

Supplementary material:

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

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1. Documentation

NT-proBNP analysis

An assay is a two-step immunoassay designed for the quantitative in vitro determination of NT-proBNP in human serum and plasma, utilizing chemiluminescent microparticle immunoassay technology on the Cobas e601 analysis instrument. The sample is first incubated with a biotinylated monoclonal NT-proBNP-specific antibody and a ruthenium-labeled monoclonal NT-proBNP-specific antibody, forming a sandwich complex with the NT-proBNP antigen. In the second incubation, streptavidin-coated microparticles are added, binding the complex to a solid phase via biotin-streptavidin interaction. The reaction mixture is then transferred into the measuring cell, where magnetically captured microparticles are immobilized on an electrode surface. Unbound substances are washed away using ProCell/ProCell M. By applying a voltage to the electrode, a chemiluminescence reaction is induced, and the emitted signal is detected using a photomultiplier. The NT-proBNP concentration is determined using a calibration curve, which is generated instrument-specifically via two-point calibration and a master curve embedded in the reagent barcode or e-barcode.

New York Heart Association classification of heart failure

| Classification | Symptoms |
|-----------------|---|
| <i>NYHA I</i> | No physical limitations. Ordinary physical activity does not cause breathlessness, fatigue, or palpitations. |
| <i>NYHA II</i> | Mild limitation of physical activity. No symptoms at rest, but ordinary physical activity (e.g., climbing stairs to the second floor, mowing the lawn, vacuuming, carrying heavier groceries) causes some breathlessness, fatigue, or palpitations. |
| <i>NYHA III</i> | Marked limitation of physical activity. No symptoms at rest, but light physical activity (e.g., walking on flat ground, dressing/undressing, climbing stairs to the first floor) causes pronounced symptoms. |
| <i>NYHA IV</i> | Symptoms may be present at rest and occur with any physical activity. |

Physical activity

| Level of physical activity | Description |
|----------------------------|---|
| <i>Sedentary</i> | Reads, watches television, or engages in other sedentary activities |
| <i>Light activity</i> | Walks, cycles, or performs other light physical activity for at least 4 hours per week (including Sunday walks, light gardening, or walking/cycling to work). |
| <i>Moderate activity</i> | Participates in recreational sports or performs heavy gardening or similar activities at least 4 hours per week. |
| <i>High activity</i> | Engages in intense exercise or sports regularly several times per week. |

2. Tables and figures

Table S1: Sensitivity analysis excluding individuals without history of CVD or beta-blockers

| Model | OR (CI: 95%) | | |
|---------|--------------------|---|-----------------------------------|
| | All participants | Without history of CVD or HF ^a | Without betablockers ^b |
| Model 2 | 5.81 (2.2; 15.34) | 3.74 (0.99; 14.21) | 6.25 (1.03; 38.08) |
| Model 3 | 5.99 (1.83; 19.61) | 9.41 (1.44; 61.33) | 9.41 (1.44; 61.33) |

Odds ratio for NT-proBNP > 125 pg/ml comparing CAN to no CAN.

^aModel adjustments: Model 2: Age, sex, diabetes duration, HbA1c, smoking status, BMI, anti-hypertensives, total cholesterol, triglycerides, systolic blood pressure. Model 3: Model 2 + eGFR.

^bModel adjustments: Model 2: Age, sex, diabetes duration, HbA1c, smoking status, BMI, anti-hypertensives, total cholesterol, triglycerides, systolic blood pressure. Model 3: Model 2 + history of cardiovascular disease, eGFR. CAN, cardiovascular autonomic neuropathy. CART, cardiovascular autonomic reflex test. NT-proBNP, N-terminal pro b-type natriuretic peptide. BMI, body mass index.

