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Neuropathy in adolescents with type 1 diabetes: Confirmatory diagnostic tests, bedside tests, and risk factors

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ABSTRACT

Aims: To estimate the prevalence of large fiber (LFN), small fiber (SFN), and autonomic neuropathy in adolescents with type 1 diabetes using confirmatory tests known from adults and to identify risk factors and bedside methods for neuropathy.

Methods: Sixty adolescents with type 1 diabetes (diabetes duration > five years) and 23 control subjects underwent neurological examination and confirmatory diagnostic tests for neuropathy, including nerve conduction studies, skin biopsies determining intraepidermal nerve fiber density, quantitative sudomotor axon reflex test (QSART), cardiovascular reflex tests (CARTs), and tilt table test. Possible risk factors were analyzed. Bedside tests (biothesiometry, DPNCheck®, Sudoscan, and Vagus®device) were compared with the confirmatory tests using ROC analysis.

Results: The prevalence of neuropathies in the adolescents with diabetes (mean HbA1c 7.6% (60 mmol/mol)) was as follows: 14% confirmed/26% subclinical LFN, 2% confirmed/25% subclinical SFN, 20% abnormal QSART, 8% abnormal CARTs, and 14% orthostatic hypotension. Higher age, higher insulin dose, previous smoking, and higher triglycerides level were found to increase the relative risk for neuropathy. The bedside tests showed poor to acceptable concordance with the confirmatory tests (all, AUC $\leq$ 0.75).

Conclusions: The diagnostic tests confirmed the presence of neuropathy in adolescents with diabetes and underscore the importance of prevention and screening.

Abbreviations: BMI, Body mass index; BP, Blood pressure; CAN, Cardiovascular autonomic neuropathy; CARTs, Cardiovascular reflex tests; COMPASS-31, The CompositeAutonomicSymptom Score; HbA1c, Hemoglobin A1c; HR, Heart rate; IENFD, Intraepidermal nerve fiber density; ISPAD, The International Society for Pediatric and Adolescents Diabetes; LFN, Large fiber neuropathy; MAP, Mean artery pressure; NCS, Nerve conduction studies; OH, Orthostatic hypotension; POTS, Postural orthostatic tachycardia syndrome; QSART, Quantitative sudomotor axon reflex test; SFN, Small fiber neuropathy; UENS, The Utah Early Neuropathy Scale.

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1. Introduction

The incidence of type 1 diabetes has increased over the past decades [1], and it is still expected to increase [2]. An estimated peak in global incidence is described for ages 10–14 years, followed closely by adolescents aged 15–19 years with an incidence of 8.1 [2]. The International Diabetes Federation estimates that globally above 1.52 million children and adolescents younger than twenty are living with type 1 diabetes [3]. Although managing diabetes can be time-consuming, proper management can enhance health, improve quality of life, and reduce the risk of complications [4,5]. Diabetes, characterized by elevated levels of blood glucose, can cause changes to cardiovascular system, eyes, kidneys, gut, central and peripheral nervous system over time [6,7].

Previous reviews have revealed that up to 88% of children and adolescents with diabetes have subclinical neuropathy [8,9], with confirmed neuropathy in 0–15% [8]. Abnormal nerve conduction can be detected in newly diagnosed children with diabetes [10], and the prevalence appears to increase rapidly over five years of follow-up [11].

Neuropathy can be classified into large fiber neuropathy (LFN), somatic small fiber neuropathy (SFN), and autonomic neuropathy, and further classified into possible, probable, subclinical, and confirmed [12]. Confirmed neuropathy is defined as having both symptoms and/or abnormal findings (signs) on neurological examination and an abnormal diagnostic test, whereas subclinical neuropathy only involves an abnormal diagnostic test with no symptoms/signs [12].

The literature highlights difficulties in estimating the prevalence of neuropathy in children and adolescents with type 1 diabetes [8,9]. This is likely due to several factors, including differences in definitions used for neuropathy, the types of nerves studied, diagnostic methods employed, and the lack of normative data in the pediatric population [8,9]. The confirmatory diagnostic tests known from adults include nerve conduction studies (NCS) for LFN, skin biopsies evaluating intraepidermal nerve fiber density (IENFD) for somatic SFN, and cardiovascular reflex tests (CARTs) as well as a quantitative sudomotor reflex test (QSART) for autonomic neuropathy [12,13].

Despite diabetic neuropathy being a significant predictor of morbidity and mortality, no clear global consensus exists on the preferred diagnostic methods [14,15]. The International Society for Pediatric and Adolescents Diabetes (ISPAD) describes possible screening tests with a low level of evidence and recommends a combination of history, clinical, examination, and clinical tests [16].

To our knowledge, no previous study has performed a comprehensive range of confirmatory tests to diagnose both large fiber-, small fiber- and autonomic neuropathy in adolescents with type 1 diabetes in the same individuals at the same time, in order to map the extent of nerve damage. Previous studies, researchers have typically investigated only one or two nerve types in the same population of adolescents, with only a few studies using confirmatory tests such as NCS, quantitative sensory testing, and CARTs to estimate the prevalence of neuropathy [8]. Different diagnostic tests and criteria have been used, and to the best of our knowledge, QSART and skin biopsy have never been applied before to adolescents with diabetes. Bedside tests and tests associated with autonomic neuropathy have been more widely used in the pediatric population. However, it is unknown whether these tests correlate with the confirmatory tests and criteria used in adults.

The aims of this study were 1) to estimate the prevalence of confirmed and subclinical LFN, SFN, and autonomic neuropathy in a selected group of adolescents with type 1 diabetes using confirmatory tests known from adults, 2) to examine potential risk factors for neuropathy, and 3) to assess the diagnostic accuracy of available bedside methods for neuropathy compared to the applied confirmatory tests.

