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RESEARCH REPORT

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Cardiovascular autonomic neuropathy in patients with type 2 diabetes with and without sensorimotor polyneuropathy

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Abstract

Background and Aims: Cardiovascular autonomic neuropathy (CAN) in patients with diabetes is associated with poor prognosis. We aimed to assess signs of CAN and autonomic symptoms and to investigate the impact of sensorimotor neuropathy on CAN by examining type 2 diabetes patients with (DPN [distal sensorimotor polyneuropathy]) and without distal sensorimotor polyneuropathy (noDPN) and healthy controls (HC). Secondarily, we aimed to describe the characteristics of patients with CAN.

Methods: A population of 374 subjects from a previously described cohort of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) were included. Subjects were examined with the Vagus™ device for the diagnosis of CAN, where two or more abnormal cardiovascular autonomic reflex tests indicate definite CAN. Autonomic symptoms were assessed with Composite Autonomic Symptom Score 31 (COMPASS 31) questionnaire. DPN was defined according to the Toronto consensus panel definition.

Results: Definite CAN was present in 22% with DPN, 7% without DPN and 3% of HC, and 91% of patients with definite CAN had DPN. Patients with DPN and definite CAN reported higher COMPASS 31 scores compared to patients with noDPN (20.0 vs. 8.3, $p < 0.001$) and no CAN (22.1 vs. 12.3, $p = 0.01$). CAN was associated with HbA1c and age in a multivariate logistic regression analysis but was not associated with IEFND or triglycerides.

Interpretation: One in five patients with DPN have CAN and specific CAN characteristics may help identify patients at risk for developing this severe diabetic complication. Autonomic symptoms were strongly associated with having both DPN and CAN, but too unspecific for diagnosing CAN.

KEYWORDS

cardiovascular autonomic neuropathy, COMPASS-31, diabetic autonomic neuropathy, diabetic peripheral neuropathy, small fiber neuropathy

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1 | INTRODUCTION

Diabetes affects an increasingly large number of people, with an estimated number of 425 million patients suffering from the disease worldwide. One of the most common long-term complications of diabetes is neuropathy, where distal sensorimotor polyneuropathy (DPN) is the most common variant.[1] The symptoms of DPN are either negative somatosensory with reduced sensation to different sensory modalities or positive symptoms with pain and tingling in the lower limbs or a combination thereof.[2] The peripheral nerve damage can also involve the autonomic nervous system. Patients may suffer from length dependent autonomic neuropathy with reduced sweat, color, and skin temperature changes in a glove and sock distribution or a more generalized autonomic neuropathy with multi organ involvement. The generalized autonomic neuropathy can result in cardiovascular, urogenital, gastrointestinal, pupillomotor, and thermoregulatory dysfunctions.[2–4] Patients suffering from cardiovascular autonomic neuropathy (CAN) may be asymptomatic but can experience severe and disabling symptoms with, for example, orthostatic intolerance or syncope. Patients with CAN have increased mortality and risk of cardiovascular and renal complications.[5–7] Common clinical factors linked to CAN are higher age, diabetes duration, triglycerides, HbA1c, BMI, and presence of retinopathy and nephropathy.[6] Prevalence estimates of CAN in patients with diabetes vary between 20% and 73% in patients with type 2 diabetes and 1%–90% in type 1 diabetes.[8] These studies differ both regarding methods used for diagnosing CAN and in included patient characteristics (e.g., duration of diabetes, age, gender distribution). The diagnostic methodology for the assessment of CAN as stated by The CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy includes signs, cardiovascular autonomic reflex tests (CARTs) and blood pressure monitoring.[6] The gold standard tests are CARTs, measuring heart rate, and blood pressure responses to provocative physiological maneuvers, including deep breathing, Valsalva maneuver, standing and tilt table test.[9] These methods are usually time-consuming and only performed at specialized centers. A simple tool for the diagnosis of CAN is the handheld Vagus™ device combined with a modified Schellong test. The modified Schellong test includes blood pressure and heart rate measured during rest and during 3 min of passive standing. Vagus™ includes three CARTs corresponding to the gold standard tests described by the Toronto Consensus panel. The device is easy to implement and use in a clinical setting[10] and shares many similarities with the gold standard tests regarding examination of the cardiovagal domain of the autonomic nervous system. The device has compared favorably with a traditional stationary device in a small cohort and shown moderate to high reproducibility.[11,12]

The relationship between CAN and DPN is not clear. Prevalence studies are not all supportive of a clear association between DPN and CAN. Some studies have found that CAN is more common in patients with DPN than noDPN in type 1 and type 2 diabetes[13–16] while others have not.[17] None of these studies have used the current definitions of DPN as proposed by the Toronto Diabetic Neuropathy Expert Group.[14] Small nerve fiber damage may affect both peripheral and autonomic small nerve fibers, potentially leading to autonomic dysfunction.[15] Previous studies have investigated small fiber dysfunction in CAN, using simple bedside tools,[15] quantitative sensory testing (QST),[18,19] and corneal confocal microscopy (CCM).[20] While these studies suggest that small fiber dysfunction is characteristic of CAN the association between intra epidermal nerve fiber density (IENFD) and CAN has not been assessed. Assessment of autonomic symptoms has been suggested as a valid approach to diagnose CAN.[6] The first validation study of the COMPASS questionnaire for the diagnosis of CAN found that COMPASS-31 scores were higher both in patients with CAN and DPN, and suggested that COMPASS 31 is an easy and reliable assessment tool for autonomic symptoms of diabetic neuropathy and can be used as a screening tool for CAN.[21] When studying CAN and DPN, there is a lack of systematic appraisal of autonomic symptoms[14,22] and many studies do not assess autonomic symptoms at all.[13,15,17,23,24]

