Cardiovascular autonomic neuropathy and indices of heart failure in type 2 diabetes: The CANCAN Study

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Abbreviations

BMI: Body mass index

CAN: Cardiovascular autonomic neuropathy

CART: Cardiovascular autonomic reflex test

CVD: Cardiovascular disease

ECG: Electrocardiogram

eGFR: Estimated glomerular filtration rate

HbA1C: haemoglobin A1C

HF: Heart failure

HFpEF: Heart failure preserved ejection fraction

HFrEF: Heart failure reduced ejection fraction

HRV: Heart rate variability

NYHA classification: New York Heart Association classification

NT-proBNP: N-terminal pro-brain natriuretic peptide

rHR: Resting heart rate

T2D: Type 2 diabetes

Article highlights

- **Why did we undertake this study?**

Cardiovascular autonomic neuropathy (CAN) is a common diabetic complication and an independent predictor of major cardiovascular outcomes including heart failure (HF) development and progression. However, its role in the pathophysiology of HF requires further investigation.

- **What is the specific question we wanted to answer?**

Whether individuals with type 2 diabetes (T2D) and CAN have higher levels of HF indicators than those without CAN. Additionally, we investigated whether CAN provides clinical value for HF screening beyond established tools such WATCH-DM risk score, or symptoms.

- **What did we find?**

We found that in this cohort of T2D participants, CAN was associated with higher NT-proBNP levels, WATCH-DM and NYHA scores, including in asymptomatic individuals

- **What are the implications of our findings?**

Our findings suggest that CAN detection may help identify individuals with T2D at a higher risk of early-stage, asymptomatic HF that is not captured by conventional tools. This finding supports the potential role of CAN in complementing established biomarkers to identify individuals with earlier HF stages, for timelier treatment initiation.

Abstract

Objective

To quantify the association between cardiovascular autonomic neuropathy (CAN) and heart failure (HF) in individuals with type 2 diabetes (T2D).

Research Design and Methods

Two hundred T2D individuals were recruited from two Danish outpatient clinics between 2021-2024. CAN was defined by abnormal cardiovascular autonomic test reflex results. HF outcomes included the primary outcome of elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) levels >125 pg/mL, WATCH-DM risk score. Symptomatic HF was defined by New York Heart Association (NYHA) classification score ≥ II. We assessed the association between measures of CAN and HF using logistic and linear regressions, adjusting for confounders and testing for effect modification between CAN and NYHA and WATCH-DM risk scores.

Results

Among 176 individuals with NT-proBNP assessments, the median (interquartile range [IQR]) age was 63 (IQR: 55, 70) years, 61% were men, and median diabetes duration was 17 years (IQR: 11, 24). Among 136 individuals with valid CAN assessment, 40% had CAN and 52% of those had elevated NT-proBNP compared to 23% of individuals without CAN. In fully adjusted model, CAN was associated with 5.7 times higher odds (95% CI: 2.0, 18.5) of elevated NT-proBNP levels compared to individuals without CAN. The association remained statistically significant in asymptomatic individuals, and in individuals with a low-to-moderate WATCH-DM risk score.

Conclusion

CAN is associated with elevated NT-proBNP levels in individuals asymptomatic for HF. This suggest that CAN may complement established biomarkers to identify individuals with earlier HF stages, for timelier treatment initiation.

Introduction

Cardiovascular autonomic neuropathy (CAN) is an important but often overlooked complication of diabetes. CAN is an independent predictor of high cardiovascular disease (CVD) risk, heart failure (HF), diabetic kidney disease, and all-cause mortality[1-6]. Assessment of CAN may identify individuals with diabetes who are at a higher risk of severe complications and premature death. CAN is characterized by impaired autonomic regulation of the cardiovascular system. This condition stems from damage to the autonomic nerve fibres that regulate heart and blood vessel function, leading to disruptions in heart rate regulation and vascular dynamics[7].

HF has also emerged as one of the most prevalent CVD complication in people with type 2 diabetes (T2D) with serious consequences on morbidity, mortality and quality of life[8]. Although most current guidelines recommend proactive screening for HF in T2D [8, 9], most individuals with HF continue to be diagnosed only after experiencing HF symptoms, that unfortunately reflect more advanced HF stages that have a very poor prognosis[8, 10]. Identifying HF in earliest stages, that present with asymptomatic myocardial wall stress is important given that it would enable earlier implementation of guidelines directed therapies that have been shown to substantially reduce the risk of HF hospitalizations and death in T2D [8, 10, 11].