2. Research design and methods

2.1. Cross-sectional study

2.1.1. Study population

Inclusion criteria were adolescents aged 15–18 years with a history of type 1 diabetes of at least five years. Participants were recruited from outpatient clinics at Danish hospitals in Randers, Aarhus, and Aalborg and Aarhus Steno Diabetes Centers Aarhus, and North Denmark, between August 2020 and December 2021. Exclusion criteria were adolescents who were taking medication or had diseases that could impact the central or peripheral nervous system. However, the presence of associated well-treated autoimmune disorders (such as thyroid disease, and celiac disease) or complications to diabetes (such as microalbuminuria) was allowed. Healthy adolescent controls were enrolled via advertisement at a boarding school and through social media.

Information regarding diabetes duration, total and basal daily insulin dose, time-in-range, glucose-monitoring system, hemoglobin A1c (HbA1c) values for the last five years, events of severe hypoglycemia and ketoacidosis in the past year, and the most recent test results for retinopathy and nephropathy (albumin/creatinine ratio) was obtained from the patient's electronic clinical records.

2.1.2. Standard protocol approvals, registrations, and patient consent

Informed oral and written consent were obtained from each participant and the accompanying parents. All procedures in the study protocol were approved by the Danish Ethics Committee (Project ID M−2019−211−19) and Legal Office, Central Denmark Region (1-16-02-42-21). Data were safely stored in REDCap, which is a secure web application for online surveys and databases.

2.1.3. Data collection

All participants underwent an examination day at Aarhus University Hospital. The adolescents with type 1 diabetes took their normal basal insulin dose on the day of examinations and all available clinical and biochemical data were extracted from their patient records. In cases of missing data, supplementary data were extracted on the day of the tests. A fasting blood sample was taken from all participants for later analysis, after which they were given 200 ml. of water.

The weight and height of each participant were measured, and their body mass index (BMI) was calculated as weight divided by the square of height. Hip and waist measurements were taken, and blood pressure (BP) and heart rate (HR) were recorded using an automatic BP monitor. The stage of puberty was determined through self-assessment using illustrations of different Tanner stages. Activity levels, alcohol consumption, and smoking status were obtained through self-reporting.

2.2. Definition and diagnostic tests for neuropathy

2.2.1. Diabetic sensorimotor polyneuropathy

According to the definitions of diabetic neuropathy established by the Toronto expert panel, distal sensorimotor polyneuropathy was classified into four categories: possible, probable, confirmed, and subclinical neuropathy [8]. Confirmed cases of LFN and SFN were defined as the presence of symptoms and/or abnormal findings (signs of neuropathy) from the more well accepted confirmatory test; NCS for LFN and IENFD for SFN.

LFN

The neurological examination was performed to assess signs of large fiber dysfunction in length-dependent areas. Touch sensation was tested using cotton wool, the sharp wooden end of a broken cotton swab, and a monofilament 10 g on the big toes. Vibration sense was tested using a tuning fork at 128 Hz at the big toes, and proprioception was tested by moving the big toes five times. The patellar - and achilles reflexes were tested using a reflex hammer, and the muscles of the hands and feets were tested for strength and checked for atrophy.

Probable LFN was defined as having reduced or absence of reflexes, reduced touch sensation in a length-dependent distribution, reduced muscle strength, and/or vibration sensation lasting less than 10 s.

NCS was used as the confirmatory diagnostic test for LFN. Sensory and motor NCS were performed using standard neurophysiological methods and surface electrodes to evaluate conduction velocity (CV), sensory and motor action potential amplitudes, distal motor latencies (DML), and minimum F-wave latencies [17]. Five nerves were tested: the peroneal, and tibial nerves for motoric functions and the peroneal, sural and cutaneous dorsalis lateralis nerves for sensory functions. Abnormal NCS was defined as at least two abnormal tests according to best evidence recommendations [18].

SFN

A neurological examination was performed to identify signs of small fiber dysfunction in the length-dependent areas. Touch sensation was tested on the big toes using a Neuropen® with NeuroTip (Owen Mumford, Oxford, UK) and temperature sensation was tested with rollers (Rolltemp II, Somedic, Sweden) at predetermined temperature levels at 25 and 38 degrees Celsius. In addition, a test for allodynia was performed on the lower leg using a brush.

The absence or reduction of touch and temperature (cold and warm) sensation was considered abnormal and indicated probable SFN.

The diagnostic confirmatory test for SFN was a skin biopsy. IENFD was determined from a 4-mm punch biopsy taken 10 cm above the right lateral malleolus, which was performed under local anesthesia. The methods for fixation, washing, cutting, and immunostaining with PGP9.5 antibody are described elsewere [19]. Abnormal IENFD was defined as being below the 5th percentile of normal, based on data obtained from our 23 included healthy adolescents.

2.2.2. Definition and diagnostic tests for autonomic neuropathy

The autonomic screening tests included: QSART, CARTs, and tilt table test analyzing orthostatic parameters.

QSART

The QSART [20] was performed to measure the indirect sweat volume response induced by antidromic nerve impulses through iontophoresis with acetylcholine. The WR TestWorks Q-Sweat Quantitative Sweat Measurement System (WR Medical Electronics Co., Maplewood, MN) was used as the equipment. The test was carried on the right side of the body at four locations: the forearm, proximal leg, distal leg, and on the foot, respectively. A heat lamp was used to maintain a constant temperature around 30–32 degrees Celsius. An abnormal QSART result was defined as reduced sudomotor volume of less than 5th percentile at the foot and a length-dependent decrease, which was defined as a sudomotor volume at the foot being less than one-third of the volume at the proximal site [13].

CARTs and tilt table test

The CARTs and tilt table test were used to assess cardiovascular autonomic neuropathy (CAN), including both parasympathetic and sympathetic evaluation.