We aimed to assess signs of CAN and autonomic symptoms among patients with recently diagnosed type 2 diabetes and well-characterized DPN according to the Toronto classification in order to investigate the impact of distal sensorimotor neuropathy on CAN. We hypothesize that these two conditions have a close relationship. Secondarily, we aimed to describe the characteristics of patients who had both CAN and DPN including the association with small fiber dysfunction.

2 | MATERIALS AND METHODS

2.1 | Study population and study design

The study population in this study was part of a previous cross-sectional clinical study of 389 Danish type 2 diabetes patients and 97 healthy controls without diabetes conducted in 2016–2018[25] (Figure A1). These patients had participated in a large questionnaire study assessing neuropathy and neuropathic pain and were invited to participate in the clinical study. We included patients with noDPN, probable and definite DPN and all healthy controls. Subjects underwent neurological examination to establish a diagnosis of DPN. This included a detailed evaluation of symptoms and signs of neuropathy, nerve conduction studies (NCS), quantification of IENFD, quantification of corneal nerve fiber length (CNFL), fiber density (CNFD), branch density (CNBD), QST with quantification of cold detection (CDT), and warm detection (WDT) thresholds and evaluation of CAN using the Vagus™ device. The study population is described in detail previously.[25,26]

2.2 | Definition of diabetic polyneuropathy

DPN was defined according to the Toronto Diabetic Neuropathy Expert Group.[27] This classification system is divided by increasing diagnostic certainty into possible, probable, and definite DPN. Patients in this study had either probable DPN, defined as having at least two of either sensory symptoms, signs, or reduced ankle reflexes; or definite DPN, defined as having one of the three as well as abnormal NCS and/or IENFD. We quantified the severity of neuropathy with the Toronto Clinical Neuropathy Score (TCNS).[28,29]

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2.3 | Vagus™ and definition of cardiovascular autonomic neuropathy

The handheld Vagus™ device was used to assess CAN. It measures resting heart rate and changes in heart rate during three cardiovascular autonomic reflex tests (CARTs): 15 and 30 s after changing position from supine to upright (30:15), during deep rhythmic expiration and inspiration (Deep breathing, E:I), and during forced expiration (Valsalva). These tests reflect parasympathetic cardiovascular function.[10]

Vagus™ was not performed if the subject had confirmed atrial fibrillation, pacemaker, or if they reported symptoms of atrial fibrillation (e.g., palpitations), and the Valsalva test was not performed in patients with diabetic retinopathy. We included only those that were able to perform all three tests in the analysis of CAN. We used reference values from a normal age-matched population supplied by the manufacturer (Vagus 2015) to categorize the tests as normal or abnormal. We used the reference values for the 70–79 age-group for seven subjects older than the age-matched population, only two of these were included in our analyses as they had complete Vagus™ tests.

2.3.1 | CAN definition

We defined CAN using the definition proposed by The CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy. The three CARTs described above were used to diagnose CAN, where one abnormal test indicates possible CAN, at least two abnormal tests indicate definite CAN and the additional finding of orthostatic hypotension indicates advanced CAN.[9]

2.4 | Modified Schellong test measuring orthostatic hypotension

Blood pressure change in response to standing was measured using a brachial blood pressure cuff to assess the presence of orthostatic hypotension. Blood pressure and heart rate were measured three times: two times after 5 min at rest while supine, and 3 min after standing. We measured blood pressure difference between the average of the two supine recordings and the recording after standing for 3 min. Orthostatic hypotension was defined as a drop in systolic blood pressure of ≥20 mmHg and/or drop in diastolic blood pressure of ≥10 mmHg of baseline within 3 min in upright position.[30,31]

2.5 | COMPASS 31

The Composite Autonomic Symptom Score 31 (COMPASS 31) questionnaire was used to assess autonomic symptoms.[32] COMPASS 31 is a validated 31-item self-assessment instrument, addressing six domains of the autonomic nervous system: orthostatic, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor functions. The output is a global autonomic severity score and domain scores. COMPASS 31 has previously been validated for the assessment of symptoms of autonomic neuropathy in patients with diabetes.[21] We used the validated Danish translation.[33]

