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External validation of a clinical risk score for the presence of cardiovascular autonomic neuropathy in type 1 diabetes

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ABSTRACT

Aims: To validate in an independent external population a CAN Risk Score previously developed in type 1 diabetes (T1D) and validated for cardiovascular autonomic neuropathy (CAN) with a good diagnostic accuracy.
Methods: Forty-seven participants with T1D (age 47.7 ± 13.2 years, duration of diabetes 30.0 (19.0–40.5) years, 24 males) underwent 4 cardiovascular reflex tests (CARTs) to diagnose early and confirmed CAN (according to 1 or 2 abnormal results). CAN Risk Score was calculated from resting heart rate, HbA1c, retinopathy and/or nephropathy, cardiovascular disease, HDL cholesterol, systolic blood pressure and smoking (range 0–10).
Results: Eleven participants (23.4 %) had CAN. The CAN Risk Score was higher in subjects with overall CAN (early and confirmed) ($P = 0.0498$) and with confirmed CAN ($P = 0.0142$) compared to those without, and correlated with CARTs severity (rho = 0.32, $P = 0.026$), Expiration/Inspiration ratio ($r = -0.33$, $P = 0.0258$) and Valsalva ratio ($r = -0.47$, $P = 0.0015$). A CAN Risk Score ≥ 4 was found in 19 participants and was associated with the presence of confirmed CAN ($P = 0.0129$). The CAN Risk Score showed an area under the ROC curve (AUC) of 0.802 ± 0.080 for confirmed CAN, and at the cut-off of 4, sensitivity, specificity and negative predictive values of 85.71 %, 67.50 % and 96.43 %.
Conclusions: This study confirmed the diagnostic value of the CAN Risk Score and supports its inclusion in a diagnostic algorithm to identify candidates for CARTs, thereby reducing universal screening. Using routinely available clinical data as categorical variables, the score is easy to calculate and implement in clinical settings.

1. Introduction

Cardiovascular autonomic neuropathy (CAN), defined as the impairment of autonomic control of the cardiovascular system in the setting of diabetes or prediabetes after the exclusion of other causes,[1,2] has a prevalence ranging from 20 % to 54 % in individuals with type 1 diabetes (T1D).[2,3] CAN is associated with a more than three-fold increase in the risk of all-cause mortality and cardiovascular events.[4] This epidemiological and prognostic impact supports the strong recommendations of scientific societies for screening and diagnosis of CAN, despite non-unanimous approaches.[1,5–8] However, CAN remains widely underdiagnosed.

The cardiovascular autonomic reflex tests (CARTs) are the gold-standard for the diagnosis of CAN but require specialized equipment, standardized protocols and trained personnel, which makes them resource-intensive and time-consuming. Among the attempts to address the burden of CAN screening and diagnosis the guidelines have suggested narrowing the candidate pool to those at greater risk.[2,5,8] In this line, a few clinical scoring models for CAN have been developed mainly in type 2 diabetes possibly useful to identify patients with a priority for CAN screening and performance of CARTs.[9–12]

Recently, we validated a simple risk score for the presence of CAN in a population of 115 individuals with T1D.[13] This scoring system was based on easily detectable clinical parameters used as categorical variables, i.e., resting heart rate (HR) of ≥80 bpm, HbA1c of ≥8 %, presence of diabetic retinopathy and/or diabetic nephropathy, systolic blood pressure (BP) ≥140 mmHg, HDL cholesterol ≤40/50 mg/dl according to the sex, presence of cardiovascular disease, and current smoking (range 1–10). This score demonstrated a good diagnostic accuracy for detection of confirmed CAN with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.89, and at the cut-off of 4 a sensitivity of 88 % and a negative predictive value for ruling out CAN of 96 %.

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We aimed to validate this previously developed CAN Risk Score[13] in a cross-sectional, single-center retrospective study involving an independent, unselected external population with T1D.

2. Subjects, materials and methods

2.1. Subjects

We conducted a retrospective evaluation of outpatients who underwent routine diagnostic assessment of diabetic neuropathy at the diabetes clinic of the University of Rome Tor Vergata (Rome, Italy) between 2011 and 2024.