Several scores have been developed to estimate HF risk using demographics and clinical characteristics (e.g. age, sex, blood pressure, biochemical measures, ECG, and CVD history)[12, 13]. The WATCH-DM risk score identifies individuals with T2D at high risk of incident HF, aiding in the detection of asymptomatic cases[12, 14]. This score has been externally validated in several cohorts, demonstrating good discrimination[14]. The New York Heart Association (NYHA) functional classification defines HF severity based on physical activity limitations from score I to IV, but its inability to capture asymptomatic HF reduces its utility for early HF detection [15]. Data from multiple large, well-phenotyped longitudinal cohorts of individuals with and without diabetes have recognized natriuretic peptides, such as N-terminal pro-brain natriuretic peptide (NT-proBNP), as a valid biomarker for HF[16]**.** NT-proBNP is released in response to increased myocardial wall stress and elevated ventricular filling pressures, which characterize early physiological changes in the development of HF [16]. Elevated NT-proBNP levels (above 125 pg/mL) has demonstrated high sensitivity for identifying HF of both preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF)[11].

Simple bedside techniques can be used to determine CAN, including heart rate variability (HRV) from standard ECG recordings [17] and the gold-standard cardiovascular autonomic reflex tests (CARTs)**,** which measure heart rate responses to physiological challenges such as changing position from lying to standing, during deep breathing, and performing the Valsalva maneuver. As CAN is prognostically linked with HF [18], CAN detection may play a role in future strategies for early HF detection. Therefore, screening for CAN shows potential for inclusion in HF detection algorithms. However, the role of CAN in identifying asymptomatic HF and its potential for enhancing risk scores like WATCH-DM was not evaluated yet.

The objective of this study was to describe the distribution of HF indicators including NT-proBNP levels, WATCH-DM risk score, and NYHA classification score among individuals with T2D with and without CAN, and to quantify the association between CAN and these HF indicators. We further evaluated the interactions between CAN, NYHA classification, and WATCH-DM risk scores, to assess the potential role of CAN in addition to established biomarkers and risk scores to identify those with earlier HF stages.

Research Design and Methods

Study design and participants

The Danish cross-sectional study *Cardiovascular Autonomic Neuropathy for risk stratification in type 2 diabetes* (CANCAN study) assessed CAN and indicators of HF and glucometric indices from continuous glucose monitoring (CGM) in individuals with T2D treated in secondary care between 2021 and 2024. The CANCAN study aimed to assess whether identifying CAN detects a high-risk T2D population with higher prevalence of HF indicators and adverse glucose profiles. Results from the CANCAN study on glucometric indices will be analyzed and presented separately. Participants were recruited from two hospital outpatient clinics located in the Central Region of Denmark (Regional Hospital Central Jutland, Viborg and Gødstrup Hospital). We included 200 consecutive individuals (aged >18 years) with T2D for more than one year. Participants were either long-term T2D individuals followed in the outpatient clinic, or they were referred for optimization of their diabetes management. Main exclusions were: arrhythmia precluding CAN assessment, laser treatment for diabetic retinopathy within the past 3 months, pregnancy or breastfeeding, a life-threatening illness with a remaining life expectancy of less than one year, or cognitive impairments that hindered their ability to provide informed consent.

Cardiovascular autonomic neuropathy

CAN was diagnosed using CARTs. Ratios of normal RR-intervals were derived from an ECG using the Vagus™ device (Medicus Engineering, Aarhus, Denmark). Three standardized CARTs were performed to assess R-R intervals in responses to postural changes from lying-to-standing, during deep breathing, and under the Valsalva maneuver [19]. Testing was conducted in a quiet, isolated examination room between 8:00 a.m. and 2:00 p.m. following a standardized protocol. Participants rested supine for 10 minutes before testing. Smoking and caffeine consumption were prohibited for at least two hours before testing. Each CART was performed once per participant by a trained examiner. Manifest CAN was defined by the presence of two or more abnormal CARTs using recently established age-based formulas using normative material for the Vagus™ device [19]. We also tested for orthostatic hypotension, defined as a sustained reduction in systolic blood pressure of at least 20 mmHg or diastolic blood pressure of 10 mmHg within 3 min from changing position from lying to standing [20].

Assessment of indicators of heart failure

NT-proBNP was measured in plasma using a two-step chemiluminescent microparticle immunoassay on the Cobas e601 analyzer (Roche Diagnostics). The analyses were conducted at the Department of Blood Samples

and Biochemistry, Aarhus University Hospital, Denmark. Detection threshold was set at 50 pg/ml. Further description of NT-proBNP analysis is presented in supplementary material. We defined HF as elevated NT-proBNP levels greater than 125 pg/mL, a threshold indicative of structural heart disease, and elevated filling pressures, consistent with previous literature [21].

The WATCH-DM risk score was developed to estimate the 5-year risk of incident HF hospitalization in individuals with T2D based on a previously published risk model incorporating clinical, laboratory, and ECG parameters [12]. For the current study, we adapted the WATCH-DM score to align with available data, using a modified version from a validation study that incorporated haemoglobin A1C (HbA1C) instead of fasting plasma glucose [14]. This version excludes the ECG measurement of QRS duration. The adjusted WATCH-DM risk score includes nine variables: two binary variables (history of myocardial infarction and coronary artery bypass grafting), and six continuous variables (age, body mass index (BMI), systolic- and diastolic blood pressure, levels of serum creatinine, high-density lipoprotein (HDL) cholesterol, and HbA1c). The score ranged from 0 to 39 points, and risk categories were defined as follows: very low (≤11 points), low (12–13 points), moderate (14–15 points), high (16–18 points), and very high (≥19 points) [14].