2.6 | NCS, IENFD, QST, and CCM

All participants had skin biopsies taken from the distal leg (10 cm above the lateral malleolus) according to international guidelines.[34] Using a bright-field immunohistochemistry protocol, intraepidermal nerve fibers were stained using PGP 9.5—antibodies where IENFD counts under the fifth centile for age and gender were considered abnormal.[34,35] NCS included examination of the sural nerve bilaterally and the median (motor and sensory), peroneal, and tibial nerves unilaterally.[36] Abnormal values were defined compared with laboratory control material according to published guidelines.[37–39] We assessed cold and warm detection thresholds on the dorsum of the right foot using standardized thermal stimuli according to a reduced version of the QST standardized protocol of the German Research Network on Neuropathic Pain (DFNS),[40] using the limit method, then data were transferred into standard normal distribution and abnormal values, adjusting for age, sex, and body localization.[41,42] CCM was performed using a Heidelberg, Retina Tomograph III laser scanning confocal microscope (Heidelberg Engineering GmbH, Heidelberg, Germany) and images were selected according to guidelines and analyzed using the fully automated software ACC-Metrics (CCMetrics: M.A. Dabbah, Imaging Science, University of Manchester, UK). Corneal nerve fiber density (CNFD): number of main fibers per mm^2, CNFL: the total length of main fibers and branches per mm^2, and the corneal nerve fiber branch density (CNBD): the total number of primary branches per mm.[43,44] Normative values for CCM were obtained from the 97 healthy controls.[45]

2.7 | Ethics

All study participants gave written, informed consent and the study was approved by the Regional Research Ethics Committee of Central Denmark Region (#1-10-72-130-16). The study was registered at Aarhus University with internal notification number 62908-250.

2.8 | Statistical analysis

We used Stata version 17 (StataCorp LLC, TX, USA). Non-normally distributed data were described by the median and interquartile range (IQR) and compared using the Mann–Whitney or Kruskal Wallis test. Categorical data was compared using Fisher's Exact test. Significance was set at $p < 0.05$. We used a logistic regression model to examine the association between age, HbA1c, triglycerides and having abnormal IENFD, and definite CAN in the subgroup of patients with DPN.

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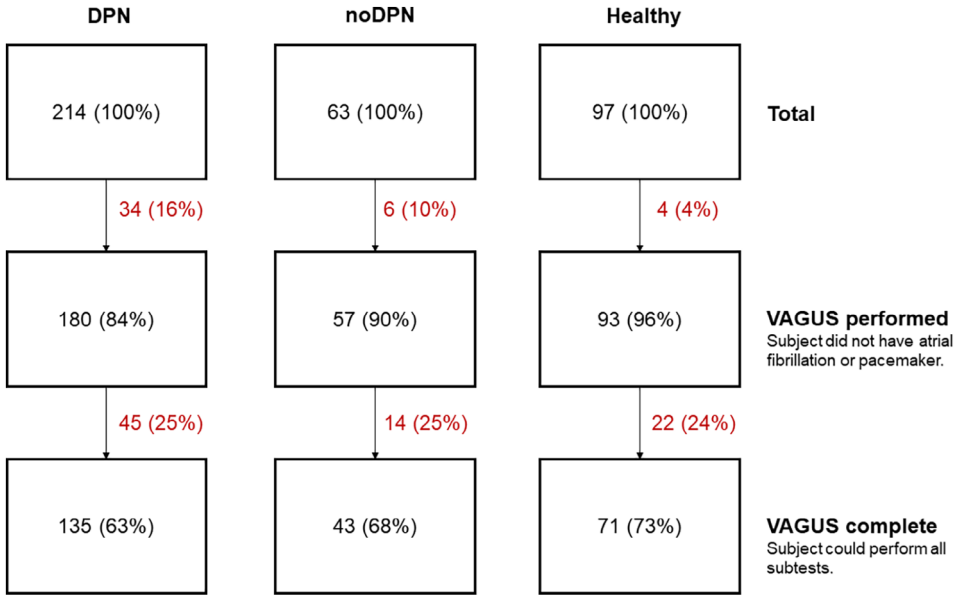

FIGURE 1 VAGUS test participation in the three groups. Subjects with atrial fibrillation or pacemaker could not perform the test. Subjects with diabetic retinopathy could not perform the Valsalva sub-test. Exclusion of patients due to incomplete VAGUS test was due to either insufficient pressure for Valsalva test, data errors, or detection of unstable heartbeat.

3 | RESULTS

3.1 | Study population

We included 374 subjects in this study, of those 214 had at least probable DPN (DPN), 63 had type 2 diabetes but not DPN (noDPN) and 97 were healthy controls (Figure A1). Characteristics of the population are described in detail elsewhere[25] as well as in the appendix (Table A1). There were no significant demographic differences between patients with DPN and noDPN. Healthy controls differed on several points from patients with diabetes; they were younger, less often male and had a lower BMI.

Vagus™ was not performed in 44 subjects due either atrial fibrillation or pacemaker (Figure 1) and 81 subjects were unable to perform all three tests (primarily due to insufficient expiration during Valsalva, data errors or unstable heartbeat detection). Thus, 125 subjects were excluded from Vagus™ testing and therefore from the resulting analysis of CAN. The total number of excluded subjects, and in particular the exclusion due to incomplete Vagus™, was similar in the three groups (Figure 1). Those who were excluded were significantly older (68.6 vs. 63.5, $p < 0.001$) than those who were included in the analysis of CAN, but they had similar BMI (28.7 vs. 29.6, $p = 0.1$) and gender distribution (males excluded 50% vs. 57% included, $p = 0.2$).