We included subjects with T1D, a minimum diabetes duration of 5 years and an age ranging from 18 to 80 years. Exclusion criteria comprised severe comorbidities, including recent cardiovascular events, heart failure, and renal failure [estimated glomerular filtration rate (eGFR) <45 mL/min/1.73 m^2], peripheral or autonomic neuropathies from causes other than diabetes, advanced peripheral arterial disease requiring revascularization, active limb ulcers, major amputations, and serious psychiatric disorders, or conditions preventing neuropathy assessment or accurate completion of questionnaires.

The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki (2013 revision) and received approval by the Lazio Area 2 Territorial Ethics Committee (approval no. 91/24). All participants provided written informed consent to be included in the study. We collected detailed clinical history, focusing on diabetes and any potential causes of polyneuropathy. Anthropometric, clinical, and metabolic parameters were measured and recorded, including body mass index (BMI), waist and hip circumferences, smoking and alcohol consumption habits, physical activity, glycated hemoglobin (HbA1c), serum cholesterol (total, HDL, LDL), triglycerides, and serum creatinine levels. Capillary blood glucose was measured during neurologic testing. Three BP measurements were taken 1 to 2 min apart using an automated device and with participants seated with uncrossed legs and remaining silent during the measurements.

Participants who engaged in physical activity for at least 1 h per week were classified as physically active. Those who smoked at least one cigarette per day were categorized as current smokers, and those who consumed at least one alcoholic drink per day as alcohol consumers. Microvascular and macrovascular complications, including previous coronary and/or cerebrovascular events, were searched for in medical history and records. Chronic kidney disease diagnosis was based on the presence of micro- and macroalbuminuria (according to 24-hour albuminuria levels of 30–299 mg/24 h and ≥ 300 mg/24 h, or an albumin-creatinine ratio of 30–299 mg/g and ≥ 300 mg/g, respectively) and/or a reduced eGFR.[14] Diagnosis of diabetic retinopathy was based on a comprehensive and dilated eye examination by an ophthalmologist, with additional procedures if deemed necessary according to standard of care. Peripheral vascular disease was assessed by clinical criteria (i.e., presence of claudication and/or absence of palpable dorsalis pedis and/or posterior tibial pulses) or by instrumental reports (Doppler sonography).

2.2. Neurological examinations

We used the Michigan Neuropathy Screening Instrument Questionnaire (MNSI-Q) and the Michigan Diabetic Neuropathy Score (MDNS)[15] to assess symptoms and signs of diabetic polyneuropathy (DPN), respectively. Responses to MNSI-Q were checked by the clinician to clarify apparent incongruences. Vibration perception thresholds (VPT) at the hallux dorsum and lateral malleolus were tested using the limits method, while warm (WTT) and cold thermal perception thresholds (CTT) were measured at the dorsum of the feet according to the levels method by means of the Neurosensory Analyzer TSA-II (Medoc, Ramat Yishai, Israel). DPN was classified as probable DPN in the presence of at least two abnormalities among neuropathic symptoms, signs, VPT, or thermal thresholds.[1,16]

Autonomic function was assessed using four CARTs: HR response to deep breathing, lying-to-standing, and the Valsalva maneuver, which provided the Expiration/Inspiration ratio, 30:15 ratio, and Valsalva ratio, along with the orthostatic hypotension test. CARTs were conducted using the DAN computerized system for data acquisition and analysis (Microlab Elettronica Sas, Padua, Italy) following standard procedures and applying age-related reference values for the HR-based CARTs. A CARTs score was calculated from the sum of scores assigned to each test (0 for normal, 1 for borderline, and 2 for abnormal results), yielding a total range of 0–8.[2,17] Early and confirmed CAN were defined by the presence of one and two abnormal tests, respectively.[1,2,17]

Resting HR as a component of CAN Risk Score was measured from the average HR of the last 10-s ECG sequences of two 1-min ECG recordings taken at the end of a 2–5 min rest with the participant lying down.

2.3. Statistical analysis

The Shapiro-Wilk test of normality was applied to test the distribution of data. Data were specified as means ± SD or medians with interquartile ranges based on data distribution. Descriptive statistics were used to characterize clinical variables of the participants.

