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Assessment of neuropathy subtypes in type 1 diabetes

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

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PK and MBS contributed equally.

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[1]Danish Pain Research Center, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[2]Core Center for Molecular Morphology, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[3]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark
[4]Steno Diabetes Center North Denmark, Aalborg University Hospital, Aalborg, Denmark
[5]Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark
[6]Steno Diabetes Center Copenhagen, Herlev, Denmark
[7]Department of Neurology, Aarhus University Hospital, Aarhus, Denmark
[8]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 (≥1 abnormal test); model 2 (≥2 abnormal tests); and model 3 (≥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.

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.

INTRODUCTION

Diabetic polyneuropathy (DPN) is a common complication of diabetes with a lifetime prevalence of up to 50%.[1] 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.[1] Ultimately, DPN can result in intolerable pain, 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%.[2]

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

small fiber neuropathy (SFN), involving Aδ and C fibers; large fiber neuropathy (LFN), involving large Aβ fibers; and mixed fiber neuropathy (MFN), involving both large and small nerve fibers.[3] Previous studies indicate that DPN predominantly manifests as MFN, with affected individuals exhibiting symptoms related to both small and large nerve fibers.[4 5] 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.[6 7] However, currently there is no consensus on a gold standard for the diagnosis of SFN,[8] 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 ≥5 years. Exclusion criteria included non-diabetes-related neuropathy, significant non-neuropathic pain, alcohol/substance abuse, and specific health conditions. Pain participants needed ≥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 ≥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 ≤5); *mild neuropathy* (TCNS=6–8); *moderate neuropathy* (TCNS=9–11); and *severe neuropathy* (TCNS ≥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*[4] in a study investigating neuropathy subtypes in T2DM-associated DPN (methodological differences: include thermotester vs thermo rollers and full nerve conduction study vs DPNCheck[4]). 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 µS; 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 ≤4 µV and/or velocity ≤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, ≥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, ≥1 abnormal result was required in the large fiber measures and 0 abnormal results in the small fiber measures. MFN was defined as ≥1 abnormal result in the small fiber measures and ≥1 abnormal result in the large fiber measures.

Model 2

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

Model 3

A diagnosis of DPN with more prevalent SFN required ≥3 abnormal results in the small fiber measures and ≤2 abnormal results in the large fiber measures. Conversely, a diagnosis of DPN with more prevalent LFN required ≥3 abnormal results in the large fiber measures and ≤2 abnormal results in the small fiber measures. MFN was defined as ≥3 abnormal results in the small fiber measures and ≥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*.[9] For a pure SFN diagnosis, the Besta criteria require ≥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.[10 11]

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 ≥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.[12] 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*×*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*×*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). Colinearity was checked, and no variance of inflation factor was found to be >5, indicating no significant issues with colinearity 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,

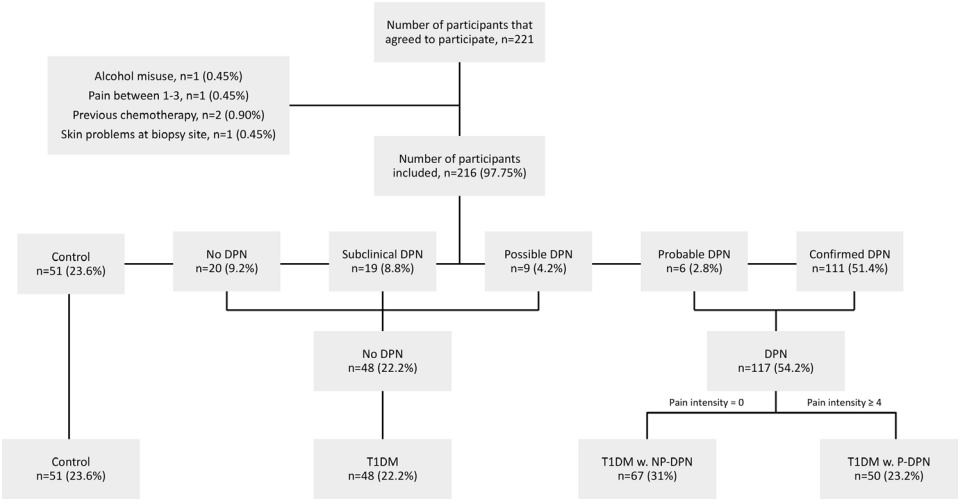

Figure 1 Flow chart of patient inclusion and patient groupings. Groupings were based on a T1DM diagnosis as well as the Toronto criteria. 221 individuals agreed to participate from which 5 (2.25%) did not meet the inclusion criteria. Reasons for exclusion: alcohol misuse (n=1; 0.45%), pain intensity 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 ≥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 ≥4 (NRS ≥4), respectively (figure 1).

Participants with DPN (n=117) were subsequently regrouped according to DPN severity: no neuropathy (TCNS ≤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 ≥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^2)	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^2)	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
10 g 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.	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<0.0
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 (≥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.[13] Additionally, our results suggest a role of smoking in P-DPN while other studies have been unable to show this association.[13 14] Moreover, we found higher HbA1c in T1DM with P-DPN, which has not been consistently reported in previous studies.[14 15]

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.[8 16]

Neuropathy subtypes

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

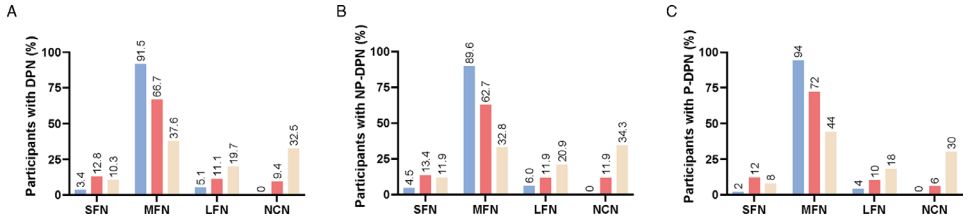

Figure 2 Bar charts visualizing the distribution of neuropathy subtype based on the criteria of the three previously defined models. Distributions were investigated in three groups: (A) combined group consisting of all T1DM with NP-DPN participants and all T1DM with P-DPN participants, DPN (first panel); (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, ≥1 abnormal tests (blue); model 2, ≥2 abnormal tests (red); and model 3, ≥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.[4] 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%)).[4] 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,[4] thereby agreeing with previous findings.[17–19] 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[4] as well as diabetes duration.[20]

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 ≥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,[9] 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: ≥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[9] 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.[21] 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.[22] 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,[23] 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.[24 25]

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 ≥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.[26–31] 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.[32–34] 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.

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ORCID iD

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

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