3.2 | Cardiovascular autonomic neuropathy

Based on the results from the complete VAGUS™ tests and modified Schellong-test we categorized subjects as having either no, possible, definite, or advanced CAN (Figure 2 and Tables A2, A3). We found a prevalence of definite CAN of 22%, 7%, and 3% in the DPN, noDPN and healthy groups respectively ($p = 0.03$ for DPN vs. no DPN; Figure 2) and in 18.5% of all type 2 diabetes patients irrespective of neuropathy status. We did not define any cases as having advanced

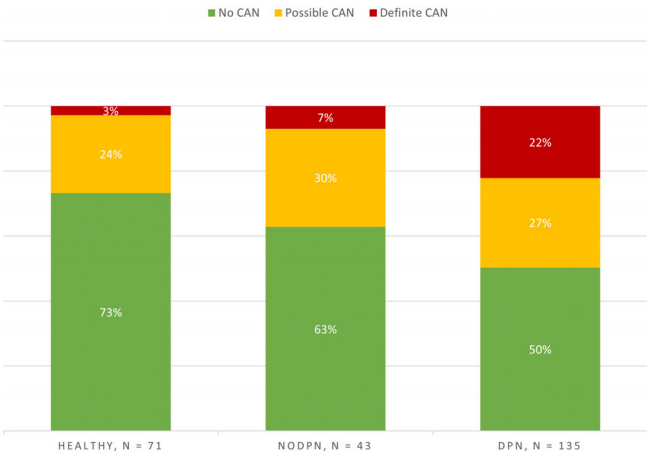

FIGURE 2 CAN prevalence by neuropathy status.

CAN in this cohort, as orthostatic hypotension was only found in three patients with DPN, who were excluded due to either incomplete Vagus™ test or AFLI. Of these, two patients had normal lying-to-standing test (RS) and deep breathing (E:I) but could not complete Valsalva maneuver, and one patient did not perform Vagus™ test due to AFLI and pacemaker.

We assessed patient characteristics in CAN subgroups among patients with type 2 diabetes (Table 1). When compared to patients without CAN, patients with definite CAN were younger ($p < 0.001$), had a higher BMI ($p = 0.01$), higher HbA1c ($p = 0.002$) and higher triglycerides ($p = 0.03$). Most patients with definite CAN also had DPN (91% vs. 72%, $p = 0.03$). More patients with definite CAN had abnormal IENFD (57% of patients with CAN vs. 29% in no CAN, $p = 0.008$; see Table 1). Patients with definite CAN tended to have history of acute myocardial infarction (AMI) or angina ($p = 0.06$) and to use antihypertensives ($p = 0.07$) and strong opioids ($p = 0.08$) while duration of diabetes, neuropathy severity (TCNS scores), and tricyclic antidepressant use were similar in the groups.

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TABLE 1 Comparison of patient characteristics in CAN subgroups in patients with diabetes.

N = 178	No CAN *n* = 95	Possible CAN *n* = 50	Definite CAN *n* = 33	*p*-value Definite CAN versus No CAN
Age, years	65.3 (55.9–71.4)	62.6 (56.2–69.8)	58.3 (50.8–64.15)	<0.001
Sex, male (%)	61 (64)	33 (66)	17 (52)	0.22
BMI, kg/m^2	30.7 (27.7–34.4)	32.3 (28.2–35.4)	34.0 (29.3–39.5)	0.011
Duration of diabetes, years	5.8 (4.4–6.6)	5.6 (3.8–7.0)	5.9 (4.0–6.9)	0.88
Ever smoking, yes (%)	66 (69)	29 (58)	17/32 (53)	0.13
More than 7/14 units of alcohol per week, yes (%)	11 (12)	2 (4)	2 (6)	0.51
HbA1c, mmol/mol	49 (45–54) (*n* = 94)	49 (44–55) (*n* = 49)	53 (49–62) (*n* = 33)	0.002
TCNS, total score	5 (2–8)	5 (2–10)	6 (2–9)	0.35
Total-Cholesterol, mmol/L	4.1 (3.6–4.8)	4.2 (3.7–4.6) (*n* = 49)	3.9 (3.6–4.3)	0.59
Triglyceride, mmol/L	1.8 (1.4–2.6)	1.9 (1.4–2.8)	2.5 (1.7–3.4)	0.026
AMI/Angina, *n* (%)	7/83 (8)	5/41 (12)	8/31 (26)	0.062
Neuropathy status				
DPN, *n* (%)	68 (72)	37 (74)	30 (91)	0.031
Small fiber parameters				
Abnormal CDT and/or WDT, *n* (%)	27 (28)	10 (20)	8 (24)	0.82
Abnormal CNFL, *n* (%)	12 (14) *n* = 87	1 (2) *n* = 48	7 (23) *n* = 30	0.26
Abnormal CNBD, *n* (%)	6 (7) *n* = 87	2 (4) *n* = 48	2 (7) *n* = 30	1.00
Abnormal CNFD, *n* (%)	7 (8) *n* = 87	3 (6) *n* = 48	4 (13) *n* = 30	0.47
Abnormal IENFD, *n* (%)	23 (29) *n* = 80	19 (45) *n* = 42	17 (57) *n* = 30	0.008
Abnormal NCS, *n* (%)	20 (22) *n* = 93	12 (24)	12 (36)	0.11
Medications				
Antihypertensive drugs, *n* (%)	65 (68)	30 (60)	28 (85)	0.074
Tricyclic antidepressants, *n* (%)	2 (2)	1 (2)	2 (6)	1.0
SNRI, *n* (%)	2 (1)	1 (1)	2 (6)	0.27
Strong opioids, *n* (%)	10 (11)	5 (10)	8 (24)	0.078