New York Heart Association (NYHA) Functional Classification for HF stage I-IV was defined by a physician to determine cardiac functional status (see documentation in supplementary material). We defined NYHA stages II-IV as HF symptoms.

Covariates

We collected data on self-reported lifestyle factors, including smoking status (smoker/ non-smoker), leisure physical activity (sedentary / non-sedentary) (see supplementary material), and average weekly alcohol consumption (number of units). Information regarding CVD history and the use of antihypertensive, glucose-lowering, lipid-lowering, and antithrombotic medications was obtained and verified through electronic health records. Blood and urine samples were collected to measure levels of HbA1C, total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, creatinine, estimated glomerular filtration rate (eGFR) (based on the CKD-EPI equation [22]), and urine albumin-to-creatinine ratio. Anthropometrics included height, weight, BMI, waist circumference, and clinical assessments of systolic and diastolic blood pressure.

Ethics

The study was approved by the Committee on Health Research Ethics in the Central Denmark Region (#93931 and #1-10-72-221-21). The study was conducted in accordance with the ethical principles for medical research stated in the 1996 Declaration of Helsinki, and all study participants gave oral and written informed consent.

Statistical Analysis

Population characteristics were summarized by CAN status and reported as median and interquartile ranges (IQR) for continuous variables and numbers and percentages for categorical variables. To examine differences in HF risk, we compared the distribution of categorized HF indices (NT-proBNP, WATCH-DM, NYHA) according to CAN status.

HF outcome was defined by an NT-proBNP level >125 pg/mL. We defined symptomatic HF by NYHA score ≥ II. The association between CAN and HF was assessed by logistic regression to estimate the odds ratio (OR) of CAN as the determinant for the outcomes of elevated NT-proBNP. Participants without a valid measure of NT-proBNP measurement or CAN assessments were excluded from the complete case analysis. Model 1 was adjusted for age, sex, and duration of diabetes, while Model 2 also included adjustments for smoking status, alcohol consumption, BMI, HbA1c, triglycerides, total cholesterol, and antihypertensive medication. Model 3 was additionally adjusted for history of CVD. Model 4 was further adjusted for eGFR. To assess the impact of beta-blocker treatment and a history of CVD, we conducted a sensitivity analysis. To assess whether the association between CAN and elevated NT-proBNP persisted in the absence of symptoms, defined by NYHA score = I, or among individuals with low-to-moderate WATCH-DM risk, we included interaction terms in separate models for binary NYHA classification (NYHA score = I vs NYHA score ≥ II) WATCH-DM risk (very-low-to-moderate [0-15 points] vs. high-to-very-high risk [≥16 points])**.** Logistic regression was used to estimate the OR of CAN as the determinant for the outcome of HF symptoms, defined by NYHA score ≥ II, with adjustments in models 1–4. We used linear regression to analyse the difference in WATCH-DM risk score between those with and without CAN.

Analyses were performed using RStudio (4.3.2, RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA)[23], with complete case and multiple imputation of chained equation (MICE) approach to handle missing data from CARTs or other covariates. All individuals underwent CARTs, and a technical report was produced when recordings were incomplete. As missingness was related to observed variables, like patient characteristics or report information, we minimized systematic exclusion risk. Therefore, we considered MICE assumptions met.

Results

Participants characteristics

In this study, 176 participants had valid measurement of NT-proBNP of which 108 (61%) were males, median age was 63 (IQT: 55; 70) years, median diabetes duration was 17 (IQT: 11; 24) years, and mean HbA1c was 64 (IQT: 56; 80) mmol/L (Figure S1). Among these individuals, 40 did not complete all CART assessments, with 21 of these unable to perform the Valsalva maneuver. Among individuals with valid CARTs assessment (n=136), the prevalence of CAN was 40% (Figure 1A), 48 (27%) had a history of CVD (myocardial infarction, stroke or peripheral artery disease), and 20 (11%) had been previously diagnosed with HF. Elevated levels of NT-proBNP (>125 pg/ml) were seen in 66 (38%), 45 (26%) had NYHA score ≥ II and 56 (33%) had high-to-

very-high risk of HF based on the WATCH-DM risk score (see Figure S2). Among individuals with elevated NT-proBNP, 33% had symptoms indicating HF with NYHA score ≥ II and 52% had high risk of HF based on the WATCH-DM score. Complete participants' characteristics are presented in Table 1 contrasting those with CAN with no CAN.