Comparisons between two groups of continuous variables were made with unpaired Student's *t*-test or Mann-Whitney test for normally or non-normally distributed data, respectively. Pearson's correlation coefficient (r) and Spearman's correlation coefficient (rho) were employed to assess reciprocal relationships between normally and non-normally distributed variables, respectively.

Moreover, the CAN Risk Score was calculated by using binary variables and assigning a score of 2 to the presence of resting HR ≥80 bpm, HbA1c ≥8 %, and retinopathy and/or nephropathy, and a score of 1 to the presence of systolic BP ≥140 mmHg, HDL ≤40 or ≤ 50 mg/dl in males and females, respectively, cardiovascular disease, and current smoking status (Supplementary Table 1). The choice of these variables in our previous study[13] was based on the results of univariate and multivariate logistic regression analysis for overall and confirmed CAN, where the components of the CAN Risk Score had been included as binary variables, being age, sex, diabetes duration, and BMI the additional independent variables in multivariate analysis. The different weighted score was based on their strength of association with overall and confirmed CAN in multivariate analysis, by giving a score of 2 to the variables with the highest degree of significance for both overall and confirmed CAN and 1 to those with significance of minor degree and/or limited to overall or confirmed CAN.[13]

We employed the ROC analysis and measured the AUC to evaluate the diagnostic accuracy of the CAN Risk Score in distinguishing between individuals with and without CAN. We used the Chi square test to calculate the sensitivity, specificity, positive and negative predictive values, and the Youden's J index (as sensitivity + specificity - 1) for the diagnosis of CAN of the CAN Risk Score at the cut-off of 4. Two-sided 95 % confidence intervals (CI) were determined.

All statistical analyses were performed using the StatView IV programme (SAS Institute Inc., Cary, NC, USA) and Statistics/Data Analysis (STATA) (StataCorp LP, TX, USA). A two-tailed value of $P < 0.05$ was considered significant. Fisher's exact *P* value was chosen for the Chi-square test.

3. Results

Among the 51 subjects with T1D who were recruited, 47 (24 males) were included according to the selection criteria. Participants' mean age was 47.7 ± 13.2 years, their mean diabetes duration was 30.0 (19.0–40.5) years, and 16 were on beta blocker agents (Supplementary Table 2).

We divided participants into two groups according to their

calculated CAN Risk Scores <4 or ≥ 4. The former group had a mean CAN Risk Score of 1.39 ± 0.99, while the latter had a score of 5.26 ± 1.33 ($P < 0.0001$). When considering their clinical and neurologic characteristics, the 19 subjects with a CAN Risk Score of ≥4 showed higher HbA1c levels, lower HDL-cholesterol levels, higher systolic BP compared to the 28 with a CAN Risk Score < 4, in addition to a significantly higher proportion being affected by retinopathy and/or nephropathy (Table 1). These findings were expected, given that these clinical variables are components of the CAN Risk Score.

Furthermore, neurologic impairment was consistently more severe in the group with a CAN Risk Score of ≥4 compared to those with a CAN Risk Score < 4 (Table 1). Specifically, a CAN Risk Score value of ≥4 was associated with the presence of confirmed CAN ($P = 0.0129$), as well as with lower Expiration/Inspiration ratio ($P = 0.0248$), 30/15 ratio ($P = 0.0472$) and Valsalva ratio ($P = 0.0108$) (Table 1).

Moreover, the CAN Risk Score was significantly higher in subjects with overall CAN (i.e., early and confirmed) (4.18 ± 2.27) and in those with confirmed CAN (5.14 ± 2.19) compared to those without CAN (2.58 ± 2.10) ($P = 0.0356$ and $P = 0.0037$, respectively).

In addition, the CAN Risk Score correlated positively with CARTs impairment expressed by CARTs score (rho = 0.32, $P = 0.026$), and inversely with Expiration/Inspiration ratio ($r = -0.33$, $P = 0.0258$) and Valsalva ratio ($r = -0.47$, $P = 0.0015$), while it showed borderline statistical significance with 30/15 ratio (rho = −0.29, $P = 0.0501$) (Supplementary Fig. 1).

