	Small fiber tests		**Large fiber tests**	
Criteria (Besta)	*(≥2 abnormal results)*		*(No abnormal results)*	
Tests	Besta criteria	This study	Beta criteria	This study
1	Pinprick **and** Thermal sensory loss **and/or** Allodynia **and/or** Hyperalgesia	Pinprick (Neurotip) **and** Thermo rollers (25°C/40°C) **and/or** Brush **and/or** N/A	Light touch **and/or** Vibration sensation **and/or** Proprioception sensation **and/or** Deep tendon reflexes	10g monofilament (Neuropen) **and/or** Biothesiometry **and/or** Proprioception sensation **and/or** Ankle and knee reflexes
2	Warm threshold **and/or** Cold threshold (Assessed by QST)	Warm threshold **and/or** Cold threshold (Assessed by QST)	Motor fiber impairment (waste and/or weakness)	Atrophy **and/or** Dorsal flex **and/or** 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 $p_{adj} < 0.001$
DPNCheck, amplitude	r(153) = -0.52 $p_{adj} < 0.001$
DPNCheck, conduction velocity	r(158) = -0.43 $p_{adj} < 0.001$

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)
Model 1: ≥ 1 abnormal tests						
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
Model 2: ≥ 2 abnormal tests						
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
Model 3: ≥ 3 abnormal tests						
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
p value (Besta vs. model 2 (adapted#))	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***	
IENFD (abnormal)	28 (73.7%)	20 (74.1%)	30 (93.8%)	14 (100.0%)	0.021*	0.178
IENFD, ancle (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***
DPNCheck (abnormal)	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***
Biothesiometry (abnormal)	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 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). 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**
Model 1: ≥ 1 abnormal tests					
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
Model 2: ≥ 2 abnormal tests					
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**
Model 3: ≥ 3 abnormal tests					
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.*

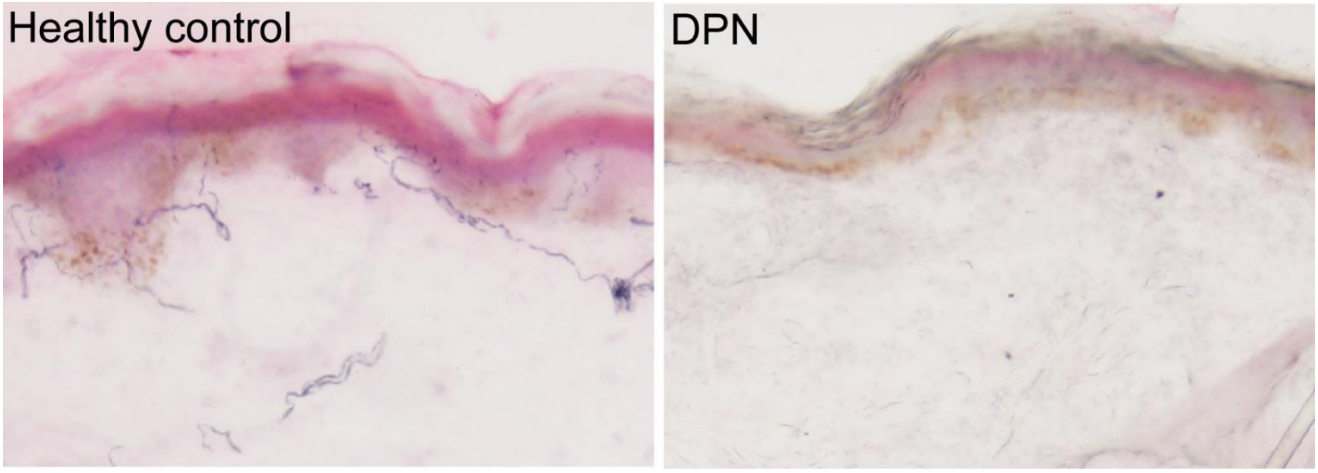

Figure S1 – Immunohistological stainig of PGP9.5⁺ cutaneous nerve fibers in skin biopsies
Cutaneous nerve fiber labelling in a skin biopsy section from a healthy control (left) and from a participant with diabetic peripheral neuropathy (DPN).