Table 1: Baseline characteristics by CAN status

	Missing	**Overall**, N = 176	**CAN missing**, N = 40	**No CAN**, N = 82	**CAN**, N = 54
Sex (Women)	0	68 (39%)	19 (48%)	27 (33%)	22 (41%)
Age (years)	0	63 (55; 70)	68 (61; 75)	61 (52; 69)	62 (56; 68)
BMI (kg/m²)	2	32 (28; 37)	30 (26; 34)	33 (28; 38)	33 (30; 36)
Duration of diabetes (years)	0	17 (11; 24)	20 (13; 30)	15 (9; 21)	19 (13; 24)
HbA1c (mmol/mol)	0	64 (56; 80)	65 (56; 85)	64 (55; 78)	64 (57; 76)
Total cholesterol (mmol/L)	2	3.90 (3.23; 4.78)	3.70 (3.33; 4.18)	4.10 (3.33; 4.88)	3.75 (3.03; 4.98)
LDL (mmol/L)	3	1.80 (1.40; 2.40)	1.70 (1.30; 1.90)	2.00 (1.50; 2.58)	1.70 (1.10; 2.73)
HDL (mmol/L)	2	1.00 (0.88; 1.20)	1.00 (0.90; 1.30)	1.00 (0.90; 1.20)	0.97 (0.80; 1.18)
Triglycerides (mmol/L)	3	2.00 (1.30; 2.90)	2.10 (1.10; 2.80)	1.95 (1.30; 2.90)	2.05 (1.40; 2.98)
eGFR (ml/min/1.73 m^2) categories	6				
< 30		16 (9%)	5 (14%)	6 (7.4%)	5 (9%)
30-59		33 (19%)	7 (19%)	12 (15%)	14 (26%)
60-89		41 (24%)	12 (33%)	18 (22%)	11 21%)
> 90		80 (47%)	12 (33%)	45 (56%)	23 (43%)
Systolic blood pressure (mmHg)	1	133 (123; 143)	135 (127; 147)	131 (123; 142)	133 (120; 143)
Diastolic blood pressure (mmHg)	1	76 (68; 82)	73 (66; 79)	78 (72; 83)	74 (66; 82)
Resting heart rate (bpm)	6	77 (66; 84)	68 (62; 80)	78 (69; 84)	80 (67; 89)
CARTS					
Lying to standing (RR ratio)	8	1.02 (1.01; 1.06)	1.03 (1.02; 1.05)	1.05 (1.01; 1.08)	1.01 (1.00; 1.02)
Deep breathing (RR ratio)	4	1.13 (1.07; 1.26)	1.15 (1.10; 1.28)	1.18 (1.11; 1.30)	1.07 (1.03; 1.08)
Valsalva maneuver (RR ratio)	45	1.24 (1.13; 1.36)	1.20 (1.14; 1.25)	1.32 (1.25; 1.45)	1.11 (1.08; 1.16)
NT-proBNP (pg/ml) categories	0				
< 50		72 (41%)	10 (25%)	47 (57%)	15 (28%)
50-124		38 (22%)	11 (28%)	16 (20%)	11 (20%)

	Missing	Overall, N = 176	CAN missing, N = 40	No CAN, N = 82	CAN, N = 54
125-300		28 (16%)	7 (18%)	10 (12%)	11 (20%)
> 300		38 (22%)	12 (30%)	9 (11%)	17 (31%)
WATCH-DM risk score	4	14.0 (11.0; 16.0)	15.0 (12.8; 17.0)	13.0 (10.0; 16.0)	15.0 (12.0; 18.0)
NYHA classification	1				
I		130 (74%)	27 (69%)	72 (88%)	31 (57%)
II		31 (18%)	9 (23%)	9 (11%)	13 (24%)
III		13 (7%)	3 (8%)	1 (1%)	9 (17%)
IV		1 (1%)	0 (0%)	0 (0%)	1 (2%)
Smoking status (smoker vs non-smoker)	1	28 (16%)	6 (15%)	12 (15%)	10 (19%)
Alcohol consumption (units per week)	1	0.0 (0.0; 2.0)	0.0 (0.0; 3.5)	0.0 (0.0; 2.0)	0.0 (0.0; 0.0)
Leisure physical activity (Sedentary lifestyle vs non-sedentary lifestyle)	15	68 (42%)	17 (50%)	27 (36%)	24 (45%)
Any antihypertensive medication (yes)	0	140 (80%)	33 (83%)	61 (74%)	46 (85%)
ACE inhibitors (yes)	0	54 (31%)	9 (23%)	25 (30%)	20 (37%)
AT2 antagonist (yes)	0	65 (37%)	16 (40%)	30 (37%)	19 (35%)
Calcium antagonist (yes)	0	71 (40%)	14 (35%)	28 (34%)	29 (54%)
Beta-blockers (yes)	0	52 (30%)	11 (28%)	19 (23%)	22 (41%)
Aldosterone antagonist (yes)	0	18 (10%)	3 (8%)	5 (6%)	10 (19%)
Glucose-lowering medication					
Metformin (yes)	0	123 (70%)	26 (65%)	56 (68%)	41 (76%)
SGLT2-inhibitors (yes)	0	81 (46%)	12 (30%)	40 (49%)	29 (54%)
DPP4 inhibitors (yes)	0	13 (7%)	2 (5%)	8 (10%)	3 (6%)
GLP1 RAs (yes)	0	91 (52%)	15 (38%)	47 (57%)	29 (54%)
Insulin (yes)	0	140 (80%)	33 (83%)	66 (80%)	41 (76%)
Lipid-lowering medication (yes)	0	155 (88%)	34 (85%)	74 (90%)	47 (87%)
Antiplatelet medication (yes)	0	76 (43%)	18 (45%)	29 (35%)	29 (54%)