Parasympathetic

CAN was defined as two or more abnormal results from the HR responses during the following tests: 1) deep breathing with a calculation of the difference between HR during expiration and inspiration (the delta HR), 2) forcefully exhaling with an expiratory pressure of 40 mmHg for 15 sec in a 20-degree tilt position and calculation of the Valsalva maneuver (VM) ratio, and 3) position change to standing and calculation of the 30:15 ratio.

The CARTs were performed in a standardized methodology with a Task Force Monitor® (CNSystems Medizintecknik AG, Graz, Austria), which included ECG monitoring and real-time measurement of respiratory pressure and volume through a mouthpiece connected to a digital transducer.

Sympathetic

The tilt table test involved tilting the adolescents from the supine to erect posture (70 degrees) for 10min. Beat-to-beat continuous BP was measured during the entire test, both in the supine (10-minute period) and head-up tilted position, and was compared to absolute oscillometric BP, which was obtained simultaneously.

Orthostatic hypotension (OH) was defined as a sustained reduction of at least 20 mmHg of systolic BP or 10 mmHg of diastolic BP within 3 min of head-up tilt-table testing [21]. Orthostatic intolerance was defined as the occurrences of symptoms and an excessive HR increase during tilting that was relieved by lying down, including postural orthostatic tachycardia syndrome (POTS) [13].

2.2.3. Bedside methods

The bedside tests for diabetic sensorimotor neuropathy included in this study were as follows 1) Biothesiometer (Biomedical Instruments, Ohio) to measure vibration sensation, with the mean of three readings on the big toe being used, and 2) DPNCheck® (NeuroMetrix, U.S.) to measure the conduction velocity and response amplitude of the sural nerve [22,23]. The bedside tests for autonomic neuropathy included 3) Sudoscan (Impeto Medical, Paris, France), which assessed sweat response to electrochemical stimulation by extracting chloride ions from the duct of the sweat glands [24], and 4) Vagus® (Medicus Engineering, Aarhus, Denmark), a handheld device that measured HR response to exercises as a CART [25].

Additionally, the participants answered the Composite Autonomic Symptom Score (COMPASS)-31 [26] at home in the weeks before the test day, and the answers were directly stored in REDCap. The Utah Early Neuropathy Scale (UENS) was also calculated based on neurological examination [27].

2.3. Statistical analysis

All statistical analyses were carried out using the software program R (R Core Team (2022), Vienna, Austria). The distribution of the variables listed in Table 1 was evaluated using the Shapiro-Wilk-test and QQ-plot. Descriptive data are presented as mean (SD) for variables with normal distribution, median (range) for continuous variables without normal distribution, and number (percentage) for categorical variables. Differences between groups were compared using Student's *t*-test for normally distributed continuous variables, Wilcoxon rank-sum test for non-parametric continuous variables, and Fisher's exact test for categorical variables. A p-value of less than 0.05 was considered statistically significant, and we estimated that our sample size will comply with this. Missing data was excluded from the analysis. Linear regression in R was used to analyze the associations between parameters.

The abnormality in confirmatory- and bedside tests was defined as below the 5th or above the 95th percentile compared to normative data obtained from healthy subjects included in this study. The relative risk ratios were calculated using 2-by-2 count data presented in a table. The pROC package in RStudio was used to visualize receiver characteristic (ROC) curves and calculate the area under the ROC curve (AUC). AUC was used to evaluate the quality of screening tests compared to confirmatory tests: 0.5 not useful, 0.5–0.7 poor quality, >0.7–0.8 acceptable quality, >0.8–0.9 excellent quality, >0.9 outstanding quality.

3. Results

A total of 60 adolescents with type 1 diabetes and 23 controls participated in the study, as part of the T1DANES cohort. The selection process is descripted in Fig. 1. The clinical and biochemical characteristics of the two groups are presented in Table 1. Among the adolescents with type 1 diabetes, 18% had HbA1c less than 7% (53 mmol/mol), 62% had HbA1c between 7 and 9% (53–70 mmol/mol), and 20% had HbA1c > 9% (70 mmol/mol).

3.1. Results of diagnostic nerve tests

None of the enrolled patients expressed symptoms in the length-

Table 1
Characteristics of the study population.

Variable	Control, N = 23	Diabetes, N = 60	p-value
Sex (female)	16 (70%)	30 (50%)	0.14
Age (years)	16.60 (15.40–18.20)	16.90 (15.00–18.90)	0.45
Diabetes duration (years)		8.5 (4.6–17.4)	
HbA1c % (mmol/mol)	5.2 (4.6–5.8) (33 (27–40))	7.6 (5.9–10.6) (60 (41–93))	<0.01
BMI (kg/mˆ2)	21.12 (16.90–30.40)	22.74 (17.63–29.61)	**0.03**
BMI-SDS	0.03 (-1.80 – 1.68)	0.57 (-2.30–1.85)	**<0.01**
Height (cm)	174 (158–188)	173 (150–191)	0.97
Hip circumference (cm)	98 (65–112)	98 (76–114)	0.47
Waist circumference (cm)	74 (59–92)	75 (53–100)	0.49
Tanner (Stage)			0.43
4	5 (22%)	19 (32%)	
5	18 (78%)	41 (68%)	
SBP (mmHg)	114 (98–130)	118 (68–147)	0.22
DBP (mmHg)	71 (59–89)	77 (55–96)	**0.03**
Heart rate (BPM)	70 (55–99)	77 (48–106)	0.21
Retinopathy (yes)		3 (5.0%)	
Nephropathy (yes)		2 (3.3%)	
Cholesterol (mmol/L)	3.80 (2.80–5.10)	4.10 (3.00–6.40)	0.15
LDL (mmol/L)	2.10 (1.40–3.50)	2.10 (0.50–4.10)	0.90
HDL (mmol/L)	1.30 (0.68–2.20)	1.50 (0.97–3.70)	**0.04**
Triglycerides (mmol/L)	0.70 (0.30–1.10)	0.90 (0.30–3.80)	**0.01**
Alcohol (units/week)			**0.02**
0	1 (4.3%)	6 (10%)	
1–3	20 (87%)	28 (47%)	
4–7	2 (8.7%)	17 (28%)	
8–14	0 (0%)	5 (8.3%)	
>15	0 (0%)	4 (6.7%)	
Smoking (Status)			0.63
Never	18 (78%)	47 (78%)	
Previous	4 (17%)	6 (10%)	
Current	1 (4.3%)	6 (10%)	
NI	0 (0%)	1 (1.7%)	
Activity (hours/week)			0.17
0	0 (0%)	5 (8.3%)	
1–3	2 (8.7%)	14 (23%)	
4–7	8 (35%)	19 (32%)	
>7	13 (57%)	22 (37%)	
HbA1c mean 5 years % (mmol/mol)	NA	7.5 (5.8–10.8) (59 (40–95))	
Total daily insulin units/kg/day	NA	0.85 (0.40–1.65)	
Basal insulin units/kg/day	NA	0.39 (0.14–0.87)	
Basal / total daily insulin ratio	NA	0.46 (0.24–0.72)	
Time in range* (%)	NA	55 (23–85)	
Time in hypoglycemia (%)	NA	5.0 (0.0–15.0)	