With regard to the diagnostic accuracy of the CAN Risk Score, the ROC curve showed an AUC of 0.802 for the diagnosis of confirmed CAN and an AUC of 0.696 for overall CAN (Fig. 1). Furthermore, at a cut-off score of 4, the CAN Risk Score exhibited a sensitivity of 85.71 %, a specificity of 67.50 % and a negative predictive value of 96.43 % for confirmed CAN diagnosis, and a sensitivity of 63.64 %, a specificity of 66.67 % and a negative predictive value of 85.71 % for overall CAN diagnosis (Table 2). To evaluate if the use of beta-blockers might influence the diagnostic performance of the CAN RIsk Score, we performed an exploratory analysis excluding the sixteen subjects on beta-blocker therapy. We obtained an AUC of 0.935 ± 0.049 for confirmed CAN and 0.790 ± 0.115 for overall CAN (Supplementary Fig. 2). Moreover, at the cut-off of 4 the CAN Risk Score exhibited a sensitivity of 100 %, a specificity of 77.8 % and a negative predictive value of 100 % for confirmed CAN diagnosis, and a sensitivity of 66.7 %, a specificity of 76.0 %, and a negative predictive value of 90.5 % for overall CAN diagnosis.

Table 1
Clinical and neurologic characteristics of participants according to CAN Risk Score cut-off of 4. In bold significant values of P.

Parameter	CAN RS <4	CAN RS ≥4	P
Number	28	19	-
Sex (M:F)	15:13	9:10	0.9043
Age (years)	47.79 ± 11.94	47.68 ± 15.11	0.9796
Diabetes duration (years)	27.00 ± 13.36	35.21 ± 15.33	0.0576
BMI (kg/m^2)	25.00 ± 3.74	26.20 ± 5.10	0.3534
HbA1c (%)	7.15 (6.58-7.50)	8.55 (8.15-9.70)	**<0.0001**
HbA1c (mmol/mol)	54.00 (48.75-58.00)	69.50 (65.25-83.00)	**<0.0001**
eGFR (ml/min/ 1.73 m^2)	94.92 ± 19.41	94.26 ± 24.27	0.9248
Total cholesterol (mg/dl)	162.50 (134.50-174.75)	160.00 (128.00-206.00)	0.9030
HDL cholesterol (mg/dl)	58.00 (52.50-68.50)	48.00 (39.25-56.50)	**0.0092**
Triglycerides (mg/dl)	66.00 (49.00-87.00)	80.00 (64.00-125.00)	0.0671
Systolic blood pressure (mmHg)	128.61 ± 17.96	138.74 ± 15.61	0.0518
Diastolic blood pressure (mmHg)	78.18 ± 8.22	79.68 ± 8.12	0.5388
With retinopathy (n, %)	6, 21.42	14, 73.68	**0.0011**
With nephropathy (n, %)	3, 10.71	9, 47.37	**0.0157**
With hypertension (n, %)	11, 39.29	13, 68.42	0.0962
With cardiovascular disease (n, %)	3, 10.71	6, 31.58	0.1596
Smokers (n, %)	4, 14.29	6, 31.58	0.2898
Physical activity (n, %)	16, 57.14	7, 36.84	0.2310
Alcohol consumption (n, %)	2, 7.14	0, 0.00	0.6496
Heart rate (bpm)	68.41 ± 11.61	73.18 ± 17.43	0.3040
With beta-blockers (n, %)	7, 25.00	9, 47.37	0.2025
Expiration/Inspiration ratio	1.39 ± 0.19	1.26 ± 0.19	**0.0248**
30:15 ratio	1.24 (1.14-1.30)	1.10 (1.03-1.27)	**0.0472**
Valsalva ratio	1.66 ± 0.32	1.41 ± 0.23	**0.0108**
Orthostatic hypotension (mmHg)	13.50 (10.00-17.00)	14.00 (7.00-24.00)	0.9826
CARTs score	0.00 (0-1.25)	1.00 (0-6.00)	0.1020
With CAN (early and confirmed) (n, %)	4. 14.29	7, 36.84	0.0912
With CAN (confirmed) (n, %)	1, 3.57	6, 31.58	**0.0129**
MNSI-Q	1.00 (0.00-2.00)	3.00 (0.50-4.50)	**0.0064**
MDNS	2.00 (2.00-5.00)	5.00 (2.50-8.00)	**0.0418**
VPT (Volt)	12.12 (8.97-19.31)	26.50 (13.43-34.90)	**0.0106**
Warm perception threshold (°C)	33.80 (32.90-36.25)	37.90 (33.96-40.75)	**0.0264**
Cold perception threshold (°C)	31.30 (29.60-31.70)	26.75 (19.05-28.73)	**0.0002**
With DPN (n, %)	11, 39.29	15, 78.95	**0.0171**

