


# Assessment of neuropathy subtypes in type 1 diabetes

Pall Karlsson ,<sup>1,2,3</sup> Marie Balle Sjøgaard,<sup>1,4</sup> Karoline Schousboe,<sup>5</sup> Hatice Isik Mizrak,<sup>6</sup> Huda Kufaishi,<sup>6</sup> Troels Staehelin Jensen,<sup>1,7</sup> Jens Randel Nyengaard,<sup>2,8</sup> Christian Stevns Hansen,<sup>6</sup> Knud Bonnet Yderstræde,<sup>5</sup> Christian Selmer Buhl<sup>3</sup>

**To cite:** Karlsson P, Sjøgaard MB, Schousboe K, et al. Assessment of neuropathy subtypes in type 1 diabetes. *BMJ Open Diab Res Care* 2024;**12**:e004289. doi:10.1136/bmjdr-2024-004289

PK and MBS contributed equally.

Received 23 April 2024  
Accepted 27 June 2024



© Author(s) (or their employer(s)) 2024. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Danish Pain Research Center, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

<sup>2</sup>Core Center for Molecular Morphology, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

<sup>3</sup>Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark

<sup>4</sup>Steno Diabetes Center North Denmark, Aalborg University Hospital, Aalborg, Denmark

<sup>5</sup>Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark

<sup>6</sup>Steno Diabetes Center Copenhagen, Herlev, Denmark

<sup>7</sup>Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

<sup>8</sup>Department of Pathology, Aarhus University Hospital, Aarhus, Denmark

## Correspondence to

Dr Pall Karlsson;  
pall@clin.au.dk

## ABSTRACT

**Introduction** Diabetic polyneuropathy (DPN), a common complication of diabetes, can manifest as small, large, or mixed fiber neuropathy (SFN, LFN, and MFN, respectively), depending on the type of fibers involved. Despite evidence indicating small fiber involvement prior to large fiber involvement in type 1 diabetes mellitus (T1DM)-associated DPN, no evidence has been produced to determine the more prevalent subtype. We aim to determine the more prevalent type of nerve fiber damage—SFN, LFN, and MFN—in T1DM-associated DPN, both with and without pain.

**Research design and methods** In this cross-sectional study, participants (n=216) were divided into controls; T1DM; T1DM with non-painful DPN (NP-DPN); and T1DM with painful DPN (P-DPN). DPN was further subgrouped based on neuropathy severity. The more prevalent type of fiber damage was determined applying small and large fiber-specific tests and three diagnostic models: model 1 ( $\geq 1$  abnormal test); model 2 ( $\geq 2$  abnormal tests); and model 3 ( $\geq 3$  abnormal tests).

**Results** MFN showed the highest prevalence in T1DM-associated DPN. No differences in neuropathy subtype were found between NP-DPN and P-DPN. DPN, with prevalent SFN plateaus between models 2 and 3. All models showed increased prevalence of MFN according to DPN severity. Model 3 showed increased DPN with prevalent LFN in early neuropathy. DPN with prevalent SFN demonstrated a similar, but non-significant pattern.

**Conclusions** DPN primarily manifests as MFN in T1DM, with no differentiation between NP-DPN and P-DPN. Additionally, we propose model 2 as an initial criterion for diagnosing DPN with a more prevalent SFN subtype in T1DM. Lastly, the study suggests that in mild stages of DPN, one type of nerve fiber (either small or large) is more susceptible to damage.

## INTRODUCTION

Diabetic polyneuropathy (DPN) is a common complication of diabetes with a lifetime prevalence of up to 50%.<sup>1</sup> DPN can clinically be divided into painful DPN (P-DPN) and non-painful DPN (NP-DPN), both of which are characterized by altered sensibility to different sensory modalities and progressive loss of peripheral nerve fibers.<sup>1</sup> Ultimately, DPN can result in intolerable pain,

## WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Prior to this study, it was established that diabetic polyneuropathy (DPN) is a common complication of diabetes, affecting up to 50% of patients, characterized by the progressive loss of peripheral nerve fibers.
- ⇒ Research, primarily focused on type 2 diabetes mellitus (T2DM), indicated that mixed fiber neuropathy (MFN) was the predominant subtype; however, the extent and specific characteristics of DPN in type 1 diabetes mellitus (T1DM) were less understood, particularly the sequence of small versus large fiber involvement.

## WHAT THIS STUDY ADDS

- ⇒ This study reveals that DPN in T1DM predominantly manifests as MFN, similar to T2DM, with no significant differentiation between painful and non-painful DPN.
- ⇒ It also introduces a model as an optimal diagnostic approach for identifying a small fiber neuropathy in the early stages of DPN, enhancing earlier recognition and potential intervention opportunities.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ The findings identified a standardized diagnostic model that could be integrated into clinical practice for early subtyping of DPN in T1DM.
- ⇒ This could potentially influence treatment strategies and improve patient outcomes by targeting specific neuropathy subtypes.
- ⇒ Additionally, the study underscores the need for further research into the pathophysiological differences and progression patterns between T1DM-associated and T2DM-associated neuropathies, which could lead to tailored interventions.

foot ulceration and amputation, significantly decreasing the quality of life of the affected individual. Currently, the prevalence of type 1 diabetes mellitus (T1DM)-associated P-DPN is unknown, with studies reporting a prevalence between 0% and 55%.<sup>2</sup>

Neuropathy can be divided into three subtypes based on nerve fiber involvement:

small fiber neuropathy (SFN), involving A $\delta$  and C fibers; large fiber neuropathy (LFN), involving large A $\beta$  fibers; and mixed fiber neuropathy (MFN), involving both large and small nerve fibers.<sup>3</sup> Previous studies indicate that DPN predominantly manifests as MFN, with affected individuals exhibiting symptoms related to both small and large nerve fibers.<sup>4,5</sup> However, these studies are based on T2DM. Consequently, it is unclear if and to what extent T1DM-associated DPN mirrors the clinical manifestations of T2DM-associated DPN. Moreover, T1DM-specific studies on DPN have found that small fiber involvement precedes that of large fibers, indicating that SFN measures could play a clinical role as monitoring and screening instruments for early detection of DPN and possibly P-DPN in T1DM.<sup>6,7</sup> However, currently there is no consensus on a gold standard for the diagnosis of SFN,<sup>8</sup> potentially affecting the ability of clinicians to diagnose DPN in the early stages and the ability to determine the more prevalent subtype of DPN.

The objective of this study was to assess the extent of fiber damage in individuals with T1DM with and without DPN and with and without neuropathic pain. We aimed to investigate the optimal combination of various diagnostic tests for DPN to determine the specific neuropathy subtypes and relate them to DPN status and severity.

## METHODS

### Study participants and the definition and assessment of DPN and P-DPN

Participants were recruited from Steno Diabetes Centers in Denmark (Odense, Aarhus, Copenhagen) and through advertising between December 2019 and November 2021. Inclusion criteria for T1DM required ages 30–75 years and a T1DM duration of  $\geq 5$  years. Exclusion criteria included non-diabetes-related neuropathy, significant non-neuropathic pain, alcohol/substance abuse, and specific health conditions. Pain participants needed  $\geq 4$  average pain on a Numeric Rating Scale (NRS) of 0–10 over the last 7 days. Controls had similar criteria, excluding metabolic/neurological disorders and depression.

Participants underwent thorough clinical assessment including; collection of medical history, interviews, physical exams, and tests like DPNCheck and Sudoscan, aiming to identify DPN and P-DPN through clinical and physiological assessments. For more detailed description, see online supplemental material.

### Clinical diagnosis of DPN and P-DPN

DPN and P-DPN diagnosis involved comprehensive examinations, including reflex assessments, vibration detection thresholds, and skin biopsies for intra-epidermal nerve fiber density (IENFD) analysis. Clinical blood samples were analyzed for related biomarkers. DPN was defined using Toronto consensus criteria, while P-DPN followed the Neuropathic Pain Special Interest Group grading system, requiring a history of neurological

disease, anatomically plausible pain distribution, and sensory signs. Severity was measured using the Toronto Clinical Neuropathy Score (TCNS) and pain levels by NRS of 0–10.

Following the Toronto consensus criteria, participants were grouped by likelihood of DPN into *no DPN*, *subclinical DPN*, *possible DPN*, *probable DPN*, and *confirmed DPN* based on symptoms, signs, and test results. The participants in the groups *possible DPN* and *subclinical DPN* were assessed by a trained diabetologist and an expert in DPN and were evaluated not to have clinical DPN and did not differ substantially from those without DPN. To increase the power of the study, these three groups were therefore grouped into one group of participants without clinical DPN (*no DPN*). Probable and confirmed DPN were pooled into a DPN group and divided into non-painful and painful DPN based on NRS of 0 or  $\geq 4$ , respectively. For more detailed description, see online supplemental material.

### DPN severity

All participants with DPN were divided into four groups based on DPN severity determined from the TCNS score: *no neuropathy* (TCNS  $\leq 5$ ); *mild neuropathy* (TCNS=6–8); *moderate neuropathy* (TCNS=9–11); and *severe neuropathy* (TCNS  $\geq 12$ ).

### Definition of DPN subtypes

To determine the optimal combination of tests to diagnose the most prevalent subtype of DPN (SFN, LFN, or MFN), based on the type of nerve fibers affected, we defined three different models with specific criteria (see definition below). The models represent slightly modified versions of models 1 and 2 described by Itani *et al*<sup>4</sup> in a study investigating neuropathy subtypes in T2DM-associated DPN (methodological differences: include thermotester vs thermo rollers and full nerve conduction study vs DPNCheck<sup>4</sup>). In all models, diagnosis of the more prevalent neuropathy subtype depended entirely on the fulfilment of the SFN criteria and/or fulfilment of the LFN criteria. In all three models, non-classifiable neuropathy (NCN) was defined as cases where neither the criteria of SFN, MFN, nor LFN diagnosis were met.