Table S2: CAN diagnosis association with HF based symptoms defined as NYHA score \geq II

| | Non-cases/ cases | Model 1 | Model 2 | Model 3 |
|----------------------|---------------------|--------------------|--------------------|-------------------|
| CAN diagnosis | 78/52 | 5.44 (2.25; 13.15) | 6.43 (2.35; 17.62) | 5.51 (1.9; 15.97) |

Odds ratio for HF based symptoms defined as NYHA score \geq II by CAN diagnosis compared 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 and eGFR. CAN, cardiovascular autonomic neuropathy. BMI, body mass index.

Table S3: Study population characteristics by Valsalva maneuver

| Characteristic | Missing | Overall N = 155 | Normal VM values N = 79 | Abnormal VM values N = 52 | Low air pressure N = 24 |
|---|---------|--------------------|-------------------------------|---------------------------------|-------------------------------|
| Sex | 0 | | | | |
| Men | | 93 (60%) | 54 (68%) | 33 (63%) | 6 (25%) |
| Women | | 62 (40%) | 25 (32%) | 19 (37%) | 18 (75%) |
| Age (years) | 0 | 63 (55, 70) | 61 (52, 69) | 63 (57, 69) | 73 (63, 78) |
| Smoking status | 0 | | | | |
| Daily | | 63 (41%) | 31 (39%) | 20 (38%) | 12 (50%) |
| Weekly/Occasionally | | 70 (45%) | 36 (46%) | 23 (44%) | 11 (46%) |
| Quit/Never smoked | | 22 (14%) | 12 (15%) | 9 (17%) | 1 (4%) |
| BMI (kg/m ²) | 0 | 33 (28, 37) | 32 (28, 38) | 34 (30, 38) | 30 (27, 34) |
| Duration of type-2 diabetes (years) | 0 | 16 (11, 24) | 15 (9, 20) | 19 (14, 24) | 23 (13, 33) |
| eGFR category | 4 | | | | |
| <30 | | 15 (10%) | 6 (8%) | 4 (8%) | 5 (22%) |
| 30-59 | | 29 (19%) | 10 (13%) | 16 (31%) | 3 (13%) |
| 60-89 | | 35 (23%) | 17 (22%) | 10 (20%) | 8 (35%) |
| ≥90 | | 72 (48%) | 44 (57%) | 21 (41%) | 7 (30%) |
| Orthostatic hypertension | 0 | 22 (14%) | 9 (11%) | 8 (15%) | 5 (21%) |
| NYHA score | 0 | | | | |
| I | | 118 (76%) | 69 (87%) | 32 (62%) | 17 (71%) |
| II | | 25 (16%) | 8 (10%) | 12 (23%) | 5 (21%) |
| III | | 11 (7%) | 2 (3%) | 7 (13%) | 2 (8%) |
| IV | | 1 (1%) | 0 (0%) | 1 (2%) | 0 (0%) |
| NT-proBNP categories | 0 | | | | |
| <50 | | 65 (42%) | 46 (58%) | 15 (29%) | 4 (17%) |
| 50-124 | | 34 (22%) | 17 (22%) | 9 (17%) | 8 (33%) |
| 125-300 | | 22 (14%) | 7 (9%) | 12 (23%) | 3 (13%) |
| 300+ | | 34 (22%) | 9 (11%) | 16 (31%) | 9 (38%) |
| NT-proBNP > 125 ml/pg | 0 | | | | |
| Below | | 99 (64%) | 63 (80%) | 24 (46%) | 12 (50%) |
| Above | | 56 (36%) | 16 (20%) | 28 (54%) | 12 (50%) |
| WATCH-DM risk score | 20 | 13.0 (10.5, 16.0) | 12.0 (10.0, 16.0) | 15.0 (12.0, 16.0) | 14.5 (13.0, 17.8) |
| WATCH-DM risk score (high to very-high risk) | 20 | 41 (30%) | 19 (26%) | 16 (36%) | 6 (33%) |
| History of myocardial infarction | 0 | 18 (12%) | 6 (8%) | 11 (21%) | 1 (4%) |
| History of stroke | 0 | 18 (12%) | 8 (10%) | 8 (15%) | 2 (8%) |
| History of major cardiovascular events | 0 | 41 (26%) | 17 (22%) | 20 (38%) | 4 (17%) |
| History of heart failure | 2 | 15 (10%) | 2 (3%) | 9 (17%) | 4 (17%) |