Median (range) for continuous; n (%) for categorical.
Categorical variables, Fisher's exact test; Continuous variable with normal-distribution, Welch Two Sample *t*-test; Continuous variable with non-normal-distribution, Wilcoxon rank sum test

SBP, systolic BP; DBP, diastolic BP; HDL, high density lipoproteins; LDL, low density lipoproteins; n, number; NA, not available,
*Only available data from 44 adolescents.

dependent area on their leg.

3.1.1. LFN

During the neurological examination, 23% of the adolescents with type 1 diabetes showed at least one sign bilaterally that indicated probable LFN. In total, 40% (23 out of 57) had abnormal responses from at least two nerves on NCS. Of these, eight had one sign bilaterally on neurological examination: four showed a reduced vibration sense and four had reduced or absence of Achilles reflexes. Hence, 14% had confirmed LFN and 26% had subclinical LFN as per the Toronto criteria.

Furthermore, we found that 26% of the adolescents with type 1 diabetes had an abnormal response from just one nerve on NCS. The most commonly affected nerves were the motor nerves; tibial nerve (n = 26), and peroneal nerve (n = 25), followed by the sensory nerves in the following order: the lateral dorsal cutaneous nerve (n = 20), peroneal nerve (n = 12), and sural nerve (n = 7).

3.1.2. SFN

On neurological examination, 3% of the adolescents with type 1 diabetes had one sign bilaterally indicating probable SFN. In total, 27% (16 out of 59) of the adolescents were found to have an IENFD of 4 or less, which was assessed as abnormal based on the data obtained from the included healthy controls. Out of the adolescents with type 1 diabetes who had an abnormal IENFD, only one showed a bilaterally sign indicating small fiber damage, felt cold roller as warm. Thus, 2% had confirmed SFN and 25% had subclinical SFN.

3.1.3. Autonomic neuropathy

A total of 20% (12 out of 60) of the adolescents with type 1 diabetes showed reduced or absence of sweat response on their foot and a reduction of sweat response in the distal direction on their leg. The most affected site for abnormal sweat response was the foot (eight reduced response, five no response) followed by the forearm (n = 9), distal leg (n = 6), and proximal leg (n = 2).

The same order of affected sites was also observed in response latency, with even more of the adolescents with type 1 diabetes having delayed response latency (foot n = 26, hand n = 19, distal leg = 4, proximal leg n = 2).

Three adolescents with type 1 diabetes had abnormal sweat responses at three out of four tested sites, with one having no sweat response at three sites and delayed response at the last site, indicating severe sudomotor dysfunction.

Definite CAN with two or more abnormal CARTs was found in 8% (5 out of 60), with two of them meeting the criteria for severe CAN. In addition, 32% of the adolescents with type 1 diabetes had only one affected CART. The most affected cardiovagal parameter was HR response to deep breathing (n = 12), followed by HR response to Valsalva maneuver ratio (n = 10), and 30:15 ratio (n = 8).

The functional parameters obtained in the supine position in a 10-minute period showed that 8% had a higher resting HR, 13% had a higher systolic BP, 20% had a higher diastolic BP, 15% had a higher mean artery pressure (MAP). In the head-up-tilted position, 17% had a higher resting HR, 34% had a higher systolic BP, 19% had a higher diastolic BP, and 32% had a higher MAP.

During tilt test, 19% (11 out of 58) of the adolescents with type 1 diabetes showed symptoms and the test was stopped for ethical and safety reasons. One experienced a vasovagal syncope with a RR pause of 6.19 s, three met the criteria for POTS, and seven had a drop in BP of at least 20 mmHg in systolic or 10mmHg in diastolic before the test was stopped. Additionally, one adolescent who did not show symptoms during the tilt table test had a sustained drop in BP meeting the criteria for OH. Including the seven adolescents with drop in BP before test stop, OH was detected in 14% of the adolescents with type 1 diabetes.

3.2. Bedside tests and diagnostic ability

The comparison of the diagnostic ability of the bedside test with the confirmatory tests was assessed using ROC curves, and the results are presented in Table 2. The majority of the bedside methods (biothesiometer, DPNCheck®, Sudoscan, Vagus® device, COMPASS-31, UENS) were found to have poor discrimination between normal and abnormal results from the confirmatory test, indicating neuropathy. However, certain aspects of two of the bedside tests were found to have acceptable quality: the conduction velocity screening for LFN by DPNCheck® and the HR response to expiration and inspiration testing of autonomic nerves by Vagus®.