Small fiber involvement was determined using the following measures: IENFD, electrochemical skin conductance (ESC) (feet), pinprick (neurotip) and temperature sensation. Abnormal results were determined as follows: IENFD, below normative reference material; ESC,  $< 60$   $\mu\text{S}$ ; pinprick, reduced, or absent sensation; temperature sensation, neutral or inverse thermal sensation of 25°C (cold) and 40°C (warm). Large fiber involvement was determined based on the measures of biothesiometry, DPNCheck, ankle reflexes, and 10 g monofilament. Abnormal results were determined as follows: biothesiometry, 25 V or higher; DPNCheck, amplitude  $\leq 4$   $\mu\text{V}$  and/or velocity  $\leq 40$  m/s; ankle reflexes, absent or reduced; 10 g monofilament,  $< 8$  sensations out of 10

stimuli. Further details on the methods can be found in the online supplemental material.

### Model 1

For a diagnosis of SFN,  $\geq 1$  abnormal result was required in the small fiber measures and 0 abnormal results in the large fiber measures. Conversely, for a diagnosis of LFN,  $\geq 1$  abnormal result was required in the large fiber measures and 0 abnormal results in the small fiber measures. MFN was defined as  $\geq 1$  abnormal result in the small fiber measures and  $\geq 1$  abnormal result in the large fiber measures.

### Model 2

Here,  $\geq 2$  abnormal results in the small fiber measures and  $\leq 1$  abnormal results in the large fiber measures were required for a diagnosis of DPN with more prevalent SFN. Conversely,  $\geq 2$  abnormal results in the large fiber measures and  $\leq 1$  abnormal results in the small fiber measures were required for a diagnosis of DPN with more prevalent LFN. MFN was defined as  $\geq 2$  abnormal results in the small fiber measures and  $\geq 2$  abnormal results in the large fiber measures.

### Model 3

A diagnosis of DPN with more prevalent SFN required  $\geq 3$  abnormal results in the small fiber measures and  $\leq 2$  abnormal results in the large fiber measures. Conversely, a diagnosis of DPN with more prevalent LFN required  $\geq 3$  abnormal results in the large fiber measures and  $\leq 2$  abnormal results in the small fiber measures. MFN was defined as  $\geq 3$  abnormal results in the small fiber measures and  $\geq 3$  abnormal results in the large fiber measures.

The models (models 1–3) were applied to three different groups: NP-DPN (T1DM with NP-DPN,  $n=67$ ), P-DPN (T1DM with P-DPN,  $n=50$ ), and pooled DPN (P-DPN+NP-DPN,  $n=117$ ). The models were additionally applied to the four DPN severity groups (no neuropathy, mild neuropathy, moderate neuropathy, and severe neuropathy).

### Definition of pure SFN

Pure SFN was determined using the gold standard criteria, the 'Besta Criteria' defined by Devigili *et al.*<sup>9</sup> For a pure SFN diagnosis, the Besta criteria require  $\geq 2$  abnormal results of the small fiber tests and no abnormal results of the large fiber tests. The tests applied in the Besta criteria can be seen in online supplemental table S1. The tests from the Besta criteria were matched with the available tests in the current study in the best possible manner (see online supplemental table S1) to create a model, here termed the 'Besta model'. Only one measure, hyperalgesia, was not matched because it was not measured in the current study. This is not thought to significantly affect the model, as evoked pain such as hyperalgesia and allodynia is considered relatively rare in DPN.<sup>10 11</sup>

To compare the reliability of our proposed model 2 to diagnose SFN, we adapted the model to match the requirements of the Besta criteria. The adapted model 2

(model 2 (adapted)) required  $\geq 2$  abnormal results in the SFN tests (IENFD, ESC (feet), pinprick (neurotip), and temperature sensation) and no abnormal results of the LFN tests (biothesiometry, DPNCheck, ankle reflexes, and 10 g monofilament).

### Statistical analysis

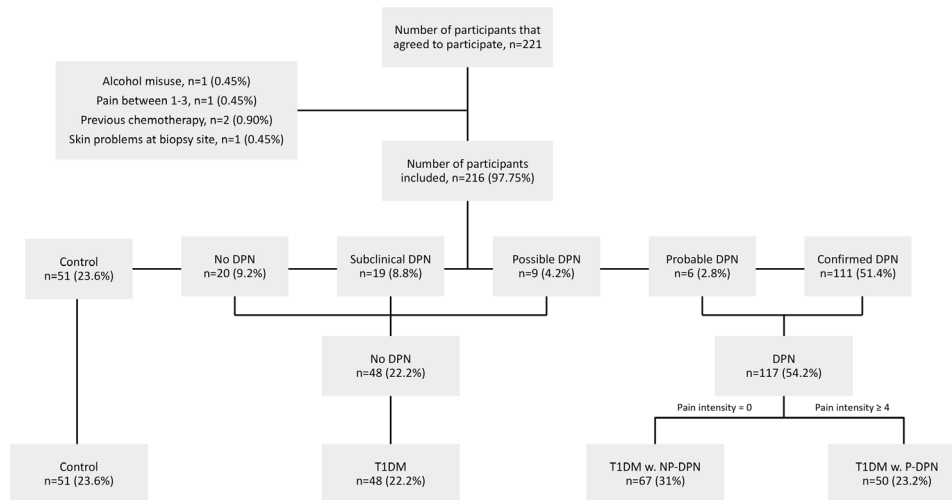
Sample size was determined based on a recent study applying the same cohort.<sup>12</sup> In the previous study, a sample size of 20–25 participants in each group was found to be sufficient with a power of 80% and an alpha of 0.05. To enhance the sensitivity in the study, we doubled the sample size to 40–50 participants per group to show group differences, incorporating various tests for DPN.

Stata SE, V.17 (StataCorp, Texas, USA) was applied for data analysis throughout this study. Normal distribution of all continuous variables was assessed, using quantile-quantile plots and histograms. None of the variables were found to be normally distributed. Data are presented as medians (IQR) for continuous variables and as frequencies with percentages for categorical variables. Multiple-group comparisons were carried out using Kruskal-Wallis H test (continuous variables) and Fisher's exact test, using ' $r \times c$ '-contingency tables (categorical variables). Two-group comparisons were carried out using Mann-Whitney U test (continuous variables) and Fisher's exact test, using ' $2 \times 2$ '-contingency tables (categorical variables). Adjustments for sex, age, hemoglobin A1c (HbA1c), and diabetes duration were carried out when appropriate, using transformed values and a linear regression model (continuous variables) or a logistic regression model (categorical variables). Collinearity was checked, and no variance of inflation factor was found to be  $>5$ , indicating no significant issues with collinearity of the confounders. Variable correlations were tested using Spearman's rank correlation analysis. The respective correlation coefficients ( $r$ ) and their  $p$  values are presented in corresponding graphs, depicting the variable's relationship. Correlation coefficients were adjusted for sex, age, HbA1c, and diabetes duration using partial correlation analysis on transformed variables. Throughout the study,  $p$  values  $<0.05$  were considered statistically significant.

### RESULTS

Out of the 221 individuals who agreed to participate in this study, 5 (2.25%) did not meet the inclusion criteria (figure 1). Participants were excluded for following reasons: alcohol abuse ( $n=1$ , 0.45%), NRS score between 1 and 3 ( $n=1$ , 0.45%), painful symptoms that may have been due to previous chemotherapy treatment ( $n=2$ , 0.90%), and skin problems at the biopsy site ( $n=1$ , 0.45%).

The remaining 216 participants were placed in either the healthy control group ( $n=51$ , 23.6%) or in groups based on the Toronto Consensus Criteria: no DPN ( $n=20$ , 9.2%); subclinical DPN ( $n=19$ , 8.8%); possible DPN ( $n=9$ ,



**Figure 1** Flow chart of patient inclusion and patient groupings. Groupings were based on a T1DM diagnosis as well as the Toronto criteria. 221 individuals agreed to participate from which 5 (2.25%) did not meet the inclusion criteria. Reasons for exclusion: alcohol misuse (n=1; 0.45%), pain intensity score between 1 and 3 on the NRS scale (n=1; 0.45%), previous treatment with chemotherapy (n=2; 0.9%), and skin problems at the biopsy site (n=1; 0.45%). The remaining 216 individuals (97.75%) underwent clinical examination and were placed in an appropriate group, 51 (23.6%) participants had been included as a healthy control group. The remaining 165 (76.4%) participants were placed in appropriate groups according to the Toronto criteria: no DPN (n=20, 9.2%); subclinical DPN (n=19, 8.8%); possible DPN (n=9, 4.2%); probable DPN (n=6, 2.8%); confirmed DPN (n=111, 51.4%). The participants in the groups no DPN, subclinical DPN, and possible DPN were pooled into a no DPN group (n=48, 22.2%), which was then termed T1DM. The participants in the groups probable and confirmed DPN were pooled into a DPN group (n=117, 54.2%), which was then subdivided into T1DM with NP-DPN (n=67, 31%) and T1DM with P-DPN (n=50, 23.2%) based on a pain intensity of 0 or  $\geq 4$  on the NRS, respectively. DPN, diabetic polyneuropathy; NP-DPN, non-painful diabetic polyneuropathy; NRS, Numeric Rating Scale; P-DPN, painful diabetic polyneuropathy; T1DM, type 1 diabetes mellitus; w., with.

4.2%); probable DPN (n=6, 2.8%); and confirmed DPN (n=111, 51.4%) (figure 1). Based on clinical evaluation, the no DPN, subclinical DPN, and possible DPN groups were combined into a no DPN group (n=48, 22.2%) and renamed T1DM. The probable DPN and confirmed DPN groups were combined into a DPN group (n=117, 54.2%) and then subdivided into T1DM with NP-DPN (n=67, 31%) and T1DM with P-DPN (n=50, 23.2%) based on pain intensity scores of zero (NRS=0) or  $\geq 4$  (NRS  $\geq 4$ ), respectively (figure 1).

Participants with DPN (n=117) were subsequently regrouped according to DPN severity: no neuropathy (TCNS  $\leq 5$ , n=38, 32.5%); mild neuropathy (TCNS=6–8, n=31, 26.5%); moderate neuropathy (TCNS=9–11, n=33 (28.2%); and severe neuropathy (TCNS  $\geq 12$ , n=15, 12.8%).

### Participant characteristics and medication

Participant characteristics are described in table 1. Between the groups, there were significant differences in age, diabetes duration, HbA1c, glucose and LDL (all  $p < 0.001$ ) (table 1). When comparing DPN participants with and without pain, only age ( $p = 0.022$ ), diabetes duration ( $p = 0.009$ ), currently smoking ( $p = 0.035$ ), and HbA1c ( $p = 0.006$ ) showed significant differences (table 1). T1DM with NP-DPN were considerably older and had longer duration of diabetes than T1DM with P-DPN. In turn, T1DM with P-DPN were more frequently smokers and had higher HbA1c compared with T1DM with NP-DPN.

