presence of postural hypotension may define a more severe stage (21).

In this study, following the recommendations for the diagnosis of CAN, we adopted the following criteria: *1*) early CAN, the presence of one abnormal or two borderline heart rate tests; *2*) confirmed CAN, the presence of two or more abnormal tests; and *3*) severe CAN, orthostatic hypotension is also present in addition to two or more abnormal tests (6).

Reproducibility of the data available in the literature provides coefficients of variation generally lower than 10% for DB and LS and slightly higher (10–15%) for VM (22). Moreover, a progressive reduction in autonomic reactivity with age has been described, and test abnormalities were defined using age-based values. The correction by age is based on data obtained by our center. Ten normal subjects for each of the following classes of age were studied: 20–29, 30–39, 40–49, 50–59, 60–69 and 70–79 years; cardiovascular autonomic tests were used to construct the age-specific reference values (23). The results are expressed as the average of three successive measurements.

Statistical Analysis

Data are presented as means ± SDs or proportions. Skewed variables were logarithmically transformed to improve normality before analysis. The one-way

ANOVA and the χ^2 test with Yates correction for continuity were used to analyze the differences among the clinical and biochemical characteristics of participants stratified by status of CAN (absent, early, and confirmed). Three forced-entry multivariate logistic regression analysis were performed to assess the factors independently associated with the presence of CAN (dependent variable). The dependent variable was included as a composite end point: CAN absent (coded as 0) and early and confirmed CAN combined (coded as 1). Covariates were chosen as potential confounding factors on the basis of their significance in univariate analysis or on the basis of their biological plausibility. Results are presented as odds ratios (ORs) with 95% CIs. Statistical analysis was performed using SPSS 19.0 statistical package software. *P* values <0.05 were considered statistically significant.

RESULTS

Among the whole cohort, the average values of LS, DB, and VR were 1.18 ± 0.14, 19.4 ± 8.9, and 1.51 ± 0.47, respectively. Only two patients were affected by orthostatic hypotension, but none had severe autonomic neuropathy. When considering at least two pathologic tests to define autonomic neuropathy (defined as confirmed CAN), the prevalence was 1.8%. When considering at least two borderline tests and one pathologic test to define early CAN, the prevalence of CAN increased to 15.3%. Table 2 shows the prevalence of CAN divided by sex; even though men tended to have a higher frequency of CAN, the differences were not statistically significant. Table 1 summarizes the main clinical characteristics of the 557 subjects with newly diagnosed type 2 diabetes stratified by levels of CAN. Age was similar in the three groups, whereas BMI was significantly higher in subjects with confirmed CAN. Glycated hemoglobin, blood pressure and pulse pressure, lipids, and estimated GFR_{MDRD} were not significantly different in the three groups. Also, the frequency of microvascular complications did not differ among groups. Subjects with confirmed CAN were more frequently receiving treatment for hypertension. The following classes of antihypertensive drugs were used by patients: central sympatholytics (7.6%), diuretics (12.4%), β-blockers (27.1%), calcium antagonists (19.8%), ACE inhibitors (30.8%), and angiotensin II antagonists (10.9%); no patients were using α-antagonists. Only the use of β-blockers and angiotensin II antagonists were significantly different among the classes of CAN, as reported in Table 1. Figure 1 shows the prevalence of each single cardiovascular autonomic test (DB, LS, VM, and orthostatic hypotension). The least frequently observed alterations were orthostatic hypotension and LS, respectively.

To identify possible predictors associated with the presence of CAN, we modeled different multivariate logistic regression models. We found that the independent and significant predictors of the presence of CAN were BMI, with an OR of 1.07 (95% CI 1.02–1.12; *P* = 0.003), and the treatment for hypertension (yes/no), with an OR of 2.12 (95% CI 1.25–3.60; *P* = 0.005) in the fully adjusted regression model (Table 3). When the categories of physical activity (available for 449 patients) were introduced in the logistic multivariate model, the results did not change. Physical activity was not a significant predictor, whereas both BMI and antihypertensive treatment maintained their significance, with ORs of 1.06 (95% CI 1.01–1.11; *P* = 0.01) and 2.42 (95% CI 1.39–4.21; *P* = 0.002), respectively.