n (%); Median (IQR)

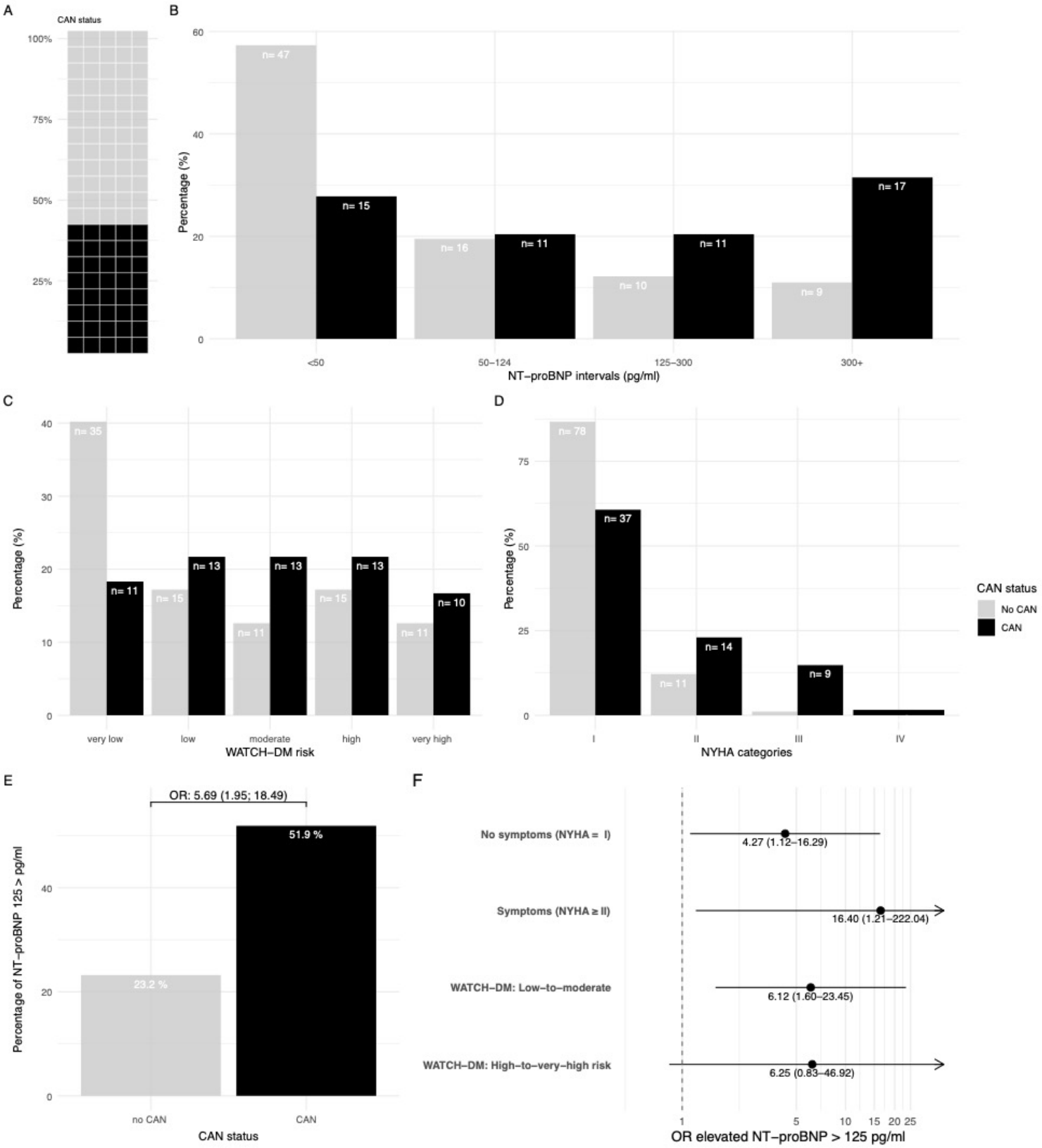
CAN, cardiovascular autonomic neuropathy. CARTs, cardiovascular autonomic reflex test. NT-proBNP, N-terminal pro b-type natriuretic peptide. bpm, beats per minute. BMI, body mass index, LDL, low density lipoprotein. HDL, high density lipoprotein. eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2). HbA1c, hemoglobin A1c.