There were differences in the application of antihypertensive and cholesterol-lowering medications (both  $p < 0.001$ ) between the groups (online supplemental table S2). There was no difference between T1DM with NP-DPN and T1DM with P-DPN (both  $p > 0.05$ ) (online supplemental table S2). As expected, T1DM P-DPN were more frequent users of peripheral and central analgesics and compounds used for neuropathic pain (gabapentinoids and antidepressants, online supplemental table S2).

### Questionnaires and tests of DPN and pain

The bedside tests, including biothesiometry, pinprick, 10 g monofilament, and temperature sensation, revealed differences between the groups (all  $p < 0.001$ ; table 2). However, there was no significant differences observed between DPN participants with and without pain. The PainDETECT score was significantly different between all groups ( $p < 0.001$ ), with T1DM with P-DPN having a median of 15.0, while in the remaining three groups had a median value of 0.0 (table 2). The same level of significance was observed in the two-group comparison between T1DM with NP-DPN and T1DM with P-DPN.

Participants with pain had the highest MNSI and TCNS scores of the four groups (all  $p < 0.001$ ) (table 2). When comparing T1DM with NP-DPN with T1DM with P-DPN, significant differences were observed in the MNSI questionnaire scores and the TCNS scores (both  $p < 0.001$ ).

**Table 1** Patient characteristics

	Control (n=51)	T1DM (n=48)	T1DM with NP-DPN (n=67)	T1DM with P-DPN (n=50)	P value	P value (NP-DPN vs P-DPN)
Sex (female)	26 (51.0%)	26 (54.2%)	24 (35.8%)	27 (54.0%)	0.133	0.061
Age (years)	58.1 (50.5; 64.5)	53.3 (45.9; 58.8)	63.9 (57.9; 70.1)	60.6 (51.8; 65.3)	0.000***	0.022*
Diabetes duration (years)	–	27.5 (15.5; 37.5)	40.0 (32.0; 51.0)	33.0 (24.0; 43.0)	0.000***	0.009**
BMI (kg/m <sup>2</sup> )	26.9 (23.9; 32.1)	25.6 (24.0; 28.3)	26.9 (23.5; 29.6)	27.2 (24.0; 31.2)	0.519	0.269
Waist (cm)	93.3 (85.0; 106.0)	91.5 (86.0; 97.0)	97.8 (88.5; 106.0)	100.8 (88.5; 108.5)	0.101	0.783
Higher education (yes)	34 (70.8%)	35 (74.5%)	43 (65.2%)	30 (63.8%)	0.639	1.000
Exercise (1–2 or more times/week, yes)	45 (93.8%)	39 (83.0%)	48 (72.7%)	35 (74.5%)	0.019*	1.000
Smoking (today, yes)	2 (4.2%)	3 (6.4%)	6 (9.1%)	12 (25.5%)	0.009**	0.035*
Alcohol (>recommended units/week, yes)	3 (6.3%)	4 (8.5%)	9 (13.6%)	6 (13.0%)	0.564	1.000
HbA1c (mmol/mol)	36.3 (34.6; 38.8)	56.0 (49.7; 61.0)	60.0 (54.0; 66.0)	67.5 (56.8; 77.0)	0.000***	0.006**
HbA1c (%)	5.5 (5.3; 5.7)	7.3 (6.7; 7.7)	7.6 (7.1; 8.2)	8.3 (7.3; 9.2)	0.000***	0.006**
Glucose (mmol/L)	5.6 (5.1; 6.3)	8.8 (6.9; 10.9)	8.5 (6.2; 11.6)	11.2 (5.8; 15.4)	0.000***	0.078
C-peptide (pmol/L)	1033.5 (689.7; 1695.0)	8.0 (8.0; 21.0)	8.0 (3.0; 8.0)	8.0 (0.0; 21.2)	0.000***	0.713
HDL (mmol/L)	1.5 (1.3; 1.9)	1.6 (1.5; 1.9)	1.7 (1.4; 2.0)	1.6 (1.4; 1.9)	0.454	0.183
LDL (mmol/L)	3.3 (2.8; 3.7)	2.2 (1.8; 2.8)	2.0 (1.5; 2.4)	2.0 (1.7; 2.4)	0.000***	0.512
Triglyceride (mmol/L)	1.3 (0.9; 1.8)	0.9 (0.7; 1.2)	0.8 (0.6; 1.3)	1.0 (0.7; 1.2)	0.000***	0.410
eGFR (mL/min/1.73 m <sup>2</sup> )	90.0 (84.4; 90.0)	90.0 (90.0; 90.0)	85.4 (75.8; 90.0)	88.3 (70.0; 90.0)	0.014*	0.368
Urine albumin/creatinine ratio (mg/mol)	1.0 (0.5; 1.7)	0.8 (0.4; 1.4)	1.3 (0.6; 3.4)	1.6 (0.8; 4.8)	0.001***	0.293
Systolic blood pressure (mm Hg)	136.7 (127.3; 147.7)	136.2 (122.2; 144.3)	138.0 (130.0; 151.0)	139.7 (130.7; 150.3)	0.296	0.766
Diastolic blood pressure (mm Hg)	84.0 (77.0; 91.3)	76.0 (71.7; 83.3)	73.3 (67.0; 81.3)	75.0 (70.3; 83.0)	0.000***	0.115

Categorical variables: presented as number of x and its percentage within each group. Continuous variables: presented as median with the 25th and 75th quartile within each group. All-group comparisons (p value) were carried out using Fisher's exact test (categorical variables) and Kruskal-Wallis H test (continuous variables). Two-group comparisons between T1DM with NP-DPN and T1DM with P-DPN (p value (NP-DPN vs P-DPN)) were carried out using Fisher's exact test (categorical variables) and Mann-Whitney U test (continuous variables). \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

†More than seven units/week for women and >14 units/week for men, according to the Danish Health Authority.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NP-DPN, non-painful diabetic polyneuropathy; P-DPN, painful diabetic polyneuropathy; T1DM, type 1 diabetes mellitus.

**Table 2** Bedside tests and questionnaires

	Control (n=51)	T1DM (n=48)	T1DM with NP- DPN (n=67)	T1DM with P-DPN (n=50)	P value	P value (NP-DPN vs P-DPN)
Pinprick, neurotip (abnormal)	1 (2.0%)	1 (2.1%)	30 (44.8%)	30 (60.0%)	0.000*	0.135
10g neurofilament, neuropen (abnormal)	0 (0.0%)	1 (2.1%)	35 (52.2%)	30 (60.0%)	0.000*	0.455
Temperature sensitivity (abnormal)	2 (4.0%)	9 (19.6%)	48 (72.7%)	36 (75.0%)	0.000*	0.832
Biothesiometer average (V)	6.7 (5.0; 9.9)	9.9 (5.5; 13.9)	27.7 (17.2; 41.7)	30.9 (18.7; 47.8)	0.000*	0.147
PainDETECT score	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 1.0)	15.0 (10.0; 22.0)	0.000*	0.000*
MNSI questionnaire score	2.0 (2.0; 3.0)	3.0 (2.0; 4.0)	5.0 (4.0; 6.0)	8.0 (7.0; 10.0)	0.000*	0.000*
MNSI clinical score	0.0 (0.0; 1.0)	0.5 (0.0; 1.5)	4.0 (3.0; 6.0)	5.0 (3.0; 6.0)	0.000*	0.170
TCNS score	0.0 (0.0; 1.0)	1.0 (0.0; 2.0)	6.0 (3.0; 9.0)	9.0 (7.0; 12.0)	0.000*	0.000*

Categorical variables: presented as number of x and its percentage within each group. Continuous variables: presented as median with the 25th and 75th quartile within each group. All-group comparisons (p value) were carried out using Fisher's exact test (categorical variables) and Kruskal-Wallis H test (continuous variables). Two-group comparisons between T1DM with NP-DPN and T1DM with P-DPN (p value (NP-DPN vs P-DPN)) were carried out using Fisher's exact test (categorical variables) and Mann-Whitney U test (continuous variables).

\*p<0.001.

MNSI, Michigan neuropathy screening instrument; NP-DPN, non-painful diabetic polyneuropathy; P-DPN, painful diabetic polyneuropathy; TCNS, Toronto Clinical Neuropathy Score; T1DM, type 1 diabetes mellitus.

(table 2). Both groups had higher scores in the T1DM with P-DPN group.

An example of IENFD-staining from a human skin biopsy is shown in online supplemental figure S1. Results from IENFD and DPNCheck are listed in table 3. Both tests exhibited significant differences between the groups (p<0.001), with a higher frequency of abnormal results in the DPN groups, before and after adjusting for sex, age, HbA1c, and diabetes duration. Comparisons between T1DM with NP-DPN and T1DM with P-DPN did not reveal significant differences for either of the measures (table 3).

### TCNS and non-clinical tests

The relationships between TCNS, as a measure of DPN severity, and IENFD and DPNCheck are depicted in online supplemental figure S2. All measures exhibited a negative correlation with TCNS. IENFD had a negative correlation coefficient of  $r(157)=-0.57$  (p<0.001, online supplemental figure S2a). The correlation coefficient of the DPNCheck amplitude was  $r(153)=-0.53$  (p<0.001, online supplemental figure S2b). The correlation coefficient of the DPNCheck conduction velocity was  $r(158)=-0.64$  (p<0.001, online supplemental figure S2c). Following adjustments for age, sex, HbA1c, and diabetes duration, all correlation coefficients remained significant (all p<0.001, online supplemental table S3). The correlation coefficient of IENFD dropped to  $r(157)=-0.36$ . For DPNCheck, the correlation coefficient of the amplitude was  $r(153)=-0.52$ , while it dropped to  $r(158)=-0.43$  for the conduction velocity.