Among 403 subjects whose smoking habit and alcohol consumption data were available, we evaluated the impact of these variables. BMI maintained its significance (adjusted OR 1.06 [95% CI 1.01–1.12]; *P* = 0.02) and neither smoking nor alcohol consumption were significant predictors of CAN.

CONCLUSIONS

This study is the largest study carried out to estimate the prevalence of CAN in patients with newly diagnosed type 2 diabetes. It suggests that very early in the course of type 2 diabetes—often just at diagnosis—an imbalance between the cardiac and vasomotor nervous systems is present in some patients. This imbalance is characterized by precocious vagal involvement, with consequent relative release of adrenergic tone. This evidence is in keeping with previous reports, suggesting that subtle autonomic function abnormalities may begin before diabetes and even before insulin resistance is displayed during the initiation of metabolic syndrome (24). Other evidence showed that autonomic impairment across different glycemic statuses is not apparent initially, for example, in impaired fasting glucose but appreciable only in impaired glucose tolerance (25).

The three rate tests (DB, LS, and VM) mainly explore parasympathetic functions, but the reflex mechanisms involved are not identical. Therefore, the diagnostic definition of CAN based on several tests allows an indication of the severity of this important diabetes complication (6,7,23).

Previous studies examining the prevalence of autonomic neuropathy in patients with newly diagnosed type 2 diabetes were mainly carried out using either very small samples of patients or different tests, thus leading to large differences in estimates. A case–control study (41 patients with newly diagnosed type 2 diabetes and 49 controls) reported significantly reduced vasoconstriction responses in patients with diabetes compared with controls (11). In 95 patients with newly diagnosed type 2 diabetes with a mean age of 49.7 years, the prevalence of three abnormal tests ranged between 2.1% and 7.3% (12). Another study of 132 patients with newly diagnosed type 2 diabetes (45–64 years old) reported a prevalence of autonomic neuropathy of 9.2% in men and 3.3% in women (13). In 145 patients with recently detected type 2 diabetes, combined parasympathetic dysfunction (abnormal and borderline) was found in 44.2% of patients, whereas combined sympathetic dysfunction occurred in 51.9% (14). In 39 patients with newly diagnosed type 2 diabetes, DB was abnormal in 28% of patients, and 6% had postural hypotension (15). Considering all these disparities in estimates of autonomic dysfunction as a result of small samples, differences in tests applied, and differences in the methodology of the interpretation of tests results, our study adds important information; we evaluated a very large

Table 2—Prevalence of confirmed and early CAN among 557 subjects with newly diagnosed type 2 diabetes, divided by sex

	Absent	Early	Confirmed
Men	305 (82.2%)	59 (15.9%)	7 (1.95%)
Women	156 (83.9%)	27 (14.5%)	3 (1.6%)

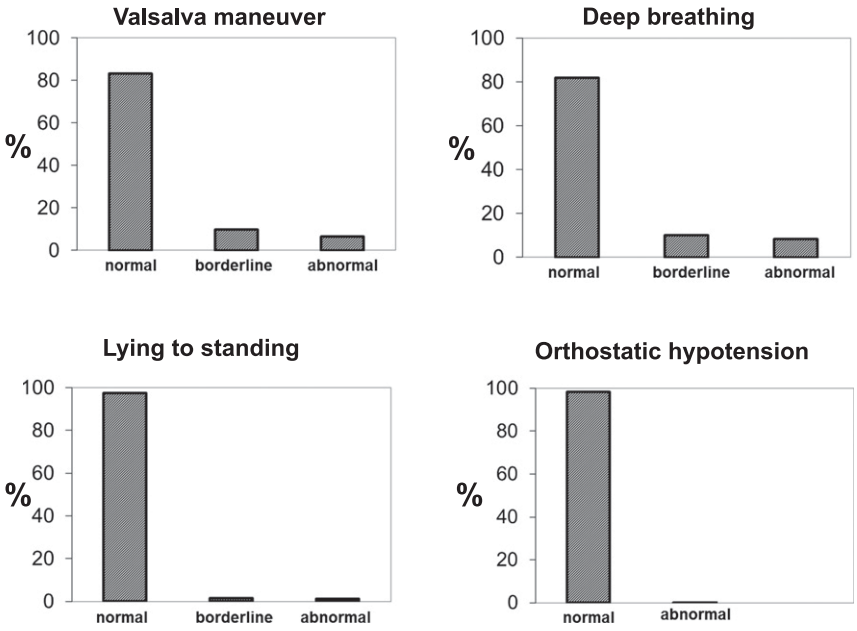

Figure 1—Percentages of normal, borderline, and abnormal results for VM, deep breathing, lying to standing, and orthostatic hypotension.

sample of patients with newly diagnosed type 2 diabetes and used a standardized methodology to assess CAN. Moreover, our findings may have clinical implications suggesting that CAN should be evaluated as soon as possible after the diagnosis of type 2 diabetes.