Figure S1: Study and analysis flowchart

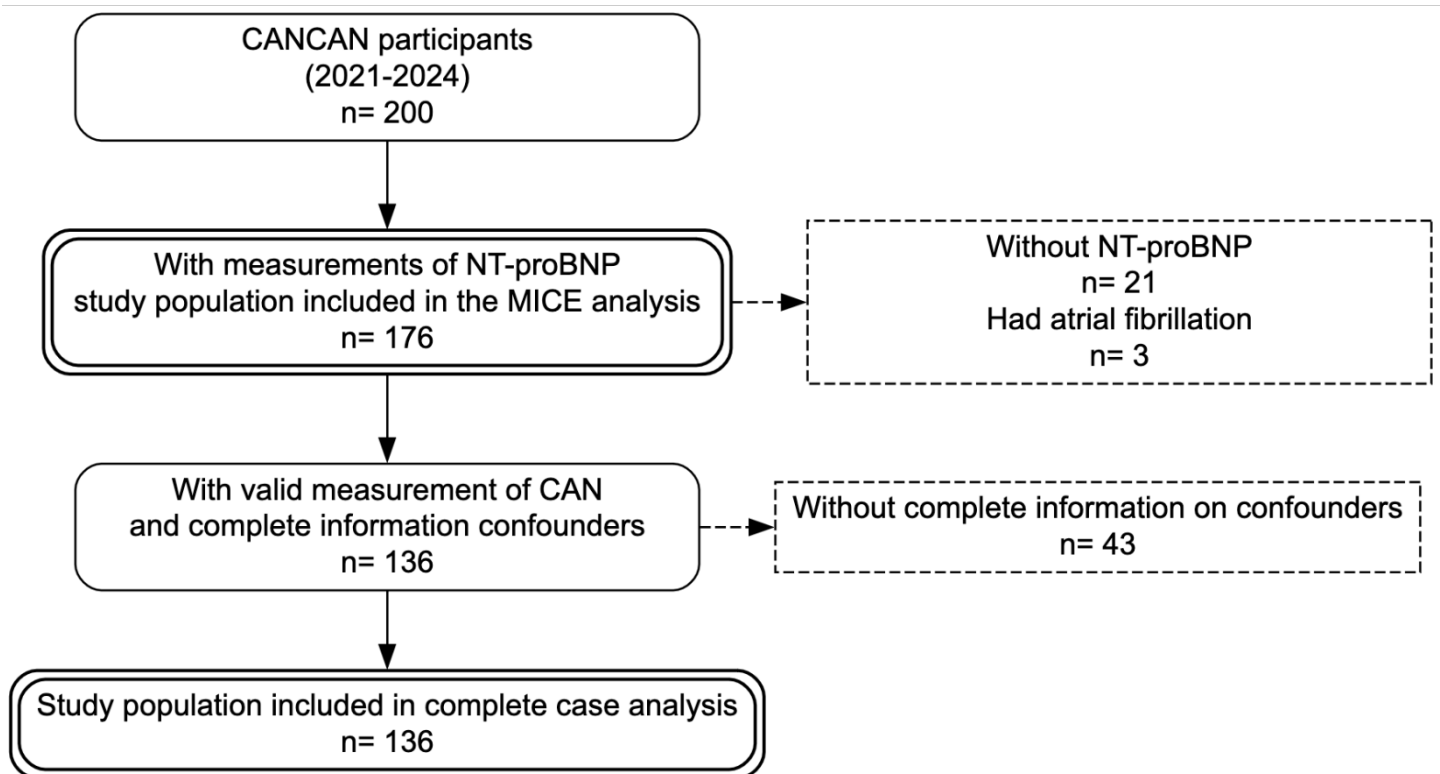


Figure S2: **Distribution of NT-proBNP, NYHA, and WATCH-DM**