### Small, large, and mixed fiber neuropathy

Figure 2 provides an overview of the neuropathy subtype distribution in a combined group of T1DM with NP-DPN and T1DM with P-DPN (DPN, figure 2A), the T1DM with NP-DPN group (NP-DPN, figure 2B), and the T1DM with P-DPN group (P-DPN, figure 2C), using the different criteria of the three models defined. In model 1 (blue), none of the participants with DPN (all three groups) were found to have NCN, while this number increased in model 2 (red) and model 3 (beige) (figure 2). Across all three groupings, models 1 and 2 classified most of the participants as having MFN, while model 3 identified a majority of the MFN subtype in the DPN and P-DPN groups. In model 3, the majority of the NP-DPN group was found to have NCN (figure 2B). The distribution of DPN with prevalent SFN did not seem to differ between model 2 and model 3 (figure 2). No significant differences were found between NP-DPN and P-DPN in any of the models (online supplemental table S4).

Online supplemental table S5 provides an overview of the prevalence of pure SFN as per the Besta model and model 2 (adapted). Comparison between the painful and non-painful groups did not yield significant results for either the Besta model or model 2 (adapted). A direct comparison between the Besta model and model 2 (adapted) did not reveal significant differences between the models in any of the groups (DPN, NP-DPN, or P-DPN).

### DPN severity

An overview of diabetes duration, HbA1c, IENFD, DPNCheck, and biothesiometer results are provided

**Table 3** Other clinical tests

	Control (n=51)	T1DM (n=48)	T1DM with NP-DPN (n=67)	T1DM with P-DPN (n=50)	P value	P value adj. (NP-DPN vs P-DPN)	P value (NP-DPN vs P-DPN)	P value adj. (NP-DPN vs P-DPN)
IENFD (abnormal)	6 (12.0%)	16 (34.0%)	54 (81.8%)	38 (84.4%)	0.000*	0.000*	0.801	0.733
IENFD, ankle (fibers/mm)	4.9 (4.0; 6.4)	3.9 (1.7; 4.7)	1.4 (0.4; 2.2)	0.6 (0.2; 2.2)	0.000*	0.000*	0.133	0.107
DPNCheck (abnormal)	6 (12.0%)	6 (12.8%)	55 (84.6%)	38 (80.9%)	0.000*	0.000*	0.619	0.882
DPNCheck, amplitude (µV)	8.0 (6.0; 12.0)	9.0 (6.0; 13.0)	3.0 (2.0; 5.0)	3.0 (2.0; 5.0)	0.000*	0.000*	0.510	0.685
DPNCheck, conduction velocity (m/s)	55.0 (51.0; 61.0)	50.0 (45.0; 54.0)	40.0 (25.0; 46.0)	38.0 (28.0; 45.5)	0.000*	0.000*	0.848	0.477

Categorical variables: presented as number of x and its percentage within each group. Continuous variables: presented as median with the 25th and 75th quartile within each group. All-group comparisons (p value) were carried out using Fisher's exact test (categorical variables) and Kruskal-Wallis H test (continuous variables). Two-group comparisons between T1DM with NP-DPN and T1DM with P-DPN (p value (NP-DPN vs P-DPN)) were carried out using Fisher's exact test (categorical variables) and Mann-Whitney U test (continuous variables). Adjustments for sex, age, HbA1c, and diabetes duration (p value adj.) were calculated using a logistic regression model (categorical variables) and a linear regression model (continuous variables).

\*P<0.05  
adj., adjusted; HbA1c, hemoglobin A1c; IENFD, intra-epidermal nerve fiber density; NP-DPN, non-painful diabetic polyneuropathy; P-DPN, painful diabetic polyneuropathy; T1DM, type 1 diabetes mellitus.

in online supplemental table S6, according to DPN severity. There were significant differences among all groups for all measures except diabetes duration and DPNCheck (abnormal) before adjustments and IENFD (abnormal) and DPNCheck (abnormal) after adjusting for confounders (all,  $p_{adj}>0.05$ ). Online supplemental table S7 provides an overview of the distribution of neuropathy subtype prevalence (SFN, LFN, and MFN) according to DPN severity. For all three models, the prevalence of MFN increased with DPN severity. For model 1, LFN and SFN decreased with DPN severity. For model 2, DPN with prevalent LFN and SFN decreased with DPN severity. For model 3, DPN with prevalent LFN ( $p<0.001$ ) and SFN ( $p>0.05$ ) increased from the no neuropathy group to the mild neuropathy group, while it decreased from mild neuropathy to moderate neuropathy and from moderate neuropathy to severe neuropathy.

## DISCUSSION

In this study, the degree of nerve fiber damage was examined in participants with T1DM, both with and without DPN, and with and without pain. The results revealed that MFN is the most prevalent subtype in T1DM-associated DPN. The results also showed that with an increasing number of tests, the prevalence shifts from MFN to either DPN with a more prevalent SFN or LFN subtype. Based on DPN severity, all three models showed an increased prevalence of MFN and a decreased prevalence of DPN with more prevalent SFN and LFN, except in model 3 ( $\geq 3$  abnormal tests) showing increased DPN with more prevalent LFN and SFN subtype, between the no neuropathy group and the mild neuropathy group.

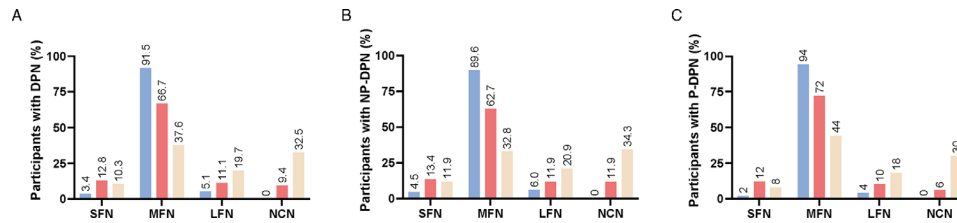
## Tests and questionnaires

As expected in carefully selected study groups, there were significant differences in several demographical measures and serum biomarkers between the groups. In contrast to our findings, longer diabetes duration has previously been associated with P-DPN.<sup>13</sup> Additionally, our results suggest a role of smoking in P-DPN while other studies have been unable to show this association.<sup>13 14</sup> Moreover, we found higher HbA1c in T1DM with P-DPN, which has not been consistently reported in previous studies.<sup>14 15</sup>

There were no differences in IENFD between DPN participants with and without pain. However, missing association could have been due to flooring effect of the IENFD as both groups have very low IENFD and thus no significant difference could be detected. Similarly, the results of DPNCheck did not differ significantly between DPN participants with and without pain. However, correlation graphs revealed a strong negative correlation with TCNS both before and after adjusting for confounders. The reported observations of IENFD and DPNCheck correspond to previous findings.<sup>8 16</sup>

## Neuropathy subtypes

To investigate subtypes of T1DM-associated DPN, we defined three models applying different criteria for the



**Figure 2** Bar charts visualizing the distribution of neuropathy subtype based on the criteria of the three previously defined models. Distributions were investigated in three groups: (A) combined group consisting of all T1DM with NP-DPN participants and all T1DM with P-DPN participants, DPN (first panel); (B) the T1DM with NP-DPN group, NP-DPN (middle panel); and (C) the T1DM with P-DPN group, P-DPN (last panel). Neuropathy subtypes were determined using one of the three models: model 1,  $\geq 1$  abnormal tests (blue); model 2,  $\geq 2$  abnormal tests (red); and model 3,  $\geq 3$  abnormal tests (beige). Subtypes were defined as large fiber neuropathy (LFN); mixed fiber neuropathy (MFN); non-classifiable neuropathy (NCN); non-painful diabetic polyneuropathy (NP-DPN); painful diabetic polyneuropathy (P-DPN); small fiber neuropathy (SFN); and type 1 diabetes mellitus (T1DM).

determination of involved nerve damage. The three models resulted in considerable variations of phenotypes of neuropathy, highlighting the importance of the criteria applied. Moreover, the models showed that most participants with DPN including both P-DPN and NP-DPN were more affected by MFN.

A recent study investigated different diagnostic models for the frequency and subtypes of neuropathy in a large T2DM cohort, including only participants with probable and confirmed DPN according to the Toronto consensus criteria.<sup>4</sup> The authors found considerable variation between the diagnostic models (MFN (51.4%–83.2%); SFN (1.4%–13.1%); LFN (9.3%–21.5%); and NCN (0.5%–14.5%)).<sup>4</sup> Here, we found the distributions of neuropathy phenotypes to be largely similar, suggesting only small differences in the distribution of neuropathy phenotypes between T1DM-associated and T2DM-associated DPN. This might be explained by the different pathologies of the two diseases or by the difference in the demographics of the included participants. Overall, our results suggest that MFN constitutes the most prevalent neuropathy subtype in both T1DM-associated and T2DM-associated DPN,<sup>4</sup> thereby agreeing with previous findings.<sup>17–19</sup> The higher prevalence of MFN found in this study may also partly be explained by DPN severity, since the participants included have been affected by T1DM and possibly DPN over many years. This explanation is supported by previous studies, showing correlations between the prevalence of MFN and DPN severity<sup>4</sup> as well as diabetes duration.<sup>20</sup>

Additionally, the models showed that as the number of abnormal tests increased, the prevalence of DPN with a more predominant LFN subtype and NCN increased, while MFN decreased. DPN with a more prevalent SFN subtype, however, showed a different pattern, with increased prevalence from model 1 to model 2 but not from model 2 to model 3, indicating that  $\geq 2$  abnormal SFN test results may be sufficient in diagnosing DPN with a more prevalent SFN phenotype in T1DM. To better compare the reliability of our model 2 with the Besta criteria as a gold standard of pure SFN,<sup>9</sup> model 2 (adapted) was generated and its ability to accurately

diagnose pure SFN was determined. In a direct comparison, no significant differences were observed between the ability of the two models to diagnose pure SFN. Based on the above findings, we propose a preliminary criteria definition for the diagnosis of DPN with a more prevalent SFN subtype in T1DM. The criteria include:  $\geq 2$  abnormal results from SFN tests: IENFD, Sudoscan (feet), pinprick, and temperature sensitivity. Moreover our model 2 (adapted) could prove a viable alternative to the Besta criteria in diagnosing pure SFN. We believe that our proposal is well-founded based on our results and the Besta criteria<sup>9</sup> and taking into consideration the high risk of false positive results, especially in model 1.