We found that BMI was the only significant factor associated with CAN. Altered autonomic function is a well-known finding in obesity (26,27), but the mechanisms by which excess weight favors autonomic dysfunction are still unclear. Alterations of several intertwined functional elements, such as inflammation (28), impaired endothelial function (29), dysfunctional leptin (30), and ghrelin regulation (31), might be involved, leading to an increase in sympathetic activity, a reduction of cardiac baroreflex sensitivity, and facilitating an increase in arterial pressure (32). Hypertension has been found to be a predictor of CAN in type 2 diabetes in cross-sectional studies (33–36), whereas the use of antihypertensive drugs was associated with autonomic indexes in the Hoorn Study (34). Consistent with this evidence, our patients with CAN were more frequently treated for hypertension. Both β-blockers and angiotensin II antagonists were proposed among the treatment options for autonomic dysfunction because they influence the sympathovagal balance by decreasing the function of the sympathetic nervous system (37). Therefore, the overall effect of these drugs could lead to a likely underestimation of the prevalence of CAN as a consequence of the release of parasympathetic tone. BMI maintained its statistical significance, however, even with antihypertensive treatment in the model.

We did not observe an association of CAN with either retinopathy or nephropathy, probably because of the small numbers of subjects affected by these complications.

Cardiovascular autonomic dysfunction, as observed in this study, can be detected very early in the natural history of type 2 diabetes, sometimes just after hyperglycemia is discovered. Hence, although the Standards of Medical Care in Diabetes 2015 (38) do not yet recommend the evaluation of CAN in patients with newly diagnosed type 2 diabetes, in agreement with our results, showing that the diagnosis of an early cardioautonomic involvement is not infrequent, we suggest performing cardiovascular autonomic tests as soon as possible for the following reasons: *1*) CAN was found to be highly prevalent 6 years after a screening-based diagnosis of type 2 diabetes (39); *2*) the Steno-2 Trial showed that in patients with longstanding type 2 diabetes intensive multifactorial intervention can reduce the risk of developing CAN by 68% (40); and, finally, *3*) Vinik and Murray (37) suggested treating autonomic dysfunction earlier because it is easier to correct during early stages since there are a greater number of therapeutic options, compared with advanced-stage autonomic neuropathic damage (18,37). Clinicians should be aware of the possibility of autonomic dysfunction soon after diagnosis in type 2 diabetes, and, consequently, researchers should intensify efforts to examine whether specific treatments to ameliorate CAN at an early stage are beneficial in the long term. If that is proven to be the case, then early screening should be considered.

The strengths of our study are the large cohort, the standardized methodology in assessing cardiovascular autonomic

Table 3—ORs (95% CIs) of predictors of CAN in subjects with newly diagnosed type 2 diabetes (*n* = 557)

	Model 1	*P* value	Model 2	*P* value	Model 3	*P* value
Age (years)	1.00 (0.98–1.02)	0.957	1.01 (0.98–1.03)	0.643	1.01 (0.98–1.03)	0.731
Male sex	1.12 (0.69–1.81)	0.643	1.25 (0.76–2.07)	0.385	1.34 (0.79–2.30)	0.278
BMI (kg/m^2)			1.08 (1.04–1.13)	<0.001	1.07 (1.02–1.12)	0.003
HbA_{1c} (% [mmol/mol])			1.11 (0.91–1.35)	0.297	1.14 (0.92–1.40)	0.232
TG-to-HDL cholesterol ratio					0.98 (0.89–1.08)	0.761
Pulse pressure (mmHg)					0.99 (0.98–1.02)	0.663
$eGFR_{MDRD}$ (mL/min/1.73 m^2)					1.01 (0.99–1.02)	0.233
Antihypertensive drugs (yes/no)					2.12 (1.25–3.60)	0.005