Association between CAN and measures of HF

Individuals with CAN showed a higher prevalence of HF assessed as described in Methods (Figure 1B–D). Specifically, a higher proportion of CAN individuals had elevated NT-proBNP (51.9%) compared to those without CAN (23.2%). In fully adjusted models (model 4) the OR for having elevated NT-proBNP were 5.69 (CI: 1.95; 18.49) compared to those without CAN (Figure 1E). Furthermore, the association between CAN and elevated NT-proBNP was similar in participants with very-low-to-moderate risk in the WATCH-DM score (OR = 6.1 [CI: 1.6; 23.5]) and those with high-to-very-high risk (OR = 6.3 [CI: 0.83; 46.9]).

The association between CAN and elevated NT-proBNP was also present in asymptomatic individuals for HF (OR = 4.3, 95% CI: 1.1; 16.3), although it was stronger in symptomatic HF individuals (OR = 16.4, 95% CI: 1.2; 222.0), although the interaction between groups was not statistically significant (p = 0.4).

Figure 1: Figure 1. Distribution of NT-proBNP, NYHA Class, and WATCH-DM Score by CAN Status, and association of CAN with Elevated NT-proBNP



A: Percentage distribution by CAN status (no CAN, CAN). B: Percentage distribution of NT-proBNP level categories stratified by individuals with and without CAN. C: Percentage distribution of WATCH-DM risk score stratified by individuals with and without CAN. C: Percentage distribution of NYHA classification stratified by individuals with and without CAN. E: Percentage of individuals with NT-proBNP > 125 pg/ml among those with and without CAN and adjusted odds ratio from Model 4. F: Effect modification of the association between CAN and NT-proBNP by symptoms defined by NYHA

classification (symptoms: NYHA ≥ II vs no symptoms: NYHA = I) and risk score defined by WATCH-DM risk (very-low-to-moderate vs high-to-very-high risk).

Imputation of missing CARTs showed an OR 2.9 (CI: 1.4; 6.6) between CAN and elevated NT-proBNP.
In sensitivity analysis, excluding participants with beta-blocker use or a history of CVD, the direction of the association between CAN and NT-proBNP remained unchanged, although exclusion of these participants reduced sample size and thus to wider confidence intervals compared to the main analysis (see Table S1). CAN was associated with 1.7 (CI: 0.3; 3.0) higher points in WATCH-DM risk, and 5.51 (1.9; 15.97) higher OR for NYHA score ≥2 (see Table S2). Among each CART, the Valsalva maneuverer showed the strongest association with elevated NT-proBNP, followed by deep breathing. Orthostatic hypotension was also associated with elevated NT-proBNP (see Table 2). Participants who were not able to perform the Valsalva maneuverer had higher NT-proBNP levels compared to those without CAN (Table S3).

Table 2 CAN diagnosis, orthostatic hypotension, and CARTs association with elevated levels of NT-proBNP (>125 pg/ml)

	Non-cases/ cases	Model 1	Model 2	Model 3	Model 4
CART					
Lying-to-standing	108/54	1.04 (0.5; 2.19)	1.21 (0.56; 2.70)	1.09 (0.49; 2.46)	0.80 (0.32; 1.97)
Deep breathing	133/33	2.76 (1.17; 6.70)	2.95 (1.18; 7.72)	2.93 (1.17; 7.72)	3.30 (1.17; 9.77)
Valsalva maneuverer	75/51	5.65 (2.42; 14.04)	8.13 (3.02; 24.81)	7.14 (2.62; 21.78)	9.00 (2.88; 33.09)
CAN diagnosis	78/52	5.26 (2.28; 12.96)	6.05 (2.41; 16.59)	5.04 (1.97; 13.94)	5.69 (1.95; 18.49)
CAN with imputed CARTs*	$127^{-\text{imputed cases}}$ /$52^{+\text{imputed cases}}$	2.61 (1.36; 5.11)	2.98 (1.48; 6.23)	2.88 (1.42; 6.04)	2.94 (1.37; 6.56)
Orthostatic hypertension	146/24	3.31 (1.25; 9.44)	3.20 (1.16; 9.51)	3.11 (1.11; 9.36)	4.04 (1.27; 13.77)

*Odds ratio for NT-proBNP > 125 pg/ml comparing orthostatic hypertension, abnormal CARTs or CAN diagnosis to normal CARTs and no CAN. Model 1: Age, sex, diabetes duration. Model 2: Model 1 + HbA1c, smoking status, BMI, anti-hypertensives, total cholesterol, triglycerides, systolic blood pressure. Model 3: Model 2 + history of cardiovascular disease. Model 4: Model 3 + eGFR. CAN, cardiovascular autonomic neuropathy. CARTs, cardiovascular autonomic reflex tests. NT-proBNP, N-terminal pro b-type natriuretic peptide. BMI, body mass index. *Analysis with imputed values for each missing CART.*