Finally, we investigated the distribution of neuropathy phenotypes according to DPN severity, including only T1DM participants with DPN. Here, MFN prevalence increased according to DPN severity in all three models. This is consistent with the general notion that DPN severity is associated with more documented signs of sensory loss or gain.<sup>21</sup> DPN with a more prevalent LFN or SFN subtype decreased in models 1 and 2. In model 3, their prevalence increased between no neuropathy and mild neuropathy, and then decreased between mild neuropathy and the more severe neuropathy groups. This might suggest that in mild/early stages of neuropathy, one fiber type (small or large) is more susceptible to damage. This contrasts with a recent study showing parallel damage to both small and large nerve fibers in individuals with newly diagnosed T1DM.<sup>22</sup> The most likely explanation for this discrepancy is the low neuropathy subtype specificity of model 3, where a diagnosis of DPN with a more prevalent SFN or LFN allows for up to two abnormal tests of LFN or SFN, respectively. Additionally, the low sensitivity (62.9%) and specificity (74.6%) of the TCNS scoring system,<sup>23</sup> which was applied as a measure of DPN severity in this study, might have affected the results. Another explanation could be the impact of diabetes duration and HbA1c, which have been shown to correlate with DPN severity.<sup>24,25</sup>



### Strengths and limitations of the study

The strengths of this study include the carefully phenotyped participants following commonly used criteria, and the relatively large sample size. The limitations include the selective inclusion of T1DM participants with and without DPN and with and without pain. These factors might affect the results by increasing the likelihood of highly distinctive differences between the groups. Moreover, the inclusion criteria of diabetes duration  $\geq 5$  years represents a limitation of the study, as this might have resulted in an underestimation of the prevalence of a specific neuropathy subtype, given that the study does not account for developments within the initial 5 years of T1DM progression. Other limitations include the T1DM group, which was composed of participants with no DPN, subclinical DPN, and possible DPN, as well as exclusion of individuals with mild pain intensity. This could result in significant differences that may not accurately represent the true population or be transferable. However, in this study, the merging of groups was based on clinical assessments, aiming to reduce bias. Additionally, the application of fixed cut-off values in both DPNCheck and biothesiometry is highly debated, where differential fixed or normative cut-off values are used.<sup>26–31</sup> The selected cut-offs affect the groupings in this study and the comparability of this study with other studies using other cut-offs. This is a major challenge in the field that should be addressed in future studies. Another limitation is the inclusion of differential tests for the diagnosis of LFN, MFN, and SFN, which vary in their diagnostic strengths for DPN. However, most of the selected tests are already applied in the clinic, except for DPNCheck and Sudoscan, and as such, the suggested model can easily be implemented in the future diabetic complication clinics and/or research. Although it is important to keep in mind the limitations of Sudoscan, whose reliability is highly debated in the scientific community.<sup>32–34</sup> The inclusion of Sudoscan in the models might impact the reliability of the proposed models, in particular model 1, as this model only requires one abnormal result from the small fiber tests to diagnose with a more prevalent SFN. Another limitation is found in the definition of models 2 and 3, as the neuropathy subtypes determined from these models are describing DPN with a more prevalent LFN or SFN subtype rather than pure SFN and LFN, rendering direct comparison with other pure SFN and LFN criteria complicated. Finally, confounders such as age, sex, diabetes duration, and HbA1c might have affected the results obtained in this study. However, this effect was minimized following confounder adjustment analyses.

### CONCLUSIONS

This study demonstrated that DPN in T1DM is predominantly manifested as mixed fiber neuropathy and that for SFN diagnosis, at least two abnormal tests of small fiber affection demonstrate the best diagnostic performance. No significant differences in DPN subtype were found

between P-DPN and NP-DPN. However, the findings indicate that in the mild stages of DPN, one fiber type is likely to be more affected than the other, supporting a complex underlying mechanism of DPN.

**Contributors** All authors meet the ICMJE uniform requirements for authorship. CSB, TSJ, CSH, KBY, JRN, and PK conceptualized the study and contributed to study design. PK, CSB, and MBS participated in data analysis. CSB, CSH, PK, KBY, KS, HIM, and HK contributed to clinical data acquisition. All authors contributed to manuscript drafting, editing, and revision for intellectual content. CSB, PK, and MBS are responsible for the integrity of the work as a whole. PK and CSB are responsible for the overall content as the guarantors.

**Funding** This research was funded by the Novo Nordisk Foundation (grant number NNF180C0052301) and by the Lundbeck Foundation (grant number R359-2020-2620).

**Competing interests** PK has received personal fees from Grünenthal, Alnylam, and Vertex Pharmaceuticals, and has received a research grant from Merck outside the submitted work.

**Patient consent for publication** Not applicable.

**Ethics approval** This study was approved by the Regional Committee on Health Research Ethics (1-10-72-103-19). All participants gave their written, informed consent in accordance with the Helsinki Declaration. All participant data were handled and stored in accordance with local rules and regulations and complied with the General Data Protection Regulation.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request. Due to restrictions under the Danish law protecting personal privacy, anonymized data can only be made available through an application to Aarhus University by contacting the corresponding author or Aarhus University (clin@au.dk).

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

### ORCID iD

Pall Karlsson <http://orcid.org/0000-0001-7082-9576>

### REFERENCES

- 1 Feldman EL, Callaghan BC, Pop-Busui R, *et al*. Diabetic neuropathy. *Nat Rev Dis Primers* 2019;5:41.
- 2 Galosi E, Hu X, Michael N, *et al*. Redefining distal symmetrical polyneuropathy features in type 1 diabetes: a systematic review. *Acta Diabetol* 2022;59:1–19.
- 3 Terkelsen AJ, Karlsson P, Lauria G, *et al*. The diagnostic challenge of small fibre neuropathy: clinical presentations, evaluations, and causes. *Lancet Neurol* 2017;16:934–44.
- 4 Itani M, Gylfadottir SS, Kroigård T, *et al*. Small and large fiber sensory polyneuropathy in type 2 diabetes: influence of diagnostic criteria on neuropathy subtypes. *J Peripher Nerv Syst* 2021;26:55–65.
- 5 Galosi E, Di Pietro G, La Cesa S, *et al*. Differential involvement of myelinated and unmyelinated nerve fibers in painful diabetic polyneuropathy. *Muscle Nerve* 2021;63:68–74.
- 6 Breiner A, Lovblom LE, Perkins BA, *et al*. Does the prevailing hypothesis that small-fiber dysfunction precedes large-fiber dysfunction apply to type 1 diabetic patients? *Diabetes Care* 2014;37:1418–24.

- 7 Ziegler D, Mayer P, Gries FA. Evaluation of thermal, pain, and vibration sensation thresholds in newly diagnosed type 1 diabetic patients. *J Neurol Neurosurg Psychiatry* 1988;51:1420–4.
- 8 Vas PRJ, Sharma S, Rayman G. Distal sensorimotor neuropathy: improvements in diagnosis. *Rev Diabet Stud* 2015;12:29–47.
- 9 Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain (Bacau)* 2008;131:1912–25.
- 10 Gylfadottir SS, Itani M, Kristensen AG, et al. The characteristics of pain and dysesthesia in patients with diabetic polyneuropathy. *PLoS One* 2022;17:e0263831.
- 11 Gylfadottir SS, Weeracharoenkul D, Andersen ST, et al. Painful and non-painful diabetic polyneuropathy: clinical characteristics and diagnostic issues. *J Diabetes Investig* 2019;10:1148–57.
- 12 Hu X, Buhl CS, Sjogaard MB, et al. Structural changes in Schwann cells and nerve fibres in type 1 diabetes: relationship with diabetic polyneuropathy. *Diabetologia* 2023;66:2332–45.
- 13 Barbosa M, Saavedra A, Oliveira S, et al. Prevalence and determinants of painful and painless neuropathy in type 1 diabetes mellitus. *Front Endocrinol (Lausanne)* 2019;10:402.
- 14 Spallone V, Morganti R, D'Amato C, et al. Clinical correlates of painful diabetic neuropathy and relationship of neuropathic pain with sensorimotor and autonomic nerve function. *Eur J Pain* 2011;15:153–60.
- 15 Ferdousi M, Azmi S, Kalteniece A, et al. Greater small nerve fibre damage in the skin and cornea of type 1 diabetic patients with painful compared to painless diabetic neuropathy. *Eur J Neurol* 2021;28:1745–51.
- 16 Petropoulos IN, Ponirakis G, Khan A, et al. Diagnosing diabetic neuropathy: something old, something new. *Diabetes Metab J* 2018;42:255–69.
- 17 Wittenberg B, Svendsen TK, Gaist LM, et al. Test-retest and time dependent variation and diagnostic values of vibratory sensation determined by Biothesiometer and the Rydel-Seiffer tuning fork. *Brain Behav* 2021;11:e2230.
- 18 Pop-Busui R, Boulton AJM, Feldman EL, et al. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes Care* 2017;40:136–54.
- 19 Yang H, Sloan G, Ye Y, et al. New perspective in diabetic neuropathy: from the periphery to the brain, a call for early detection, and precision medicine. *Front Endocrinol (Lausanne)* 2019;10:929.
- 20 Jin Y, Wang W, Chen W, et al. Corneal confocal microscopy: a useful tool for diagnosis of small fiber neuropathy in type 2 diabetes. *J Diabetes Investig* 2021;12:2183–9.
- 21 Themistocleous AC, Ramirez JD, Shillo PR, et al. The pain in neuropathy study (PINS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. *Pain* 2016;157:1132–45.
- 22 Ziegler D, Bönhof GJ, Strom A, et al. Progression and regression of nerve fibre pathology and dysfunction early in diabetes over 5 years. *Brain (Bacau)* 2021;144:3251–63.
- 23 Gylfadottir SS, Itani M, Kroigård T, et al. Diagnosis and prevalence of diabetic polyneuropathy: a cross-sectional study of Danish patients with type 2 diabetes. *Eur J Neurol* 2020;27:2575–85.
- 24 Kwai NCG, Arnold R, Poynter AM, et al. Association between glycemic variability and peripheral nerve dysfunction in type 1 diabetes. *Muscle Nerve* 2016;54:967–9.
- 25 Nisar MU, Asad A, Waqas A, et al. Association of diabetic neuropathy with duration of type 2 diabetes and glycemic control. *Cureus* 2015;7:e302.
- 26 Ramanathan S, Thomas R, Chanu AR, et al. Standard clinical screening tests, sural radial amplitude ratio and F wave latency compared to conventional nerve conduction studies in the assessment of sensorimotor polyneuropathy in patients with type 2 diabetes mellitus. *Indian J Endocrinol Metab* 2021;25:509–15.
- 27 Nolte S, van Londen M, Elting JWJ, et al. Vibration threshold in non-diabetic subjects. *PLoS One* 2020;15:e0237733.
- 28 Fukuda T, Fujii A, Akihisa T, et al. Association between diabetic peripheral neuropathy as measured using a point-of-care sural nerve conduction device and urinary albumin excretion in patients with type 2 diabetes. *J Clin Med* 2023;12:4089.
- 29 Shibata Y, Himeno T, Kamiya T, et al. Validity and reliability of a point-of-care nerve conduction device in diabetes patients. *J Diabetes Investig* 2019;10:1291–8.
- 30 Chatzikosma G, Pafili K, Demetriou M, et al. Evaluation of sural nerve automated nerve conduction study in the diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. *Arch Med Sci* 2016;12:390–3.
- 31 Young MJ, Breddy JL, Veves A, et al. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. *Diabetes Care* 1994;17:557–60.
- 32 Casellini CM, Parson HK, Richardson MS, et al. Sudoscan, a noninvasive tool for detecting diabetic small fiber neuropathy and autonomic dysfunction. *Diabetes Technol Ther* 2013;15:948–53.
- 33 García-Ulloa AC, Almeda-Valdes P, Cuatecontzi-Xochitiotzi TE, et al. Detection of sudomotor alterations evaluated by Sudoscan in patients with recently diagnosed type 2 diabetes. *BMJ Open Diabetes Res Care* 2022;10:e003005.
- 34 Duchesne M, Richard L, Vallat J-M, et al. Assessing sudomotor impairment in patients with peripheral neuropathy: comparison between electrochemical skin conductance and skin biopsy. *Clin Neurophysiol* 2018;129:1341–8.