Abbreviations: AMI, Acute Myocardial Infarction; CDT, cold detection threshold; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; CNFD, corneal nerve fiber density; DPN, distal sensorimotor polyneuropathy; IEFND, intraepidermal nerve fiber density; NCS, nerve conduction studies; SNRI, Serotonin and Noradrenaline Reuptake Inhibitor; TCNS, Toronto Clinical Neuropathy Score; WDT, warm detection threshold.
Note: Values are median (IQR) or *n* (%). Ever smoking (yes) was defined as either current smoking or previous smoking. Hypertension and AMI/Angina signifies current or previous disease. Both hypertension and antihypertensive medicine were reported, although due to missing data in the antihypertensive medicine question, hypertension is presented here. We can assume that most patients with diagnosed hypertension are in antihypertensive treatment, and that these variables are comparable. When deviating from the number of subjects in a group, missing data has been stated as *n* = x in the table. Only patients with complete Vagus™ were included in the analysis of CAN. One patient in the DPN group and 1 patient in the noDPN group were excluded due to being older than the available normal ranges for interpretation of Vagus™ tests. *p*-values were calculated with Mann Whitney-*U* for continuous variables and Fishers Exact in categorical variables. p-value for neuropathy status calculated with fishers exact for the 2 × 2 table of noDPN/DPN and definite CAN/no CAN. CAN was defined using the Toronto Consensus Criteria (definite, possible and no CAN).

We then compared patient characteristics between patients with definite CAN and no CAN among patients with DPN (Table 2). Triglycerides ($p = 0.006$), HbA1c ($p = 0.003$), IENFD ($p = 0.037$), and age ($p < 0.001$) remained significantly different while BMI was no longer significantly different ($p = 0.072$).

In a multiple logistic regression analysis for the relationship between having CAN and age, HbA1c, triglycerides and abnormal IENFD, we found that lower age (OR = 0.92, $p = 0.003$) and higher HbA1c (OR = 1.07, $p = 0.023$) were associated with having definite CAN while triglycerides and abnormal IENFD were no longer significantly associated with definite CAN (appendix).

3.3 | COMPASS 31 (autonomic symptoms)

We calculated global and domain autonomic severity scores in the neuropathy and CAN subgroups, as illustrated in Figure 3a) neuropathy groups 3b) CAN groups (only in DPN patients) and in Tables 3 and 4.

Patients with DPN reported more autonomic symptoms compared to those with noDPN (20.0 vs. 8.3, $p < 0.001$; Table 3). Domain scores showed similar relationships as the global scores, for example patients with DPN reported more orthostatic symptoms than those with noDPN (4.0 vs. 0, $p < 0.001$).

Patients with definite CAN reported more autonomic symptoms than patients with possible CAN (22.1 vs. 11.7) and no CAN (22.1

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TABLE 2 Comparison of patient characteristics in CAN subgroups in patients with DPN.

$N = 135$	No CAN $n = 68$	Possible CAN $n = 37$	Definite CAN $n = 30$	p-value Definite CAN versus No CAN
Age, years	68.0 (59.8–72.4)	66.9 (60.7–71.6)	57.8 (50.5–63.1)	<0.001
Sex, male (%)	46 (68)	27 (73)	17 (57)	0.36
BMI, kg/m^2	31.5 (27.9–35.7)	32.2 (27.7–38.8)	33.2 (29.1–40.6)	0.072
Duration of diabetes, years	5.9 (4.3–6.45)	5.8 (3.9–7.5)	5.9 (4.0–6.9)	0.78
Ever smoking, yes (%)	50 (74)	22 (59)	16/29 (55)	0.097
More than 7/14 units of alcohol per week, yes (%)	9 (13)	2 (5)	2 (7)	0.46
HbA1c, mmol/mol	49 (44–54) ($n = 67$)	48 (44–57) ($n = 36$)	55.5 (49–62)	0.003
TCNS, total score	7 (5–9)	8 (5–10)	7 (4–9)	0.44
Total-Cholesterol, mmol/L	3.95 (3.3–4.55)	4.2 (3.6–4.75) ($n = 36$)	3.9 (3.7–4.5)	0.86
Triglyceride, mmol/L	1.7 (1.4–2.4)	1.8 (1.4–3.0)	2.6 (1.8–3.5)	0.006
AMI/Angina, n (%)	6/57 (10)	5/34 (15)	7/28 (25)	0.14
Small fiber parameters				
Abnormal CDT and/or WDT, n (%)	24 (35)	8 (22)	8 (27)	0.49
Abnormal CNFL, n (%)	10 (16) $n = 63$	1 (3) $n = 35$	7 (26) $n = 27$	0.38
Abnormal CNBD, n (%)	4 (6) $n = 63$	2 (6) $n = 35$	2 (7) $n = 27$	1.00
Abnormal CNFD, n (%)	5 (8) $n = 63$	2 (6) $n = 35$	4 (15) $n = 27$	0.44
Abnormal IENFD, n (%)	23 (38) $n = 61$	19 (63) $n = 30$	17 (63) $n = 27$	0.037
Abnormal NCS, n (%)	20 (29)	12 (32)	12 (40)	0.35
Medications				
Antihypertensive drugs, n (%)	45 (66)	24 (65)	25 (83)	0.095
Tricyclic antidepressants, n (%)	2 (3)	0 (0)	1 (3)	1.0
SNRI, n (%)	2 (3)	1 (3)	2 (7)	0.58
Strong opioids, n (%)	9 (13)	4 (11)	7 (23)	0.24