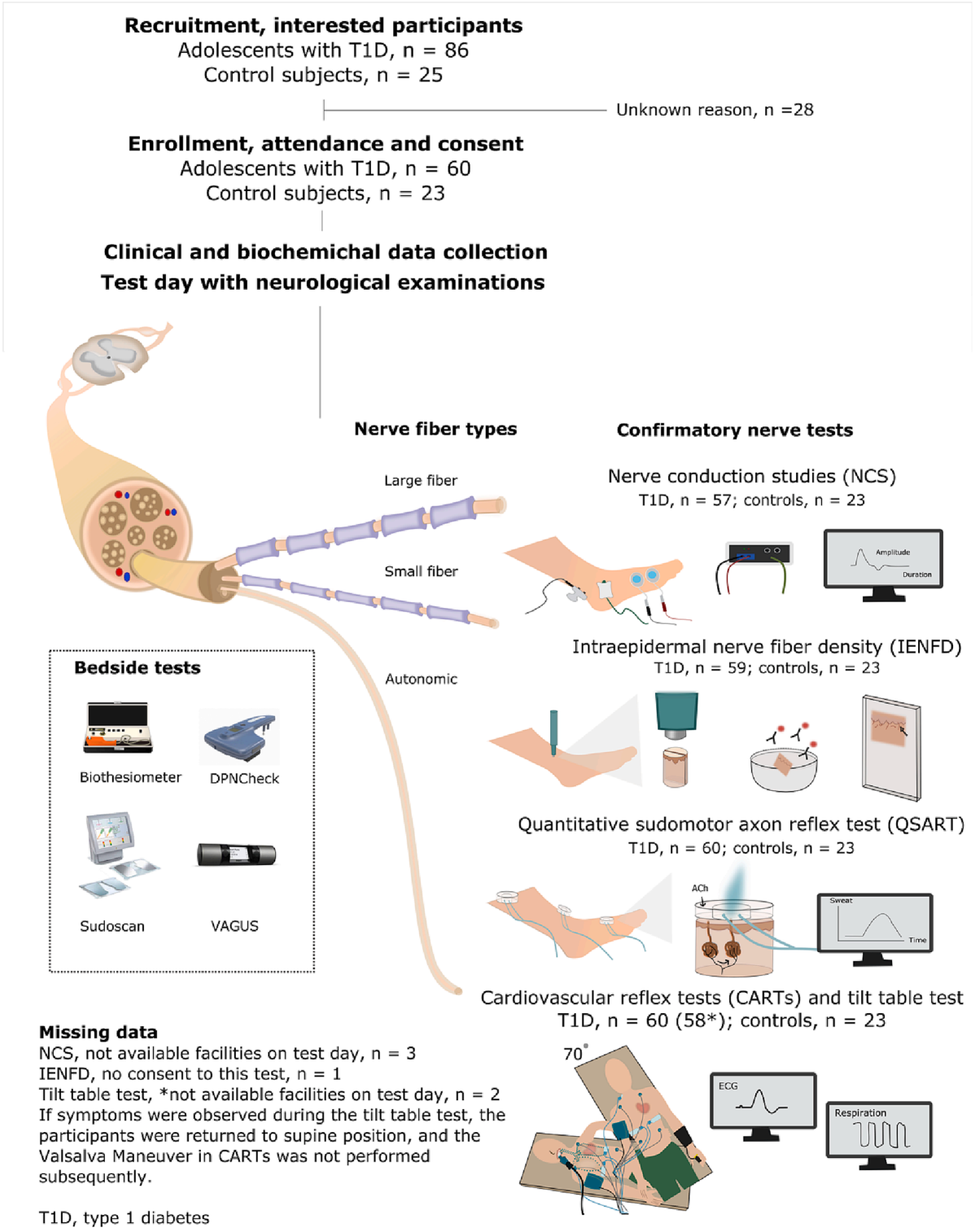

Fig. 1. Flowchart of the study population selection and illustration of tests performed.

3.3. Risk factors

Table 3 presents the clinical and biochemical variables and relative risk ratios associated with abnormal results of confirmatory test for neuropathy. Of the confirmed cases of LFN (n = 8), the mean diabetes duration was 9 years (ranging from 5.3 to 17.4 years), with a mean HbA1c of 8.3% (67 mmol/mol), and none had other microvascular complications.

3.4. Number of affected nerves

Out of the total adolescents with type 1 diabetes, 18% had two abnormal tests, 7% had three abnormal tests, and 2% had four abnormal tests indicating LFN, SFN, and autonomic neuropathy. Among those with only one affected confirmatory test (43%), the affected test was NCS (n = 11), followed by abnormal QSART (n = 6), IENFD (n = 4), OH (n = 3), and CARTs (n = 2). The five adolescents with three or more

Table 2
Bedside tests and symptom questionnaires and their diagnostic ability compared to confirmatory tests for neuropathy.