	Small fiber tests		Large fiber tests	
Criteria (Besta)	<i>(≥2 abnormal results)</i>		<i>(No abnormal results)</i>	
Tests	Besta criteria	This study	Beta criteria	This study
1	Pinprick <b>and</b> Thermal sensory loss <b>and/or</b> Allodynia <b>and/or</b> Hyperalgesia	Pinprick (Neurotip) <b>and</b> Thermo rollers (25°C/40°C) <b>and/or</b> Brush <b>and/or</b> N/A	Light touch <b>and/or</b> Vibration sensation <b>and/or</b> Proprioception sensation <b>and/or</b> Deep tendon reflexes	10g monofilament (Neuropen) <b>and/or</b> Biothesiometry <b>and/or</b> Proprioception sensation <b>and/or</b> Ankle and knee reflexes
2	Warm threshold <b>and/or</b> Cold threshold (Assessed by QST)	Warm threshold <b>and/or</b> Cold threshold (Assessed by QST)	Motor fiber impairment (waste and/or weakness)	Atrophy <b>and/or</b> Dorsal flex <b>and/or</b> Plantar flex
3	IENFD	IENFD	Nerve conduction studies	DPNCheck

Table S1 – Besta Criteria Tests

Overview of the tests applied in the Besta criteria and there corresponding tests in this study. QST; quantitative sensory testing. IENFD; intraepidermal nerve fiber density. N/A; not applicable.

	Control (n=51)	T1DM (n=48)	T1DM w. NP-DPN (n=67)	T1DM w. P- DPN (n=50)	p value	p value (NP-DPN vs. P-DPN)
Insulin (yes)	-	48 (100.0%)	67 (100.0%)	50 (100.0%)	-	-
Other antidiabetics (yes)	-	3 (6.3%)	1 (1.5%)	3 (6.0%)	0.338	0.312
Antihypertensive (yes)	4 (7.8%)	23 (47.9%)	53 (80.3%)	32 (65.3%)	0.000***	0.087
Cholesterol lowering (yes)	1 (2.0%)	28 (58.3%)	57 (86.4%)	39 (79.6%)	0.000***	0.447
Platelet inhibitors (yes)	1 (2.0%)	7 (14.6%)	31 (46.3%)	22 (44.9%)	0.000***	1.000
Anticoagulant (yes)	0 (0.0%)	0 (0.0%)	4 (6.2%)	3 (6.1%)	0.068	1.000
Antidepressants (yes)	1 (2.0%)	4 (8.3%)	7 (10.8%)	13 (26.5%)	0.002**	0.045*
Analgesics (yes)	1 (2.0%)	4 (8.5%)	11 (16.7%)	30 (65.2%)	0.000***	0.000***
Paracetamol (yes)	1 (2.0%)	1 (2.1%)	9 (13.4%)	17 (34.0%)	0.000***	0.013*
NSAIDs (yes)	1 (2.0%)	2 (4.2%)	4 (6.0%)	3 (6.0%)	0.756	1.000
Tricyclic antidepressants (yes)	0 (0.0%)	0 (0.0%)	1 (1.5%)	5 (10.0%)	0.008**	0.082
Gabapentin (yes)	0 (0.0%)	1 (2.1%)	1 (1.5%)	14 (28.0%)	0.000***	0.000***
Pregabalin (yes)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (4.0%)	0.101	0.181
SNRIs (yes)	0 (0.0%)	2 (4.2%)	0 (0.0%)	4 (8.0%)	0.015*	0.031*
Tramadol (yes)	0 (0.0%)	1 (2.1%)	0 (0.0%)	5 (10.0%)	0.003**	0.013*
Codeine (yes)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-
Morphine (yes)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (6.0%)	0.022*	0.075
Any other treatment (yes)	4 (8.5%)	11 (27.5%)	19 (35.8%)	7 (17.1%)	0.006**	0.062

Table S2 – Patient medication

Categorical variables. Presented as number of “yes”-answers and its percentage within each group. Comparisons between groups was carried out using Fisher’s exact test (p value). Comparisons between T1DM w. NP-DPN and T1DM w. P-DPN was carried out using Fisher’s exact test (p value (NP-DPN vs. P-DPN)). \*p value <0.05; \*\*p value <0.01; \*\*\*p value <0.001.

Partial correlations	TCNS
IENFD	r(157) = -0.36 p <sub>adj</sub> < 0.001
DPNCheck, amplitude	r(153) = -0.52 p <sub>adj</sub> < 0.001
DPNCheck, conduction velocity	r(158) = -0.43 p <sub>adj</sub> < 0.001

Table S3 – Partial correlation coefficients of IENFD and DPNCheck

Partial correlations, adjusting for age, sex, HbA1c levels, and diabetes duration. Tests did not include the control group.

	Control (n=51)	T1DM (n=48)	NP-DPN (n=67)	P-DPN (n=50)	p value	p value (NP-DPN vs. P-DPN)
<b>Model 1: <math>\geq 1</math> abnormal tests</b>						
SFN	10 (19.6%)	27 (56.3%)	63 (94.0%)	48 (96.0%)	0.000***	1.000
LFN	8 (15.7%)	14 (29.2%)	64 (95.5%)	49 (98.0%)	0.000***	0.635
MFN	0 (0.0%)	7 (14.6%)	60 (89.6%)	47 (94.0%)	0.000***	0.513
LFN only	8 (15.7%)	7 (14.6%)	4 (6.0%)	2 (4.0%)	0.100	1.000
SFN only	10 (19.6%)	20 (41.7%)	3 (4.5%)	1 (2.0%)	0.000***	0.635
<b>Model 2: <math>\geq 2</math> abnormal tests</b>						
SFN	0 (0.0%)	6 (12.5%)	51 (76.1%)	42 (84.0%)	0.000***	0.359
LFN	1 (2.0%)	1 (2.1%)	50 (74.6%)	41 (82.0%)	0.000***	0.377
MFN	0 (0.0%)	0 (0.0%)	42 (62.7%)	36 (72.0%)	0.000***	0.326
LFN only	1 (2.0%)	1 (2.1%)	8 (11.9%)	5 (10.0%)	0.075	1.000
SFN only	0 (0.0%)	6 (12.5%)	9 (13.4%)	6 (12.0%)	0.021*	1.000
<b>Model 3: <math>\geq 3</math> abnormal tests</b>						
SFN	0 (0.0%)	0 (0.0%)	30 (44.8%)	26 (52.0%)	0.000***	0.460
LFN	0 (0.0%)	0 (0.0%)	36 (53.7%)	31 (62.0%)	0.000***	0.451
MFN	0 (0.0%)	0 (0.0%)	22 (32.8%)	22 (44.0%)	0.000***	0.250
LFN only	0 (0.0%)	0 (0.0%)	14 (20.9%)	9 (18.0%)	0.000***	0.815
SFN only	0 (0.0%)	0 (0.0%)	8 (11.9%)	4 (8.0%)	0.004**	0.553

Table S4 – SFN, LFN, and MFN

Categorical variables. Presented as number having neuropathy subtype and its percentage within each group. Comparisons between groups was carried out using Fisher's exact test (p value). Comparisons between T1DM w. NP-DPN and T1DM w. P-DPN was carried out using Fisher's exact test (p value (NP-DPN vs. P-DPN)). \*p value <0.05; \*\*p value<0.01; \*\*\*p value<0.001.

	DPN (n=117)	NP-DPN (n=67)	P-DPN (n=50)	p value (NP-DPN vs. P-DPN)
Besta model	2 (1.7%)	1 (1.5%)	1 (2%)	1.000
Model 2 (adapted#)	3 (2.6%)	2 (3%)	1 (2%)	1.000
<b>p value (Besta vs. model 2 (adapted#))</b>	1.000	1.000	1.000	

Table S5 – Pure SFN determined by Besta model and model 2 (adapted#)

Categorical variables. Presented as number having pure SFN and percentages within each group. Comparisons were carried out using Fisher's exact test (p value). # Model 2 was adapted determine pure SFN (requires 2 abnormal SFN measures and no abnormal LFN tests). \*p value <0.05; \*\*p value<0.01; \*\*\*p value<0.001.