TG, triglyceride.

neuropathy, and the completeness of the database. The study has some limitations. The cross-sectional design of this study, using standard cardiovascular reflex tests in patients with newly diagnosed type 2 diabetes (and not more sensitive autonomic measures such as baroreflex sensitivity or spectral analysis of heart rate variability) does not allow us to draw conclusions on the natural course of autonomic control of the cardiovascular system in type 2 diabetes. The cohort comprised patients referred to the Division of Endocrinology and Metabolic Diseases at the University of Verona School of Medicine; therefore, the results of this study cannot be generalized to other diabetic populations. Diabetic polyneuropathy was not assessed.

In conclusion, cardiovascular autonomic dysfunction can be detected very early in the natural history of type 2 diabetes, just after hyperglycemia is discovered. We therefore consider it appropriate to perform cardiovascular tests in order to identify, and eventually to treat with physical activity or common medications, the sympathovagal imbalance.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. G.Z. and V.C. wrote the manuscript and analyzed data. D.R., M.G., C.N., and V.S. researched data. M.T., M.D., C.Br., I.P., C.Be., and F.B. contributed to the discussion and reviewed the manuscript. G.T., R.C.B., and E.B. reviewed and edited the manuscript. L.S. analyzed data. K.T. reviewed the manuscript. G.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Kahn R. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Clinical measures. Diabetes Care 1992;15:1081–1083
2. Vinik AI, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. J Diabetes Investig 2013; 4:4–18
3. Singh JP, Larson MG, O'Donnell CJ, et al. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). Am J Cardiol 2000;86:309–312
4. Young LH, Wackers FJ, Chyun DA, et al.; DIAD Investigators. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled study. JAMA 2009; 301:1547–1555
5. Ewing DJ, Clarke BF. Autonomic neuropathy: its diagnosis and prognosis. Clin Endocrinol Metab 1986;15:855–888
6. Ewing DJ, Marryn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985;8:491–498
7. Spallone V, Ziegler D, Freeman R, et al.; Toronto Consensus Panel on Diabetic Neuropathy. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011;27: 639–653
8. Ziegler D, Gries FA, Mühlen H, Rathmann W, Spüler M, Lessmann F; The Diacan Multicenter Study Group. Prevalence and clinical correlates of cardiovascular autonomic and peripheral diabetic neuropathy in patients attending diabetes centers. Diabetes Metab 1993;19: 143–151
9. Valensi P, Pariès J, Attali JR; French Group for Research and Study of Diabetic Neuropathy. Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and microangiopathic complications—the French multicenter study. Metabolism 2003; 52:815–820
10. Spallone V, Bellavere F, Scionti L, et al.; Diabetic Neuropathy Study Group of the Italian Society of Diabetology. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. Nutr Metab Cardiovasc Dis 2011;21:69–78
11. McDaid EA, Monaghan B, Parker AI, Hayes JR, Allen JA. Peripheral autonomic impairment in patients newly diagnosed with type II diabetes. Diabetes Care 1994;17:1422–1427
12. Ratzmann KP, Raschke M, Gander I, Schimke E. Prevalence of peripheral and autonomic neuropathy in newly diagnosed type II (noninsulin-dependent) diabetes. J Diabet Complications 1991;5:1–5
13. Lehtinen JM, Uusitupa M, Siitonen O, Pyörälä K. Prevalence of neuropathy in newly diagnosed NIDDM and nondiabetic control subjects. Diabetes 1989;38:1307–1313
14. Jyotsna VP, Sahoo A, Sreenivas V, Deepak KK. Prevalence and pattern of cardiac autonomic dysfunction in newly detected type 2 diabetes mellitus. Diabetes Res Clin Pract 2009; 83:83–88
15. Rota E, Quadri R, Fanti E, et al. Clinical and electrophysiological correlations in type 2 diabetes mellitus at diagnosis. Diabetes Res Clin Pract 2007;76:152–154