Abbreviations: AMI, Acute Myocardial Infarction; BMI, body mass index; CDT, cold detection threshold; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; CNFD, corneal nerve fiber density; DPN, distal sensorimotor polyneuropathy; IEFND, intraepidermal nerve fiber density; NCS, nerve conduction studies; SNRI, Serotonin and Noradrenaline Reuptake Inhibitor; TCNS, Toronto Clinical Neuropathy Score; WDT, warm detection threshold.
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vs. 12.3, $p = 0.009$; Table 4). Domain scores showed similar relationships as the global scores, for example patients with definite CAN reported more orthostatic symptoms than those with no CAN (12.0 vs. 0, $p = 0.024$). When analyzing the data from only patients with DPN there was a tendency for higher scores among those with definite CAN compared to no CAN. (26.0 vs. 15.5, $p = 0.077$; Figure 3).

4 | DISCUSSION

4.1 | Major findings

Using the screening device Vagus™ we evaluated CAN in patients with recently diagnosed type 2 diabetes. The prevalence of definite CAN was 22%, 7%, and 3% in the DPN, noDPN and healthy control groups respectively and 18.5% among all patients with type 2 diabetes. The majority of those with definite CAN also had DPN. Having definite CAN was associated with lower age, increased HbA1c, higher values of triglycerides, and abnormal IENFD in a univariate analysis. When examining this relationship in a multivariate analysis, only age and Hba1c were significant. Based on the COMPASS 31 questionnaire, patients with DPN reported higher global autonomic severity scores than patients with noDPN and healthy controls, which was also the case for patients with and without CAN. This difference was not significant when analyzing the subset of patients with only DPN.

4.2 | Autonomic symptoms (COMPASS 31)

We assessed self-reported autonomic symptoms depending on CAN and DPN phenotype with the COMPASS 31 questionnaire. CAN may be symptomatic or asymptomatic and autonomic symptoms can be

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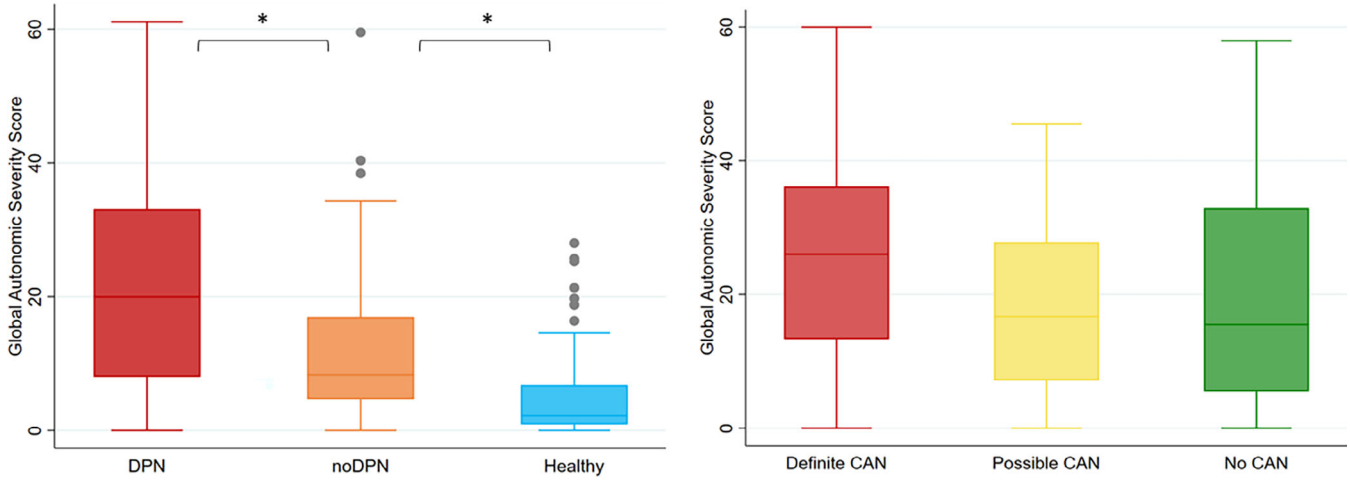

FIGURE 3 Autonomic Symptom Severity Scores stratified by neuropathy (left) and CAN status (DPN only) (right) (* = $p < 0.05$).