	No neuropathy (0-5, n=38)	Mild neuropathy (6-8, n=31)	Moderate neuropathy (9-11, n=33)	Severe neuropathy (≥12, n=15)	p value	p value adj.
Diabetes duration	36.0 (27.0;44.0)	38.0 (30.0;54.0)	38.0 (33.0;47.0)	30.0 (25.0;43.0)	0.592	
HbA1c levels	58.0 (52.0;62.3)	63.7 (57.0;71.8)	66.0 (59.7;76.9)	71.0 (59.4;85.0)	0.000***	
<b>IENFD (abnormal)</b>	28 (73.7%)	20 (74.1%)	30 (93.8%)	14 (100.0%)	0.021*	0.178
IENFD, angle (fibers/mm)	1.7 (0.8;2.7)	1.4 (0.4;3.3)	0.4 (0.1;1.5)	0.3 (0.1;0.6)	0.000***	0.000***
<b>DPNCheck (abnormal)</b>	27 (75.0%)	26 (83.9%)	29 (90.6%)	11 (84.6%)	0.411	0.624
DPNCheck, amplitude (uV)	4.0 (3.0;6.0)	2.0 (0.0;5.0)	2.0 (1.0;3.0)	2.0 (2.0;4.0)	0.000***	0.000***
DPNCheck, conduction velocity (m/s)	43.5 (39.5;49.0)	35.0 (0.0;44.0)	32.0 (2.0;42.0)	32.0 (0.0;46.0)	0.001***	0.000***
<b>Biothesiometry (abnormal)</b>	15 (39.5%)	18 (58.1%)	25 (75.8%)	13 (86.7%)	0.002**	0.003**
Biothesiometry (V)	19.9 (12.3;29.2)	28.3 (18.3;44.0)	40.0 (28.3;49.0)	47.8 (30.8;50.0)	0.000***	0.000***

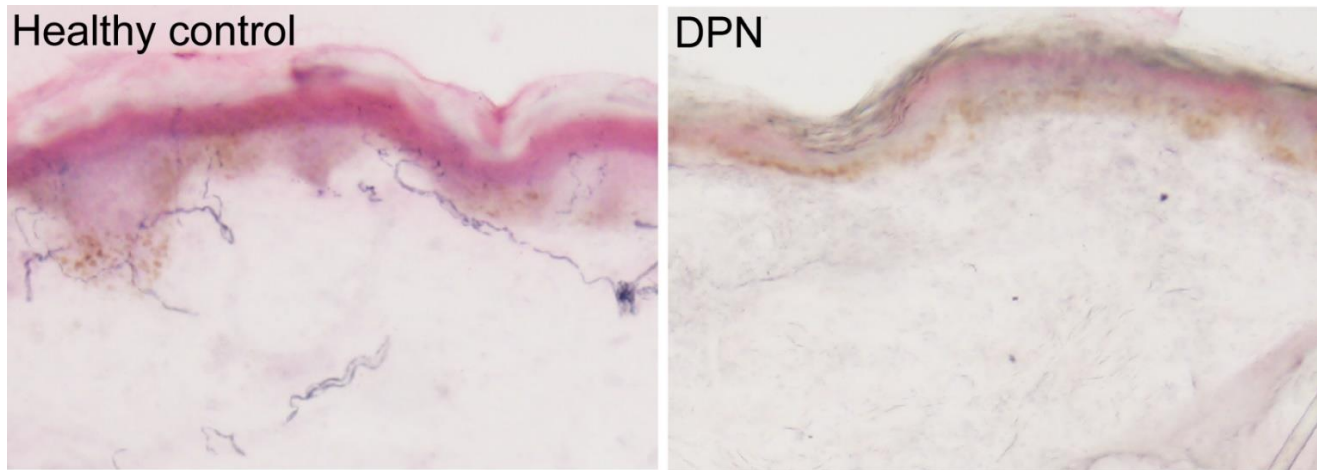
Table S6 – Other tests

Categorical variables: Presented as number of *x* and its percentage within each group. Continuous variables: Presented as median with the 25<sup>th</sup> and 75<sup>th</sup> quartile within each group. All-group comparisons (*p* value) were carried out using Fisher's exact test (categorical variables) and Kruskal Wallis *H* test (continuous variables). Adjustments for sex, age, HbA1c, and diabetes duration (*p* value adj.) were calculated using a logistic regression model (categorical variables) and a linear regression model (continuous variables). Intraepidermal nerve fiber density, IENFD. \**p* value < 0.05; \*\**p* value < 0.01; \*\*\**p* value < 0.001.

	No neuropathy (0-5, n=38)	Mild neuropathy (6-8, n=31)	Moderate neuropathy (9-11, n=33)	Severe neuropathy (≥12, n=15)	p value
<b>Model 1: ≥ 1 abnormal tests</b>					
MFN	30 (78.9%)	29 (93.5%)	33 (100.0%)	15 (100.0%)	0.007**
LFN	5 (13.2%)	1 (3.2%)	0 (0.0%)	0 (0.0%)	0.046*
SFN	3 (7.9%)	1 (3.2%)	0 (0.0%)	0 (0.0%)	0.363
<b>Model 2: ≥ 2 abnormal tests</b>					
MFN	11 (28.9%)	20 (64.5%)	32 (97.0%)	15 (100.0%)	0.000***
LFN	7 (18.4%)	5 (16.1%)	1 (3.0%)	0 (0.0%)	0.075
SFN	10 (26.3%)	5 (16.1%)	0 (0.0%)	0 (0.0%)	0.001**
<b>Model 3: ≥ 3 abnormal tests</b>					
MFN	3 (7.9%)	4 (12.9%)	23 (69.7%)	14 (93.3%)	0.000***
LFN	4 (10.5%)	15 (48.4%)	3 (9.1%)	1 (6.7%)	0.000***
SFN	4 (10.5%)	6 (19.4%)	2 (6.1%)	0 (0.0%)	0.209

Table S7 – SFN, LFN, and MFN distribution over neuropathy severity

Categorical variables. Presented as number having neuropathy subtype and its percentage within each group. Comparisons between groups was carried out using Fisher's exact test (*p* value). \**p* value < 0.05; \*\**p* value < 0.01; \*\*\**p* value < 0.001.



*Figure S1 – Immunohistological staining of PGP9.5<sup>+</sup> cutaneous nerve fibers in skin biopsies*  
Cutaneous nerve fiber labelling in a skin biopsy section from a healthy control (left) and from a participant with diabetic peripheral neuropathy (DPN).

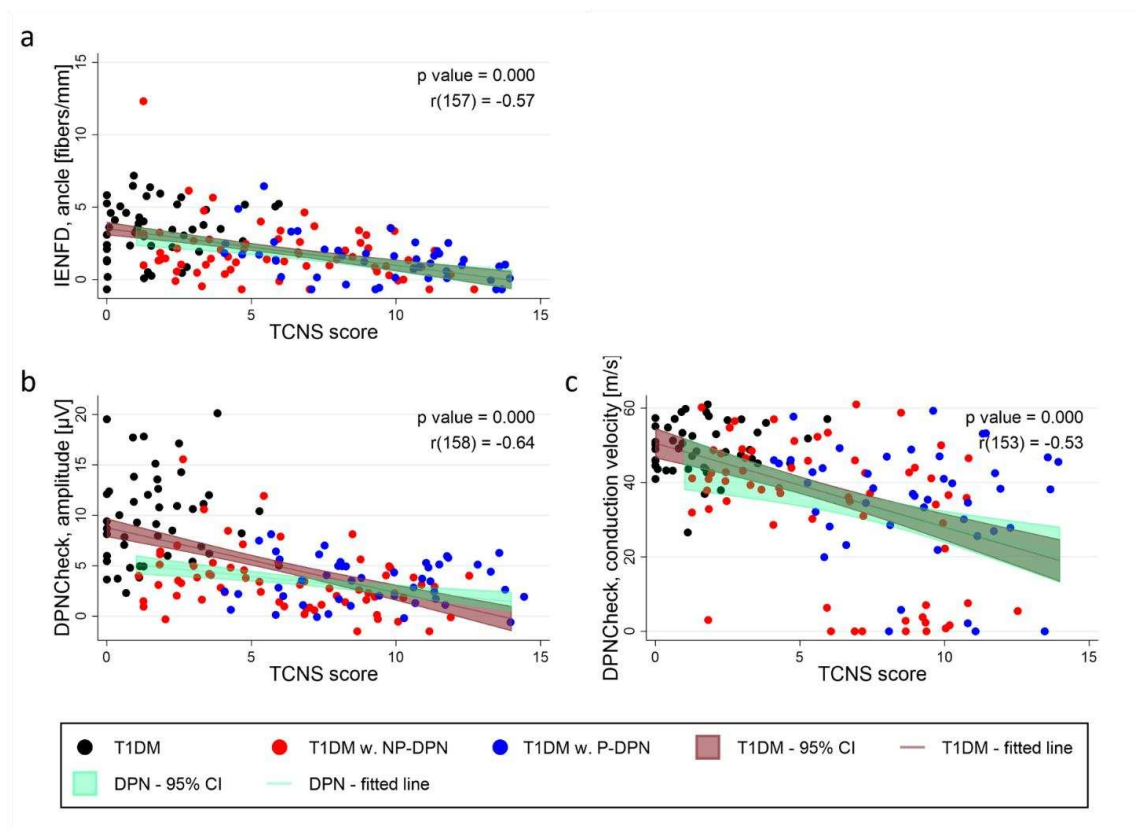


Figure S2 – TCNS Correlations with IENFD and DPNCheck

Graphs depict the correlation between TCNS score and IENFD (a); TCNS score and DPNCheck, amplitude (b); and TCNS score and DPNCheck conduction velocity (c). Spearman's correlation coefficient for each correlation is visualized in the upper right corner of each graph along with the corresponding p value. Correlation analysis is carried out on the 3 out of the four groups: type 1 diabetes mellitus, T1DM (black circles); T1DM with non-painful diabetic polyneuropathy, T1DM w. NP-DPN (red circles); and T1DM with painful diabetic polyneuropathy, T1DM w. P-DPN (blue circles). Two lines were fitted, one based on all included participants with T1DM (brown line) and one based on all participant with DPN (green line). Intraepidermal nerve fiber density, IENFD; Toronto clinical neuropathy score, TCNS.

### Study Participants and the Definition and Assessment of DPN and P-DPN

T1DM participants were recruited at the three participating Steno Diabetes Centers in Denmark, located in Odense, Aarhus, and Copenhagen, from December 2019 to November 2021. Control participants were recruited through online advertisements in Aarhus only.