Conclusions

In this cross-sectional study of contemporary individuals with T2D as followed in outpatient clinics in Denmark, we found that individuals with CAN more frequently showed signs of HF including elevated levels of NT-proBNP, higher WATCH-DM risk score, and higher NYHA classification compared to the individuals without CAN. The association between CAN and elevated NT-proBNP was also present among individuals asymptomatic for HF and in the individuals classified as low-to-moderate risk of HF using the WATCH-DM score.

This study adds to the growing body of evidence that individuals with CAN represent a high-risk T2D population for more severe cardiovascular complications and increased mortality[1-6]. Our findings extend this by demonstrating an increased risk of HF, including early-stage HF, as indicated by elevated NT-proBNP levels**.** Several lines of evidence showed that individuals with T2D and HF have a poor prognosis including higher rates of hospitalization and a 3- to-5-fold higher mortality compared to individuals with T2D without HF [8, 24]. Furthermore, these data suggest that early identification of CAN in T2D may aid in detecting individuals at particularly high risk of HF, even when asymptomatic. Sympathetic overload in CAN leads to increased cardiac stress through elevated heart rate, stroke volume, and blood pressure [25, 26]. The hemodynamic changes of CAN contribute to cardiac and arterial remodelling [27, 28]. Cardiac and arterial remodelling results in structural and functional alterations in the heart that increase the risk of HF [29, 30]. Hence, CAN may promote the progression of HF before clinical symptoms of HF become apparent.

Although, among those with elevated NT-proBNP, one-third exhibited symptoms consistent with HF, as defined by NYHA score II or higher, the association between CAN and elevated NT-proBNP remained significant in those individuals asymptomatic for HF. Additionally, among individuals with elevated NT-proBNP, half were classified as having a high-to-very-high risk according to the WATCH-DM score, while the other half were categorized as low-to-moderate risk. However, our data showed that the association between CAN and elevated NT-proBNP was similar among those with both high and low-to-moderate WATCH-DM risk. Hence, CAN may provide additional information beyond risk scores such as the WATCH-DM score and symptoms of HF defined by NYHA score and reveal a higher risk of HF than uncovered by these measures alone. These initial data support further studies with follow-up data on HF hospitalizations to confirm a possible additive predictive value of CAN when incorporated into existing risk scores or biomarkers.

Although the American Diabetes Association recommends screening for CAN [9] [8], the implementation of general screening for CAN in routine care remains limited and has not yet been adopted in Denmark. In this study, CAN prevalence was 40%, higher than the 15% reported in the ADDITION study of screen-detected T2D in Denmark and higher than CAN prevalence (19%) in another Danish outpatient clinic study[31, 32]. Thus, the population in our study falls within the higher end of the generally reported prevalence range of 12-73% for CAN in T2D[33]. The reason may be that the cohort in this study comprises individuals with T2D affiliated with a specialist diabetes clinic (secondary care) that usually handle more advanced T2D characterized by multiple complications and suboptimal glycemic control. This contrasts with the broader T2D population in Denmark generally managed in primary care**.** These factors likely explain the higher prevalence of CAN observed. The individuals with CAN did not differ in conventional cardiovascular risk factors compared to those without CAN yet showed a higher proportion of kidney disease and use of blood pressure lowering medications. The similar levels of blood pressure, triglycerides, HbA1c, lipids, and BMI, may be explained by a more intensive treatment of individuals with CAN who showed a higher cardiovascular risk due to a more prevalent CVD history. Exclusion of individuals with a history of cardiovascular disease, hospitalization for HF, or use of beta-blockers did not alter the association between CAN and elevated NT-proBNP. Of note, even within this high-risk group of individuals with T2D, we identified a subgroup with CAN who may require closer

monitoring for the early detection of HF. Importantly, this subgroup may not have been identified had they not been diagnosed with CAN.

NT-proBNP is recognized as a sensitive marker of early-stage HF that can be used to identify those individuals that will benefit from early interventions before clinical symptoms appear[11, 34, 35]. However, its specificity varies across HF phenotypes, being less specific for detecting HFpEF compared to HFrEF [11, 36]. Therefore, although we cannot link our findings to specific HF phenotypes, NT-proBNP remains a valuable indicator of early HF. The validity of NT-proBNP as a marker for HF may be confounded by atrial fibrillation, obesity, or impaired kidney function[11]. Individuals with concomitant atrial fibrillation were excluded from our analyses, and in this study controlling for BMI did not alter the magnitude of the association between CAN and elevated NT-proBNP. Impaired kidney function, as indicated by decreased eGFR, is linked to greater cardiac dysfunction but may also lead to elevated NT-proBNP levels, independently of HF[11]. After adjusting for eGFR, the association between CAN and NT-proBNP strengthened, suggesting the true link between CAN and cardiac dysfunction may have been underestimated due to the potential elevation of NT-proBNP in individuals with impaired kidney function. Adjustments for smoking status, medication, clinical risk factors, and CVD history did not change the association between CAN and elevated NT-proBNP. Therefore, we consider our findings robust and adequately adjusted for relevant confounding factors.