TABLE 3 Global and Domain Autonomic Severity Scores (COMPASS 31) by neuropathy status.

	Healthy Controls $n = 97$	noDPN $n = 63$	DPN $n = 214$	p-value DPN versus noDPN
Global Autonomic Score	2.2 (0.9–6.8)	8.3 (4.7–17.0)	20.0 (8.0–33.1)	<0.001
Orthostatic intolerance score	0 (0–0)	0 (0–8)	4.0 (0–20)	<0.001
Bladder score	0 (0–1.1)	1.1 (0–2.2)	1.1 (0–2.2)	0.049
Pupillomotor score	0 (0–1.0)	0.3 (0–1.3)	1.0 (0–2.0)	0.003
Gastrointestinal symptoms score	0.8 (0–1.8)	3.6 (1.8–5.4)	4.5 (1.8–8.0)	0.072
Sudomotor score	0 (0–2.1)	2.1 (0–4.3)	4.3 (0–6.4)	0.011
Vasomotor score	0 (0–0)	0 (0–0)	0 (0–0)	0.098

Note: Values are median (IQR). p-value were calculated using Mann–Whitney U test.

TABLE 4 Global and Domain Autonomic Severity Scores (COMPASS31) by CAN in patients with diabetes and in patients with DPN.

	No CAN		Possible CAN		Definite CAN		p-value Definite versus No CAN	
	Diabetes patients $n = 95$	DPN only $n = 68$	Diabetes patients $n = 50$	DPN only $n = 37$	Diabetes patients $n = 33$	DPN only $n = 30$	Diabetes patients	DPN only
Global Autonomic Score	12.3 (4.4–28.7)	15.5 (5.5–32.9)	11.7 (6.3–22.4)	16.7 (7.1–27.8)	22.1 (13.3–35.9)	26.0 (13.3–36.2)	0.009	0.077
Orthostatic intolerance score	0 (0–12)	0 (0–16)	0 (0–12)	0 (0–12)	12.0 (0–20)	12 (0–20)	0.024	0.070
Bladder score	1.1 (0–2.2)	1.1 (0–2.2)	0.6 (0–2.2)	1.1 (0–2.2)	1.1 (0–2.2)	1.1 (0–2.2)	0.64	0.70
Pupillomotor score	1 (0–2)	1 (0–2)	1 (0–1.7)	0 (0–2)	2.0 (1–2.7)	1.8 (1–3)	0.004	0.042
Gastrointestinal symptoms score	3.6 (1.8–6.3)	4.0 (1.8–7.6)	4.9 (1.8–7.1)	4.5 (1.8–7.1)	5.4 (2.7–9.8)	5.8 (2.7–9.8)	0.026	0.091
Sudomotor score	4.3 (0–6.4)	4.3 (0–8.6)	2.1 (0–6.4)	2.1 (0–6.4)	4.3 (0–8.6)	4.3 (0–8.6)	0.12	0.61
Vasomotor score	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0.8)	0 (0–1.7)	0.15	0.21

Note: Values are median (IQR). p-values were calculated using Mann–Whitney U test.

nonspecific, representing other conditions than CAN. In this cohort, subjects with definite CAN reported more autonomic symptoms than those with possible or no CAN even when we only included those with DPN. When we looked at patients with DPN and no DPN, DPN patients reported more autonomic symptoms than those without DPN. Our results only partly support a symptom-focused diagnosis of CAN using questionnaires such as COMPASS 31 as suggested by other studies.[21,46–48] However, as it is also strongly associated with DPN, this makes COMPASS 31 difficult to implement as a diagnostic or screening tool for CAN on its own.

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4.3 | CAN

4.3.1 | Patient characteristics

In our cohort, we found that CAN was associated with younger age and increased HbA1c. Our data supports an association between DPN and CAN. Definite CAN was found more frequently among patients with DPN (22%) than among those with noDPN (7%; $p = 0.03$) and healthy controls (3%), and 91% of patients diagnosed with definite CAN had DPN. The link between CAN and DPN seems plausible, as both conditions are characterized by damage to the peripheral nervous system. Low et al. go so far as to state that *"[…] autonomic neuropathy is an integral part of most cases of peripheral neuropathy"*.[49] The longitudinal ADDITION study[23] found increased HbA1c, hypertriglyceridemia and obesity to be risk factors of CAN both at 7- and 13-year follow-up and that patients with CAN tended to be younger. The ADDITION study has similar methodology for diagnosing CAN as they also used the Vagus™ device and examined patients with type 2 diabetes in a similar age group. Although patients in their cohort were examined for DPN, the relationship between these two parameters was apparently not analyzed. Contrary to the generally accepted link between higher age and risk of CAN[6] we found that CAN was associated with younger age. This was also found when using the Vagus™ test (ADDITION) and when using different methodology.[19] This opposite relationship may have been caused by factors specific to the Vagus™ test, however another study which also used the Vagus™ test found higher age in patients with CAN in type 2 diabetes.[50] Despite small fibers are involved in both DPN[27] and in CAN[51] we were not able to find a significant association between IENFD and CAN in multivariate analysis. Neither CCM-parameters nor QST-parameters were associated with CAN in our cohort, suggesting that other factors than small fiber affection assessed by these three measures play a role for CAN. However, further studies are necessary to clarify this issue.