The inclusion criteria for T1DM participants were age 30-75 years and T1DM duration  $\geq 5$  years. Exclusion criteria for T1DM participants were inability to understand oral instructions, neuropathy of other causes than diabetes, severe pain of other causes than neuropathy, any history of alcohol misuse ( $>14$  or  $>21$  units/week for women



and men, respectively), substance abuse, pregnancy, any skin disorder in biopsy area, prior chronic ulcers in biopsy areas, clinical intermittent claudication, very weak/non-palpable foot pulses, existing diabetic foot ulcers, and known allergy to lidocaine. Participants with pain had to have an average pain of  $\geq 4$  on a numeric rating scale (NRS) over the last 7 days to be included.

The in- and exclusion criteria for the control participants were like those of the T1DM participants except for the addition of exclusion criteria: the presence of any metabolic or neurological disorders, ongoing pain of any cause, and clinical depression.

All study participants underwent the following examinations: history taking, patient interview of pain symptoms, blood and urine samples, anthropometric measures, DPNCheck, Sudoscan, vibration (biothesiometry), clinical neurological examination (muscle strength, knee and ankle reflexes, tuning fork, sensory function (brush, neurotip, neuropen, Rolltemp), position sensation, Michigan Neuropathy Screening Instrument (MNSI) examination and questionnaire, skin biopsies and questionnaires (Toronto Clinical Neuropathy Score (TCNS), subjective and validated questionnaire on symptoms of neuropathy (COMPASS-31), numeric rating scale (NRS) of pain (0-10), PainDETECT). [1]

### **Clinical diagnosis of DPN and Pain**

The participants underwent a comprehensive examination performed by trained medical doctors. The examination techniques were trained and harmonized between the participating centers using written instructions and instruction videos demonstrating the techniques. Tests performed included assessing ankle and knee reflexes, vibration detection thresholds, using biothesiometry on the hallux (Horwell neurothesiometers, Wilford Industrial, Nottingham, results of 25 V or above were considered abnormal[2]), mechanical detection, using a Neuropen (10 g monofilament, less than 8 sensations out of 10 stimuli was considered abnormal), and pinprick sensation, using a Neurotip® on the hallux (reduced or absent sensation was considered abnormal). Temperature sensation was assessed using thermo rollers (Rolltemp II) of 25°C (cold) and 40°C (warm) on top of the hallux, with participants indicating whether they felt cold, warm, or neutral (neutral or inverse thermal sensation were considered abnormal). In addition, participants were evaluated using DPNCheck to measure sural nerve amplitude and velocity (amplitude  $\leq 4\mu\text{V}$  and/or velocity  $\leq 40$  m/s were considered abnormal[3, 4]), a 3 mm punch skin biopsy, 10cm above the lateral malleoli, for later intraepidermal nerve fiber density (IENFD) analysis, and clinical blood sample analysis, including HbA1c, triglycerides, total and LDL-cholesterol.

The Toronto consensus criteria were applied as a case definition of DPN[5], while P-DPN was defined using the NeuPSIG grading system for neuropathic pain adapted to DPN as at least probable P-DPN (i.e. history of relevant neurological disease, pain distribution being neuroanatomically plausible and associated with sensory signs in the same region)[6, 7]. Signs of DPN were determined using the tests mentioned above, with diagnosis confirmed through abnormal IENFD results, based on available normative reference values[8] and/or abnormal DPNCheck, amplitude and/or velocity. Participants were diagnosed with P-DPN if they reported pain in both feet/legs, sensory signs in the feet/legs, had a diagnosis of T1DM and abnormal IENFD and/or DPNCheck. The level of pain was measured using a NRS from 0 (no pain) to 10 (maximum pain). As the Toronto consensus criteria does not measure the severity of DPN, the TCNS from 0 to 19 was applied to assess DPN severity where TCNS 1-5 represents mild DPN, 6-11 moderate DPN, and 12-19 severe DPN.

T1DM participants were grouped into likelihood of DPN following the Toronto consensus criteria: *subclinical DPN*, characterized by the presence of no signs or symptoms of neuropathy confirmed by an abnormal DPNCheck or IENFD; *possible DPN*, characterized by the presence of at least one of either sensory symptoms, sensory signs, or abnormal ankle reflexes; *probable DPN*, characterized by the presence of a combination of two or more of either sensory symptoms, sensory signs, or abnormal ankle reflexes; and *confirmed DPN*, characterized by the presence of one of either sensory symptoms, sensory signs, or abnormal ankle reflexes confirmed by an abnormal DPNCheck or IENFD. T1DM participants without signs or symptoms of neuropathy confirmed by normal DPNCheck and IENFD were gathered in a group labeled *no DPN*.

The participants in the groups *possible DPN* and *subclinical DPN* were assessed by a trained diabetologist and an expert in DPN and were evaluated not to have clinical DPN. For *subclinical DPN*, having abnormal IENFD and/or DPNCheck (amplitude), there were no signs or symptoms of DPN. Additionally, for *possible DPN*, having just one symptom or sign, there were no confirming tests supporting the presence of DPN and in no cases was it plausible that the sign or symptom was due to DPN. Based on this and that there were little or no overall differences between the groups, these two groups were combined with the *no DPN* group into a clinically determined *no DPN* group, thereby increasing the overall power of the study. Participants with T1DM were categorized into two main groups: the T1DM group and the DPN group. The T1DM group included individuals with no or very minimal signs of DPN: namely those clinically categorized to have *no DPN*. The DPN group was composed of individuals with *probable DPN* and *confirmed DPN*. Further, the DPN group was divided into two subgroups based on NRS scores: the NP-DPN subgroup (T1DM w. NP-DPN) and the P-DPN (T1DM w. P-DPN) subgroup. Individuals in the T1DM w. P-DPN subgroup experienced neuropathic pain persisting for at least three months, with an average

pain intensity of 4 or higher on the NRS over the past week, occurring on a semi-daily basis or more frequently, and were without any severe pain unrelated to neuropathy. Those in the T1DM w. NP-DPN subgroup had no reported pain, indicated by a score of 0 on the NRS. Participants scoring between 1 and 3 on the NRS were not included in the study.

### **Skin Biopsies**

Two 3 mm punch biopsies (Miltex, York, PA) were taken at the same site, 10 cm proximal to the lateral malleolus, under sterile conditions after subcutaneous injection of 0.4-0.5 ml lidocaine solution (20 mg/ml), in accordance with published guidelines[9]. The processing and staining of the biopsies are described in detail elsewhere[10]. Briefly, the biopsies were fixed in Zamboni fixative overnight (o/n), cryoprotected, and stored at -20°C until sectioning. Fifty  $\mu$ m thick sections were stained using a free-floating protocol using PGP 9.5 (1:2000, Zytomed, Berlin, Germany) and an appropriate secondary antibody.

IENFD was determined under an x40 objective by a trained investigator blinded by the origin of the biopsies. PGP 9.5-immunoreactive nerve fibers crossing from the dermis to the epidermis were quantified. The total number of counted nerve fibers were then divided by the section length, giving an estimated IENFD [11]. Abnormal IENFD was defined based on published normative reference values when using the same methods [8].

### **Sudomotor function**

Sudoscans<sup>®</sup> was applied as a proxy measure of small nerve fiber function in the skin by evaluating sweat gland function in the skin. The measure is thought to function as a small fiber measure as the sweat glands in the skin are densely innervated by small nerve fibers, which presumably are affected by small fiber damage alongside the small nerve fibers innervating epidermis[12].

Sudoscans determine sweat gland function by determining the chloride concentration on the surface of the skin. The detailed mechanics of the method have been described previously[12]. In this study, the average sweat gland function of both feet was assessed, and abnormal results were defined as electrochemical skin conductance (ESC) values <60  $\mu$ S[12].

### **References**

- [1] Jensen TS, Karlsson P, Gylfadottir SS, et al. (2021) Painful and non-painful diabetic neuropathy, diagnostic challenges and implications for future management. *Brain* 144(6): 1632-1645. [10.1093/brain/awab079](https://doi.org/10.1093/brain/awab079)

- [2] Young MJ, Breddy JL, Veves A, Boulton AJ (1994) The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. *Diabetes Care* 17(6): 557-560. 10.2337/diacare.17.6.557
- [3] Chatzikosma G, Pafili K, Demetriou M, Vadikolias K, Maltezos E, Papanas N (2016) Evaluation of sural nerve automated nerve conduction study in the diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. *Arch Med Sci* 12(2): 390-393. 10.5114/aoms.2016.59265
- [4] NEUROMetrix (2023) Test Results. Available from <https://www.dpncheck.com/test-results/>. Accessed 31st October 2023
- [5] Tesfaye S, Boulton AJ, Dyck PJ, et al. (2010) Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 33(10): 2285-2293. 10.2337/dc10-1303
- [6] Finnerup NB, Haroutounian S, Kamerman P, et al. (2016) Neuropathic pain: an updated grading system for research and clinical practice. *Pain* 157(8): 1599-1606. 10.1097/j.pain.0000000000000492
- [7] IASP International Association for the Study of Pain. Taxonomy. Pain terms. Neuropathic pain. Available from <https://www.iasp-pain.org/resources/terminology/#Neuropathicpain>. Accessed April 21st 2021
- [8] Lauria G, Bakkers M, Schmitz C, et al. (2010) Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst* 15(3): 202-207. 10.1111/j.1529-8027.2010.00271.x
- [9] Lauria G, Hsieh ST, Johansson O, et al. (2010) European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *Eur J Neurol* 17(7): 903-912, e944-909. 10.1111/j.1468-1331.2010.03023.x
- [10] Karlsson P, Haroutounian S, Polydefkis M, Nyengaard JR, Jensen TS (2016) Structural and functional characterization of nerve fibres in polyneuropathy and healthy subjects. *Scand J Pain* 10: 28-35. 10.1016/j.sjpain.2015.08.007
- [11] Karlsson P, Nyengaard JR, Polydefkis M, Jensen TS (2015) Structural and functional assessment of skin nerve fibres in small-fibre pathology. *Eur J Pain* 19(8): 1059-1070. 10.1002/ejp.645
- [12] Casellini CM, Parson HK, Richardson MS, Nevoret ML, Vinik AI (2013) Sudoscan, a noninvasive tool for detecting diabetic small fiber neuropathy and autonomic dysfunction. *Diabetes Technol Ther* 15(11): 948-953. 10.1089/dia.2013.0129