16. Charles M, Ejskjaer N, Witte DR, Borch-Johnsen K, Lauritzen T, Sandbaek A. Prevalence of neuropathy and peripheral arterial disease and the impact of treatment in people with screen-detected type 2 diabetes: the ADDITION-Denmark study. Diabetes Care 2011;34:2244–2249
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–470
18. Zoppini G, Cacciatori V, Gemma ML, et al. Effect of moderate aerobic exercise on sympatho-vagal balance in Type 2 diabetic patients. Diabet Med 2007;24:370–376
19. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology. Neurology 1998;46:1470
20. Assessment: clinical autonomic testing report of the Therapeutic and Technology Assessment Subcommittee of the America Academy of Neurology. Neurology 1996;46:873–880
21. American Diabetes Association American Academy of Neurology. Consensus statement: Report and recommendations of the San Antonio conference on diabetic neuropathy. Diabetes Care 1988;11:592–597
22. Smith SA. Reduced sinus arrhythmia in diabetic autonomic neuropathy: diagnostic value of an age-related normal range. Br Med J (Clin Res Ed) 1982;285:1599–1601
23. Balzani I, De Masi G, Carraro M, Carenza P, Thomaseth K, Bellavere F. Valori di normalità dei test cardiovascolari per la diagnosi di neuropatia autonomica diabetica ottenuti con acquisizione computerizzata ed analisi automatica della variazioni di frequenza cardiaca. Atti del XIII Congresso Nazionale SID. Diabete 1991;3 (Suppl. 2):787–789
24. Chang CJ, Yang YC, Lu FH, et al. Altered cardiac autonomic function may precede insulin resistance in metabolic syndrome. Am J Med 2010;123:432–438
25. Wu JS, Yang YC, Lin TS, et al. Epidemiological evidence of altered cardiac autonomic function in subjects with impaired glucose tolerance but not isolated impaired fasting glucose. J Clin Endocrinol Metab 2007;92:3885–3889
26. Bergström B, Lilja B, Osterlin S, Sundkvist G. Autonomic neuropathy in non-insulin dependent (type II) diabetes mellitus. Possible influence of obesity. J Intern Med 1990;227:57–63
27. Masuo K, Mikami H, Itoh M, Ogihara T, Tuck ML. Sympathetic activity and body mass index contribute to blood pressure levels. Hypertens Res 2000;23:303–310
28. Tracey KJ. The inflammatory reflex. Nature 2002;420:853–859
29. Belin de Chantemele EJ, Stepp DW. Influence of obesity and metabolic dysfunction on the endothelial control in the coronary circulation. J Mol Cell Cardiol 2012;52:840–847
30. Belin de Chantemèle EJ, Mintz JD, Rainey WE, Stepp DW. Impact of leptin-mediated sympatho-activation on cardiovascular function in obese mice. Hypertension 2011;58: 271–279
31. Lambert E, Lambert G, Ika-Sari C, et al. Ghrelin modulates sympathetic nervous system activity and stress response in lean and overweight men. Hypertension 2011;58:43–50
32. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 1998;31: 68–72
33. Voulgari C, Psallas M, Kokkinos A, Argiana V, Katsilambros N, Tentolouris N. The association between cardiac autonomic neuropathy with metabolic and other factors in subjects with type 1 and type 2 diabetes. J Diabetes Complications 2011;25:159–167
34. Ayad F, Belhadj M, Pariés J, Attali JR, Valensi P. Association between cardiac autonomic

neuropathy and hypertension and its potential influence on diabetic complications. Diabet Med 2010;27:804–811
35. Gerritsen J, Dekker JM, TenVoorde BJ, et al. Glucose tolerance and other determinants of cardiovascular autonomic function: the Hoorn Study. Diabetologia 2000;43:561–570
36. Spallone V, Maiello MR, Cicconetti E, Menzinger G. Autonomic neuropathy and cardiovascular risk factors in insulin-dependent and non insulin-dependent diabetes. Diabetes Res Clin Pract 1997;34:169–179
37. Vinik AI, Murray GL. Autonomic neuropathy is treatable. US Endocrinol 2008;4:82–84
38. American Diabetes Association. Standards of medical care in diabetes—2015: summary of revisions. Diabetes Care 2015;38 (Suppl. 1):S4
39. Charles M, Fleischer J, Witte DR, et al. Impact of early detection and treatment of diabetes on the 6-year prevalence of cardiac autonomic neuropathy in people with screen-detected diabetes: ADDITION-Denmark, a cluster-randomised study. Diabetologia 2013;56: 101–108
40. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 2003;348: 383–393