A relatively large proportion of individuals (23%) did not complete the CARTs assessments, which is consistent with findings from the ADDITION study, where 12% to 33% did not complete the test[31]. The analysis of CAN using imputed CARTs values yielded slightly lower risk estimates and narrower confidence intervals compared to complete case analysis. The direction of the association remained consistent, supporting the validity of our findings after accounting for individuals with missingness of CARTs. Most incomplete CARTs assessments were due to individuals being unable to perform the Valsalva maneuver. These individuals had higher NT-proBNP levels than individuals within the normal Valsalva maneuver values, suggesting that individuals who cannot complete this CART are at higher risk of HF and echocardiogram could be considered in those individuals. Among the CARTs, the Valsalva maneuver followed by deep breathing showed the strongest association with elevated NT-proBNP, while orthostatic hypotension was also strongly associated with elevated NT-proBNP. Our findings show that even a single abnormal CART (excluding the lying-to-standing test) or orthostatic hypotension may indicate increased HF risk and could prompt clinical attention [33].

Strengths and limitations

Strengths of this study are : the large, well-characterized cohort of individuals with T2D, assessed in outpatient clinics and the thoroughly phenotypes for microvascular complications, including autonomic neuropathy, and the use of non-invasive reproducible assessments for CAN and biomarkers [37], making it easy to implement in clinical care. A limitation is that we did not assess echocardiography, which is gold standard for assessing HF and is central to classifying HFrEF and HFpEF[30]. However, previous studies have shown that CAN is associated with both structural and functional cardiac changes in both left atrium and the left ventricle, affecting systolic and diastolic function in individuals with type 1 and T2D[38, 39]. Future studies are warranted

to investigate the association between CAN and defined structural and functional cardiac abnormalities and to determine the prognostic value of CAN, yet we consider the use of validated indicators of HF to support an association between CAN and HF. In HF, compensatory changes in heart rhythm may cause autonomic dysfunction due to increased sympathetic activity, reflecting cardiomyopathy progression rather than diabetic neuropathy[40]. Therefore, due to the cross-sectional design of our study, we cannot exclude the possibility of reverse causality. Longitudinal epidemiological studies support that CAN primarily contributes to the development of HF, rather than being a consequence of it[18, 27]. In addition, since individuals in this study showed low NYHA scores and thus likely possess an early-stage HF less likely to explain CAN in this group of people with long-standing T2D very likely to have developed CAN due to diabetes. The WATCH-DM score is a validated tool for predicting 5-year HF risk in T2D using routine clinical data[13], however the score is not designed for diagnosing HF[18]. The CANCAN study includes secondary care patients with more advanced diabetes compared to the general population with T2D**.** It remains to be demonstrated whether these findings can be generalized to a broader T2D population without a history of CVD, such as those in primary care.

In conclusion, this study shows an association between CAN and indicators of HF, including elevated NT-proBNP levels, NYHA scores, and WATCH-DM scores in individuals with T2D. Notably, the finding between CAN and elevated NT-proBNP was evident among those individuals who had no symptoms of HF when assessed by NYHA score or had low-to-moderate risk of HF using WATCH-DM score. Our findings, supports a potential role of CAN detection to identify people at overall higher risk of complications and specifically with higher risk of early-stage HF not uncovered using conventional risk scores and HF symptom assessments as WATCH-DM risk score and NYHA score. These findings highlight the role of CAN detection at the point of care to identifying high-risk individuals with T2D who may benefit from a more timely and personalized care.

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Authors' contributions

Study concept and design: JRS, DRW, LB, RPB, AS, STA. Contributed to the data: JRS, LB, HHT, STA, AS. Planning the statistical analysis: JRS, DRW, LB, STA, CSH, RPB. Conducted the statistical analysis: JRS and LB. All authors contributed to, critically revised, and approved the final version of the manuscript. JRS is the guarantor of this work and, as such, has 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.

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Conflicts of interests

All the authors declare that there is no duality of interest associated with their contribution to this manuscript.

Availability of data and materials

The CANCAN study is managed by a steering committee at Steno Diabetes Center Aarhus, Denmark. The committee encourages interested researchers to use this resource. For further inquiries, please contact STA or JRS.

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Tables and figures

Table 1: Study characteristics
Table 2: CAN and risk of heart failure defined by NT-proBNP
Figure 1: Distribution of NT-proBNP, NYHA, and WATCH-DM by CAN status