Bedside test			Confirmatory test			ROC analysis	
Test	n	n abnormal	Test	n	n abnormal	AUC	Grade of discrimination
Large- and small fiber nerves							
Utah early neuropathy score	60	14 (score ≥ 0)	NCS	57	23	0.60	Poor
Utah early neuropathy score	60	14 (score ≥ 0)	IENFD	59	16	0.50	Poor
Biothesiometer	60	1	NCS	57	23	0.55	Poor
DPNCheck® Velocity	55	1	NCS	57	23	0.73	Acceptable
DPNCheck® Amplitude	55	16	NCS	57	23	0.49	Poor
Autonomic nerves							
Sudoscan, feet	60	1	QSART, foot	60	12	0.68	Poor
Sudoscan, hand	60	5	QSART, forearm	60	9	0.60	Poor
Vagus: HR response to standing (RS)	60	15	CARTs 30:15 ratio	60	8	0.67	Poor
Vagus: HR response to deep breathing	60	7	CARTs delta HR	60	12	0.72	Acceptable
Vagus: HR response to Valsalva Maneuver (VM)	57	5	CARTs VM ratio	60	10	0.69	Poor
COMPASS-31 OH Items	60	40 (score ≥ 0)	Tilt table test	58	12	0.45	Poor
COMPASS-31 Sudomotor Items	60	18 (score ≥ 0)	QSART	60	12	0.59	Poor
COMPASS-31 Total Score	60	58 (score ≥ 0)	QSART, CARTs, and/or tilt table test	60	24	0.75	Acceptable

In bedside test the category results (normal versus abnormal) were based on cut-off (<5 or > 95 percentiles) obtained from the included control subjects. Used equipment: Biothesiometer (Biomedical Instruments, Ohio), DPNCheck® (NeuroMetrix, U.S.), Sudoscan (Impeto Medical, Paris, France), Vagus® (Medicus Engineering, Aarhus, Denmark).
In confirmatory test the category results normal versus abnormal) were based cut-off (<5 or > 95 percentiles) obtained from the included control subjects.
In the ROC curve analysis the continuous data from bedside tests were used and compared to the binary output for the confirmatory test. If missing data of results from screening test or confirmatory test, the data was excluded. Grade of discrimination: 0.5 no discrimination, 0.5–0.7 poor, 0.7–0.8 acceptable, 0.8–0.9 excellent, >0.9 outstanding.
Abbreviation: AUC area under curve, CARTs Cardiovascular autonomic reflex test, COMPASS-31 The Composite Autonomic Symptom Score, IENFD intraepidermal nerve fiber density, NCS nerve conduction studies, OH Orthostatic hypotension, QSART Quantitative sudomotor axon reflex test.

affected confirmatory tests showed both damage in their large, small, and autonomic nerves. Having one abnormal confirmatory test indicating LFN, SFN, or autonomic neuropathy did not significantly increase the relative risk of having another abnormal confirmatory test (data not shown).

4. Discussion

In this study, 60 Danish adolescents with type 1 diabetes were included and evaluated for neuropathy using the most widely accepted confirmatory test and criteria for neuropathy typically used in adults. Our results confirmed that neuropathy is present in adolescents, even when using these tests, and more types of nerves can be affected at the same time. We found that 2–14% had confirmed sensorimotor neuropathy, 8% had CAN, 25–26% had subclinical sensorimotor neuropathy (25–26%), and 40% had at least one abnormal autonomic test (CARTs, QSART, or tilt table) when compared to data from 23 healthy adolescents.

Our included adolescents had no history of known neuropathy, and altogether, our findings emphasize the importance of early screening and prevention of various types of neuropathy to prevent severe outcomes, such as pain, amputation, decreased quality of life, and increased risk for cardiovascular events and mortality [28].

Remarkably, there is still no clear consensus on the best diagnostic test and criteria for neuropathy, particularly not in the pediatric population. Of our included confirmatory tests, NCS is the most widely used in the pediatric population [8]. Our results, with 40% of participants having abnormal NCS, are comparable to other studies showing a prevalence of abnormal NCS between 30 and 57% in adolescents [29-32]. However, we highlight that only 14% had confirmed LFN using the Toronto criteria [12].

To the best of our knowledge, no prior studies have estimated SFN based on IENFD, most likely because a skin biopsy is invasive [33]. However, we found it was well-tolerated among our participants. Blankenburg et al. found a prevalence of SFN at 62% using quantitative sensory testing [29]. Notably, mostly bedside sensorimotor tests have been performed with findings of the prevalence of probable neuropathy up to 62% [9].

Only a few studies have estimated the prevalence of CAN in adolescents with type 1 diabetes [34-36]. R Pop-Busui et al.[37] have emphasized that CARTs are the gold-standard for the diagnosis of CAN, but it may not be practical in large research studies and clinical care, and instead suggest using HR variability (HRV) indices. The SEARCH study [36], which used HRV indices to diagnose CAN (≥3 abnormal HRV indices, with the 5th percentile as cut-off), found a prevalence of CAN of 14.4% in 1746 teenagers and young adults with type 1 diabetes, similar to our findings of 8%. The prevalence of CAN defined by CARTs in adolescents with type 1 diabetes has been found to be as high as 41% when using 10th percentile as the cut-off [35].

All the above highlight the importance of establishing similar protocols and guidelines for detecting neuropathy, including reaching a consensus on the tests used and cut-off values, both in clinical practice and in research trials. This is to reduce the risk of overestimation, and for making the findings in the pediatric population comparable to findings in adults.

In general, findings of neuropathy might be evaluated critically, because other factors can influence the test results. We found that 14% had a drop in BP consistent with OH, but in seven out of the eight cases, it was not determined if the drop was sustained because the adolescents were tilted back to supine position due to symptoms. It is considered

Table 3
Clinical and biochemical variables and relative risk ratios for abnormal confirmatory tests for neuropathy.