4.3.2 | Prevalence estimates and methodological challenges

Despite a low prevalence of objective orthostatic hypotension (only three patients) and a short duration of diabetes of approximately 6 years we found that CAN was common, particularly among patients with DPN. In fact, 22% of patients with DPN had definite CAN, and 91% of those with definite CAN had DPN. Irrespective of neuropathy status, we diagnosed definite CAN in 18.5% of patients with type 2 diabetes. As stated previously, prevalence estimates of CAN in diabetes vary wildly.[8] Methodological inconsistencies regarding the autonomic test make it difficult to compare CAN prevalence across studies: The EURODIAB Prospective Complications Study (type 1 diabetes) diagnosed 17% with CAN defined as one out of two abnormal CARTs[14]; Bello et al. diagnosed 26.9% with CAN defined as two out of five abnormal CARTs[52]; de Matos et al. diagnosed 10% with definite CAN defined as at least three out of five abnormal CARTs.[53] The most similar study is the longitudinal ADDITION study which diagnosed definite CAN in only 9% at 6-year and 15.1% at 13-year follow-up using the Vagus™ device.[23] Patient characteristics (diabetes type, duration of diabetes) may also affect comparability.

4.4 | Strengths and limitations

We included a relatively high number of subjects, performed a detailed neuropathy phenotyping, and included a healthy control group. We used a simple, validated method to detect CAN which enabled detection of autonomic neuropathy in a large cohort. We also attempted to follow the current consensus for diagnosis of CAN as strictly as possible, to ensure reproducibility and comparability across studies. The cross-sectional study design precludes conclusions regarding causality, and we were not able to evaluate whether DPN preceded CAN, or whether they are two parallel conditions developed independently. In addition, patients have been shown to shift between CAN subgroups over time in the ADDITION study[23] which we were unable to account for. We used the Vagus™ device to screen a larger population for CAN than would be feasible with the traditional Ewing battery test for autonomic function. Our findings are therefore dependent on the accuracy of Vagus™ to detect CAN. Vagus™ has been tested against a stationary device in ten patients with and without diabetic autonomic neuropathy[11] and been shown to have moderate to high reproducibility,[12] but more studies evaluating the accuracy of the device are needed. Shortcomings in comparison to confirmatory standard tests include less control over whether the deep breathing exercise is performed and analyzed correctly. During confirmatory testing breathing frequency and volume are recorded and manual analysis secures that extra beats or noise are not used for analysis. We included a sympathetic measure (modified Schellong test, Orthostatic hypotension) to compensate for the fact that Vagus™ tests parasympathetic function. Despite the Vagus™ device being simple to use in theory, 81 subjects were excluded due to insufficient expiratory pressure on Valsalva, data errors or unstable heartrate detection. It is important to note, that autonomic testing usually measures the autonomic nervous system through indirect measures of heart rate and blood pressure changes which are under autonomic control. Conditions such as heart failure and myocardial infarction may alter the autonomic balance, resulting in a reduction in cardiac vagal outflow to the heart,[54] conversely CAN is known to influence various cardiac disorders including heart failure and silent myocardial infarction, and that it can lead to severe morbidity and mortality and increase the risk of sudden cardiac death.[55,56] It is difficult to establish whether CAN leads to cardiac issues or whether cardiac issues lead to misdiagnosis of CAN in this cross-sectional design. Patients across the different CAN groups tended to differ in cardiac health as indicated by more AMI/angina and use of antihypertensives among those with definite CAN. Various medications may potentially affect the autonomic tests[57,58] including medications that influence the adrenergic alfa- and beta-receptors or with anticholinergic effects (tricyclic antidepressants, alfa- and betablockers, antihypertensives etc.). Tricyclic

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antidepressant use was similar in those with definite and no CAN. Unfortunately, we did not account for use of betablockers. Pausing medicine prior to testing would eliminate this factor. Finally, other factors such as BMI may independently lead to disturbances of autonomic balance, however this appears to mainly be an issue in morbidly obese patients[58] which was not the case for this cohort.

5 | CONCLUSION

In this relatively large well-characterized cohort of recently diagnosed type 2 diabetes patients, one in five with DPN had definite CAN and almost all patients with definite CAN had DPN. The link between DPN and CAN is apparent, but not all patients with DPN develop CAN. Prospective studies are needed to determine the causal relationship between CAN and DPN. This study suggests that young patients and patients with increased HbA1c are more likely to have CAN and DPN. As for the COMPASS questionnaire, its use for the detection of CAN in the clinic is limited as it may be too unspecific.

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CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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