Abbreviations: BMI: body mass index; CAN: cardiovascular autonomic neuropathy; DPN: diabetic polyneuropathy; eGFR: estimated glomerular filtration rate; F: female; HbA1c: glycosylated hemoglobin; HDL: high-density lipoprotein; CARTs: cardiovascular reflex tests; M: male; MDNS: Michigan Diabetic Neuropathy Score; MNSI-Q: Michigan Neuropathy Screening Instrument Questionnaire; VPT: vibration perception threshold.
Values are presented as median (interquartile range), number (%), or mean ± standard deviation. The unpaired t test (parametric) and Mann-Whitney U test (nonparametric) were used as tests of significance for means and the chi-square test (with the Fisher exact P value) was used for categorical variables.

4. Discussion

We recently developed a clinical score for the detection of risk of CAN in T1D.[13] This CAN Risk Score is easily accessible because it consists of 7 routinely collected clinical parameters, well recognized as risk factors or markers for CAN,[3,18,19] here used as categorical variables, i.e., HR, HbA1c, retinopathy and/or nephropathy, systolic BP, HDL-cholesterol, cardiovascular disease and smoking status.[13] The present study was aimed at validating this clinical CAN Risk Score in an external independent unselected population with T1D and showed that, at the cut-off of 4, it maintained a good sensitivity for confirmed CAN (86 %) and a high negative predictive value for both overall and confirmed CAN (86 % and 96 %, respectively).

CARTs are still the gold standard for CAN diagnosis but their universal implementation for CAN screening appears unfeasible for several reasons including the reduced availability of necessary equipment, the limited expertise of clinicians, the short duration of routine diabetes consultations, and the non-defined cost-effectiveness. Thus, the selection of subjects at higher risk for CAN is a promising strategy for a targeted use of CARTs. Few studies have focused on this objective in T1D.

Tran and colleagues developed a web-based calculator from a large cross-sectional cohort to predict the risk of T1D-related microvascular complications, including autonomic neuropathy.[20] This tool was based on a nomogram including 4 factors, i.e., age, age at T1D diagnosis, average HbA1c and systolic BP of the last 3 clinical visits. However, the diagnosis of CAN was extracted from electronic health records and the study lacked an external validation. In addition, no cut-off for predicted probability was established for referral to CARTs as the calculator was above all intended to raise awareness about microvascular complications among clinicians and patients rather than serving as a screening tool.

To date, only one study has proposed a numerical risk score for CAN in T1D, which included total cholesterol, triglycerides, postprandial sweating, diastolic BP, abnormalities in monofilament test, retinopathy, and nephropathy.[12] However, this score was based on continuous