Variable	Relative risk ratio (Confidence interval 95%)				
	Abnormal test				
	NCS (n = 23/57)	**IENFD (n = 15/59)**	**QSART (n = 12/60)**	**CAN (n = 6/60)**	**OH (n = 8/58)**
HbA1c, current (% (mmol/mol)					
> 9 (75) (n = 7)	2.20 (0.66,7.31)	0.86 (0.21, 3.54)	3.42 (0.38, 31.32)	1.71 (0.13, 23.32)	0.86 (0.09, 7.83)
7–9 (53–70) (n = 41)	1.67 (0.60,4.61)	0.75 (0.29, 1.97)	2.63 (0.37, 18.76)	0.88 (0.10, 7.69)	0.33 (0.05, 2.12)
< 7 (53) (n = 12)	1	1	1	1	1
HbA1c, mean of all values last 5 yrs (% (mmol/mol))					
> 9 (75) (n = 5)	1.38 (0.39, 4.82)	6.60 (0.89,48.8)	4.40 (0.51, 37.97)	NaN*	0.73 (1.10, 1.40)
7–9 (53–70) (n = 42)	1.35 (0.49, 3.72)	3.22 (0.47, 36.0)	2.36 (0.33, 16.67)	1.05 (0.13, 8.46)	0.37(0.10, 1.40)
< 7 (53) (n = 11)	1	1	1	1	1
HbA1c, highest value last 5 yrs (% (mmol/mol))					
> 10 (86) (n = 10)	1.14 (0.48, 2.70)	1.57 (0.64, 3.86)	1.60 (0.52, 4.88)	1.20 (0.15, 9.65)	0.53 (0.26, 6.52)
≤ 10 (86) (n = 48)	1	1	1	1	1
Diabetes duration (yrs)					
>10 (n = 17)	0.65 (0.29, 1.47)	0.84 (0.32, 2.25)	2.53 (0.95, 6.76)	NA	0.96 (0.22, 4.23)
5–10 (n = 43)	1	1	1	1	1
Age (yrs)					
17 to < 19 (n = 17)	0.82	1.61	2.53	0.63	4.78 (1.30,17.62)
15 to < 17 (n = 43)	(0.43,1.58)	(0.70, 3.71)	(0.95, 6.76)	(0.08, 5.26)	1
Sex					
Male (n = 30)	1.81 (0.91,3.58)	0.97 (0.42, 2.23)	1.40 (0.50, 3.92)	0.67 (0.12, 3.71)	1.07 (0.30, 3.88)
Female (n = 30)	1	1	1	1	1
Time in range (%)					
<50 (n = 19)	0.98 (0.45, 2.15)	0.63 (0.22, 1.78)	1.32 (0.44, 3.90)	2.63 (0.26, 26.92)	0.49 (0.06, 4.33)
≥ 50–70 (n = 25)	1	1	1	1	1
Total insulin dose/kg/day					
> 1 U/kg/day (n = 13)	1.66 (0.88, 3.12)	0.51 (0.13, 1.95)	0.33 (0.05, 2.32)	14.5 (1.76,188.5)	0.55 (0.07, 4.03)
≤ 1 U/kg/day (n = 47)	1	1	1	1	1
Basal insulin dose/kg/day					
> 0.5 U/kg/day (n = 15)	2.57 (1.46, 4.53)	0.42 (0.11, 1.63)	1.00 (0.31, 3.22)	12.0 (1.45, 99.17)	1.05 (0.24, 3.46)
≤ 0.5 U/kg/day (n = 45)	1	1	1	1	1
Basal/total insulin					
> 0.5 (n = 23)	1.36 (0.73, 2.53)	0.97 (0.41, 2.30)	1.61 (0.59, 4.39)	0.40 (0.05, 3.38)	0.91 (0.24, 3.46)
≤ 0.5 (n = 37)	1	1	1	1	1
Other microvascular complications					
Yes (n = 5)	0.60 (0.11,3.39)	NaN	1.00 (0.16, 6.24)	2.75 (0.38, 20.14)	NaN
No (n = 55)	1	1	1	1	1
BMI-SDS					
≥ 1 (n = 15)	0.46 (0.16, 1.32)	1.33 (0.55, 3.21)	0.60 (0.15–2.44)	0.75 (0.09–6.20)	0.96 (0.22, 4.23)
<1 (n = 45)	1	1	1	1	1
Waist circumference / height					
≥ 0.5 (n = 8)	2.68 (0.68,2.55)	0.93 (0.26, 3.34)	1.62 (0.42, 6.32)	1.62 (0.21, 12.76)	2.08 (0.51, 8.58)
<0.5 (n = 52)	1	1	1	1	1
Cholesterol (mmol/l)					
≥ 5 (n = 12)	1.29 (0.66,2.55)	NaN*	0.31 (0.04, 2.24)	2.56 (0.48, 13.61)	NaN*
< 5 (n = 46)	1	1	1	1	1
LDL (mmol/l)					
> 3 (n = 10)	0.57 (0.16,1.98)	0.36 (0.05, 2.37)	0.48 (0.07, 3.34)	1.20 (0.15, 9.63)	NaN*
≤ 3 (n = 48)	1	1	1	1	1
Triglycerides (mmol/l)					
≥ 2 (n = 3)	1.81 (0.91, 3.58)	2.57 (1.03, 6.44)	NaN	4.58 (0.72, 29.38)	2.52 (0.44, 14.42)
< 2 (n = 55)	1	1	1	1	1
Smoking status					
Current (n = 6)	1.31 (0.54, 3.14)	0.56 (1.03, 6.44)	0.80 (0.12, 5.20)	NaN	1.28 (0.18, 8.88)
Previous (n = 6)	1.31 (0.46,3.71)	0.56 (1.03, 6.44)	0.80 (0.12, 5.20)	5.33 (1.10, 25.77)	1.28 (0.18, 8.88)
Never (n = 48)	1	1	1	1	1
Alcohol (units/week)					
≥ 8 (n = 9)	0.98 (0.43,2.22)	1.03 (0.36, 2.93)	2.05 (0.65, 6.44)	7.56 (0.77, 74.26)	4.12 (0.68, 24.99)
4–7 (n = 17)	0.59 (0.23,1.47)	0.39 (0.10, 1.54)	0.72 (0.17, 3.14)	4.25 (0.42, 43.50)	3.88 (0.79, 19.10)
≤ 3 (n = 34)	1	1	1	1	1
Activity (hrs/week)					
≤ 3 (n = 19)	0.56 (0.23,1.35)	1.74 (0.57, 5.25)	0.93 (0.29, 2.96)	1.11 (0.17, 7.09)	1.40 (0.36, 5.46)
4–7 (n = 19)	1.00 (0.51,1.95)	1.83 (0.61, 5.51)	0.69 (0.19, 2.53)	0.55 (0.05, 5.62)	0.35 (0.04, 3.09)
≥ 8 (n = 22)	1	1	1	1	1

*NaN not a number. If no adolescents with an abnormal test were in the risk category.

likely that the drop was due to volume status rather than neurogenic failure for two reasons: 1) the included adolescents were fasting and only consumed 200 ml of water two hours prior to the tilt table test, and 2) OH indicates end-stage autonomic failure, which not is expected to be present in asymptomatic adolescents.