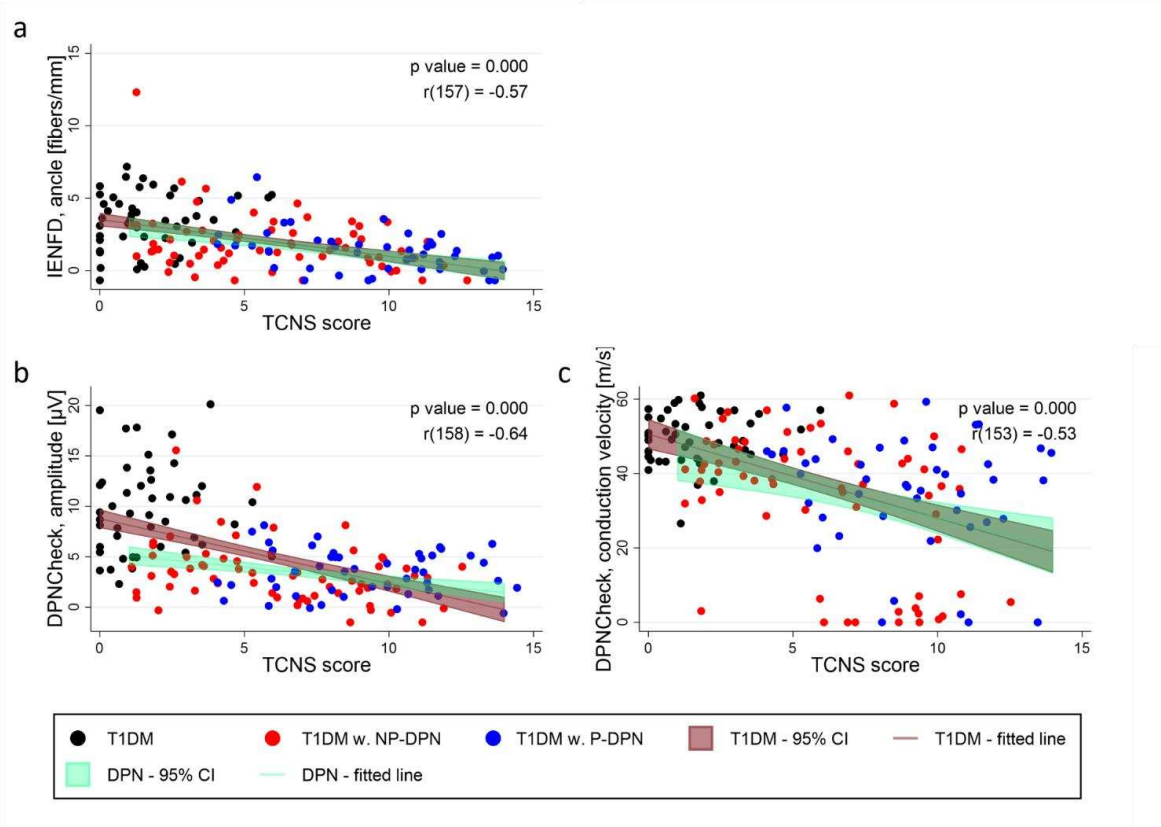

Figure S2 – TCNS Correlations with IENFD and DPNCheck
Graphs depict the correlation between TCNS score and IENFD (a); TCNS score and DPNCheck, amplitude (b); and TCNS score and DPNCheck conduction velocity (c). Spearman's correlation coefficient for each correlation is visualized in the upper right corner 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 ≥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 ≥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, neuroptip, 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 ≤4μV and/or velocity ≤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 µm 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

Sudoscan® 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].

Sudoscan determines 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 µS[12].

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