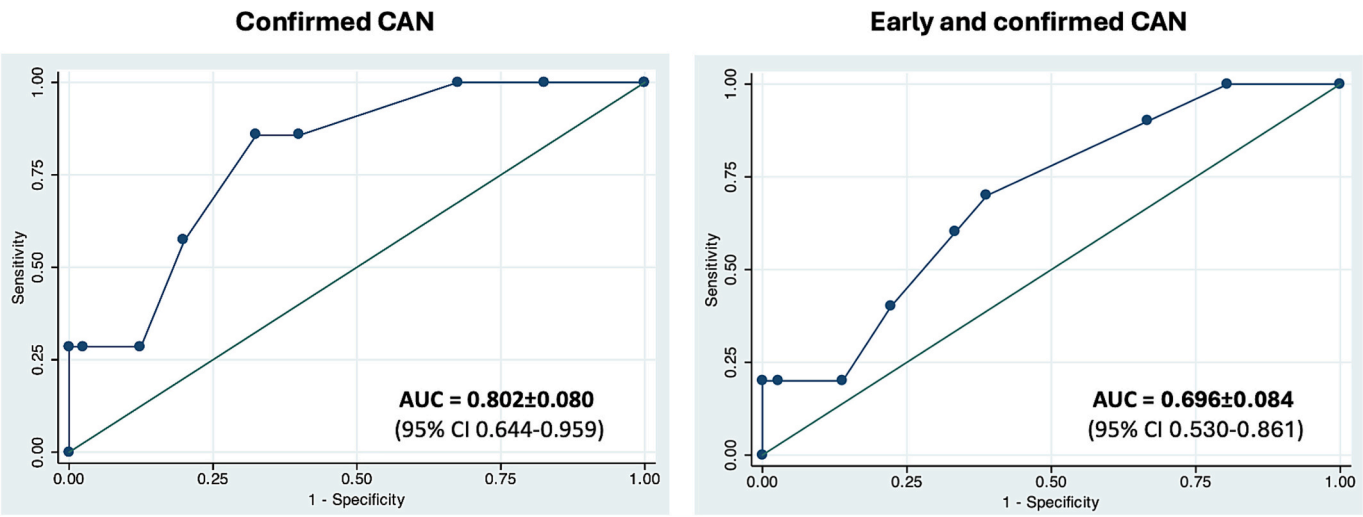

Fig. 1. Diagnostic accuracy of CAN Risk Score for confirmed CAN and overall (early and confirmed) CAN measured using area under the ROC curve (AUC).

Table 2
Diagnostic performance of CAN Risk Score at the cut-off of 4 for CAN and confirmed CAN. In bold values over 80%.

Cut-off ≥4	CAN (early and confirmed)	Confirmed CAN
Sensitivity	63.64% (CI 35.21% - 92.07%)	**85.71**% (CI 59.78% - 111.64%)
Specificity	66.67% (CI 51.27% - 82.07%)	67.50 % (CI 52.98% - 82.02%)
Positive predictive value	36.84% (CI 15.15% - 58.53%)	31.58% (CI 10.68% - 52.48%)
Negative predictive value	**85.71**% (CI 72.75% - 98.67%)	**96.43**% (CI 89.56% - 103.30%)
Youden's J	0.30	0.53

Abbreviations: CI, Confidence intervals.

variables and a complex formula, which is likely to restrict its use, and its diagnostic performance failed to be confirmed when applied to an external population being the sensitivity only 50 %. Moreover, parameters such as postprandial sweating and monofilament testing are not routinely recorded in diabetes consultations.

Another risk calculator, the Steno Type 1 Risk Engine (ST1RE), was developed primarily to estimate the risk of cardiovascular events in T1D in a retrospective longitudinal study, but it also demonstrated significant associations with microangiopathic complications including autonomic neuropathy.[21] This online risk calculator is based on 10 variables (age, sex, diabetes duration, smoking status, HbA1c, systolic BP, LDL-cholesterol, albuminuria, eGFR, physical exercise) and allows stratification of individuals into three categories, i.e., low risk, moderate risk, and high risk. However, diagnosis of autonomic neuropathy relied solely on symptoms and physical examination findings (e.g. orthostatic hypotension, severe hypoglycemia, asymptomatic hypoglycemia, symptoms suggestive of gastroparesis, erectile dysfunction without another identifiable cause, or retrograde ejaculation), which could limit the diagnostic performance of this tool. Moreover, this kind of risk calculators have mainly the value of prognostic prediction of development of complications,[22] whereas the CAN Risk Score used here is primarily aimed at screening in the diagnostic pathway of CAN.[19]

Machine learning models have also been used for the prediction of microvascular complications, including CAN, in type 2 diabetes (T2D). For example, a cross-sectional study conducted in a Bangladeshi population with T2D (n = 96) analyzed the associations between demographic, clinical, laboratory features and CAN using a chi-squared test.[23] Significant variables were input into machine learning models, and the random forest (RF) model provided the highest accuracy for predicting CAN (98.67 %). A 'prior probability' model was used to adjust for class imbalances, as the vast majority of participants were diagnosed with CAN (65 out of 96, including 21 with no available test results). However, this might have increased the level of approximation in the study findings. Conversely, the risk score validated in this study is made up of binary variables and hence is easy to use, straightforward to calculate and reliant on readily available clinical and/or laboratory parameters, which facilitates its adoption in clinical practice.