In this study, we evaluated sudomotor (QSART), vagal function (CARTs), and the adrenergic function (tilt table) as separate entities, which could also be discussed instead of using any abnormality on

autonomic function testing as a combined test. The composite autonomic scoring scale (CASS) for quantifying generalized autonomic failure has been found useful in grading the degree of autonomic failure and distinguishing between asymptomatic and symptomatic cases in adults [38], which supports the importance of considering an overall autonomic picture from a clinical perspective.

Based on our limited understanding of the pathogenesis and the condition of treatment-induced-neuropathy, there is a need to focus on neuropathy from the time of diagnoses. The time frame for initiating screening and diagnostic tests in pediatrics has been discussed, with recommendations ranging from two to five years after the onset of diabetes [9,16]. The current screening protocols recommend a neurological examination as the first step, followed by confirmation with NCS in positive cases [8,9]. An optimal screening program for neuropathy should include tests for both LFN, SFN, and autonomic neuropathy, as all types of nerves can be affected. Among the adolescents in this study who had only one abnormal confirmatory test for neuropathy, most showed signs of large fiber damage, although small fiber and autonomic nerves could also be affected, and multiple types of nerves can be affected simultaneously.

However, it is important to note that the more tests performed and variables considered, the greater the risk of false positives. Confirmatory diagnostic tests require specialized equipment and trained healthcare professionals. In the clinic, bedside tests with high sensitivity and specificity are preferable.

In our opinion, there is insufficient attention to bedside methods for detecting neuropathy. Bedside methods can be effective for detecting neuropathy to some extent as we have shown for DPNCheck® and Vagus®. However, further research might be conducted to evaluate the efficacy of different methods in larger pediatric populations.

Given that treating neuropathy can be challenging, early prevention and management of risk factor are considered crucial. Some well-known but untreatable risk factors for diabetic neuropathy include the duration of diabetes, age, and height, while the level of HbA1c can be modified [39,40]. In this study, 80% of the adolescence did not meet the ISPAD criteria for good metabolic control. Intensive therapy in the early years of diabetes has been shown to reduce the risk of neuropathy later in life [41]. In our study, none of the analyzed risk factors were consequently associated with multiple abnormal confirmatory tests for neuropathy. Previous research has identified an unhealthy lipid profile and other microvascular complications as risk factors for diabetic neuropathy in adolescents [9]. Additionally, our study showed that a higher total and basal insulin dose were risk factors, which could indicate high blood glucose levels and decreased insulin sensitivity. Recent evidence suggests that insulin plays a crucial role in Schwann cells and in proper neuronal function, so disruption in insulin availability could harm peripheral nerves [42]. Further research into risk factors and the underlying mechanisms (inflammatory, biochemical, genetic, and epigenetic modifiers) of neuropathy is needed, along with longitudinal studies evaluating the effect of improved metabolic control and lifestyle changes on the pediatric population.

The strengths of our study include the use of confirmatory tests in a standardized manner in all participants, with definitions and criteria for neuropathy based on the best available evidence. All tests were performed with the same equipment and by the same healthcare professionals.

One of the main limitations of the study is the small population size, which restricts the ability to adjust for risk factors. Additionally, our small control group included a greater number of females than males. Each confirmatory test had limitations, and various factors such as surrounding tissue and molecules, organ failure, vascular damage, and external factors like temperature and measurement inaccuracies, can affect the results. Also, the lack of psychosocial factors, gene and protein expression profiles, and imaging of the brain is a limitation as it would have made it possible to compare our findings with clinical presentation, genetic causes, and changes in the central nervous system.

In conclusion, LFN, SFN, and autonomic neuropathy are not uncommon in adolescents with type 1 diabetes, even with the use of diagnostic confirmatory tests known from adults. Through education of healthcare professionals in youth-friendly approaches and the use of advanced technologies, the goal of good metabolic control can be achieved, potentially reducing the risk of neuropathy in the future. A multidisciplinary approach, with a specialized diagnostic center for neuropathy connected to diabetic centers, would be favorable. Improving screening methods, educating healthcare professionals on the best available diagnostic tests, educating patients on neuropathy-related symptoms, and conducting research on the pathogenetic mechanism, risk factors, and treatment options for diabetic neuropathy may enhance the quality of life for those affected in the future.

Author Contributions and Guarantor Statement

V.F.R, E.T.V, A.J.T, J.R.N, and Ku.K. were involved in the conception and design of the study. V.F.R. was the project leader and she, E.T.V, Ku. K., and M.M were the managers for the recruitment of participants in collaboration with the pediatric diabetes teams at Randers Region Hospital, Aarhus University Hospital/Steno Diabetes Center Aarhus, and Aalborg University Hospital/Steno Diabetes Center North. V.F.R. and M. T. performed all included tests, except NCS, which H.T. performed. P.K. stained and counted the IENFD. V.F.R. wrote the first draft of the manuscript, and all authors edited, reviewed, and approved the final version of the manuscript. V.F.R. is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, and the right to publish the data.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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