Because of its very high negative predictive value at the proposed cut-off score of 4 or greater, this CAN risk score represents a useful tool for identifying low-risk individuals in whom CARTs are unlikely to be advantageous and cost-effective. Moreover, it exhibited a good sensitivity especially for confirmed CAN cases, which would aid in identifying individuals at higher risk for CAN.

The CAN Risk Score and its proposed use align with Bang's editorial on biomarker scores in T2D risk prediction,[24] where the author highlights that a biomarker risk score should be cost-effective, have a clear "next step" (i.e., CARTs), and address a recognized need in the real world (i.e., the underdiagnosis of CAN) and potential users.

4.1. Limitations and strengths

Our study has some limitations. Firstly, the sample size was relatively small. Secondly, the prevalence of CAN in this external validation study (23.4 %) was lower than in the original validation study (36.5 %), which can affect generalizability, despite the prevalence being consistent with reported figures in the literature for T1D. Our single-center, cross-sectional and retrospective design is another limitation.

Nevertheless, gold-standard CARTs were employed for the diagnosis of CAN and the participants were well-characterized for clinical variables. Furthermore, this tool is easily accessible because of the use of parameters, used as binary variables, which are routinely collected in clinical practice. The present validation study showed that the CAN Risk Score previously developed is applicable across various populations with T1D, even with different prevalences of CAN.

Given that this validation study was from the same clinic and geographical area as the original study,[13] further validation in a wider population of another country and ethnicity is also needed.

In the original study[13] we excluded patients on beta-blockers to avoid their possible confounding effect on resting heart rate and then to be able to consider this accessible autonomic index as a reliable component of the CAN Risk Score. In this study, we did not keep this exclusion criterion to test the applicability of the CAN Risk Score in a wider population in a real-world setting and nevertheless we got a

confirmation of its diagnostic validity for CAN. However, we also performed an exploratory analysis to assess the diagnostic value of CAN Risk Score after excluding the sixteen participants using beta-blockers and found that diagnostic accuracy and performance of the CAN Risk Score remained high and slightly improved. This strengthens the external validation and raises the question of whether the use of beta-blockers might lessen the diagnostic performance of the CAN Risk Score given their effect on resting HR, which is a component of this scoring system.

4.2. Application in clinical practice

The integration of this easily accessible CAN Risk Score in a diagnostic algorithm[19] can help identify subjects at greater risk for CAN, candidates for CARTs performance, thus reducing by more than half the burden of universal screening and possibly the magnitude of underdiagnosis.

Moreover, due to its simplicity, the implementation of the CAN Risk Score could increase awareness of CAN among clinicians and patients and therefore contribute to broadening the diagnosis of CAN. Finally, as the CAN Risk Score consists of some factors that are potentially modifiable (HbA1c, systolic BP, HDL-cholesterol, smoking), its application could motivate clinicians and patients to address these risk factors more proactively through lifestyle modifications and/or pharmacological therapy.

4.3. Conclusions

In conclusion, this CAN Risk Score for T1D is the first with diagnostic properties supported by external validation. Given the limited sample size, a further validation in a larger and more diverse population is warranted. Due to its characteristics, this CAN Risk Score can be effectively included in a recommended diagnostic algorithm for CAN, with the potential benefits of enhancing awareness of this diabetic complication among health practitioners and patients, encouraging intervention on modifiable risk factors, and enabling a more targeted and cost-effective implementation of CARTs for CAN diagnosis.

Prior presentation

This study was presented at the 34th Annual Meeting of Diabetic Neuropathy Study Group - NEURODiab, held in Rome Italy from 5 to 8 September 2024.

CRediT authorship contribution statement

Pietro Pertile: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ilenia D'Ippolito:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beatrice De Santis:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aikaterini Andreadi:** Writing – review & editing, Validation, Methodology, Conceptualization. **Davide Lauro:** Writing – review & editing, Validation, Methodology, Conceptualization. **Vincenza Spallone:** Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethical approval

The study was approved by the Lazio Area 2 Territorial Ethics Committee (approval no. 91/24).

Guarantor

VS 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.

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Declaration of competing interest

The Authors declare that there are no potential conflicts of interest relevant to the subject of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2025.109066.

Data availability

The data generated and analyzed during the current study are available from the corresponding author on a reasonable request.

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