Diagnostic Accuracy of Perception Threshold Tracking in the Detection of Small Fiber Damage in Type 1 Diabetes

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Abbreviations

AUC: Area under the curve

BMI: Body mass index

CCM: Corneal confocal microscopy

CDT: Cold detection threshold

CNAP: Center for Neuroplasticity and Pain

CNBD: Corneal nerve branch density

CNFD: Corneal nerve fiber density

CNFL: Corneal nerve fiber length

DFNS*:* German Research Network on Neuropathic Pain

DN4: Douleur Neuropathique 4 Questionnaire

DPN: Diabetic peripheral neuropathy

HbA1c: Glycated hemoglobin A1c

HC: Healthy controls

HDT: Heat detection threshold

IVCM: In-vivo corneal confocal microscopy

IENFD: Intra epidermal nerve fiber density

IQR: Interquartile ranges

mA: Milliampere

MNSI: Michigan neuropathy screening instrument

NCA: Nerve conduction amplitude

NCV: Nerve conduction velocity

NPV: Negative predictive value

PDPN: Painful diabetic peripheral neuropathy

PPV: Positive predictive value

PTT: Perception threshold tracking

ROC: Receiver operating characteristic

SD: Standard deviation

T1DM: Type 1 diabetes mellitus

VAS: Visual analog scale

Abstract

Aim: An objective assessment of small nerve fibres is key to the early detection of diabetic peripheral neuropathy (DPN). The present study investigates the diagnostic accuracy of a novel perception threshold tracking technique in detecting small nerve fibre damage.

Methods: Participants with type 1 diabetes (T1DM) without DPN (n=20), with DPN (n=20), with painful DPN (n=20) and 20 healthy controls (HC) underwent perception threshold tracking on the foot and corneal confocal microscopy. Diagnostic accuracy of perception threshold tracking compared to corneal confocal microscopy was analysed using logistic regression.

Results: The rheobase, corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD), and corneal nerve fibre length (CNFL) (all $p<0.001$) differed between groups The diagnostic accuracy of perception threshold tracking (rheobase) was excellent for identifying small nerve fibre damage, especially for corneal nerve fibre length with a sensitivity of 94%, specificity 94%, positive predictive value 97% and negative predictive value 89%. There was a significant correlation between rheobase with CNFD, CNBD, CNFL and MNSI (all $p<0.001$).

Conclusion: Perception threshold tracking had a very high diagnostic agreement with corneal confocal microscopy for detecting small nerve fibre loss and may have clinical utility for assessing small nerve fibre damage and hence early DPN.

Clinical trials: NCT04078516

Introduction

Diabetic peripheral neuropathy (DPN) affects more than 50% of people with diabetes mellitus and is associated with considerable morbidity due to painful diabetic neuropathy and foot ulceration as well as increased mortality [1–5]. Currently advocated screening methods for the detection of DPN e.g. monofilament testing or vibration perception thresholds only diagnose advanced disease or large fibres e.g. nerve conduction testing [6]. Small nerve fibre abnormalities occur early and may precede large fibre dysfunction in DPN [7]. Quantitative sensory testing can be used to assess small Aδ- and C-fibre function, but have limited precision and reproducibility [8]. More objective methods like corneal confocal microscopy (CCM) and skin biopsies with quantification of intra-epidermal nerve fibre density (IENFD) are considered the gold standard for evaluating small fibre damage in DPN [9–11]. However, they require advanced equipment and expertise for analysis and evaluate structural rather than functional abnormalities.

We have recently developed a novel perception threshold tracking technique, which utilizes weak electrical currents to selectively stimulate peripheral large (Aβ) and small nerve fibres (Aδ) [12]. It differed between people with and without diabetes and correlated with thermal thresholds obtained from quantitative sensory testing [12]. However, the limited accuracy and reproducibility of thermal thresholds compared to CCM or skin biopsies, limits the strength of this comparison.

In the present paper, we aimed to 1) compare the outcomes of perception threshold tracking and CCM, 2) investigate the diagnostic accuracy of perception threshold tracking for the detection of small fibre neuropathy using CCM as a reference, and 3) investigate the relationship between structural and functional measures of small nerve fibre damage.

Methods

Study design and Participants

The study was conducted between August 2019 and February 2022 in participants from the "*MEDON*" (*Methods for Early Detection Of diabetic peripheral Neuropathy*)-cohort (clinicaltrials.gov: NCT04078516)[12–14]. This population is described in details elsewhere, but in short, the original cohort consisted of 80 participants equally divided into four groups: participants with T1DM and painful diabetic peripheral neuropathy (PDPN), participants with T1DM and DPN, participants with T1DM and no DPN, and healthy controls (HC) without diabetes or pain [12–14]. The four groups were matched 1:1:1:1 on age (+/- 2 years) and sex. PDPN was diagnosed based on the Douleur Neuropathique 4 Questionnaire (DN4)-score ≥ 4 and DPN was diagnosed according to the clinical Toronto consensus (neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes) [15,16]. The population was extensively screened to exclude other causes of neuropathy including vitamin deficiencies, hematologic or immune diseases, thyroid, or parathyroid disease, chronic kidney-disease, previous alcohol or drug abuse, previous chemotherapy, severe or chronic viral infection, and active cancer. Subjects with current eye-infections, corneal abrasions, a history of bilateral refractive surgery or anterior segment trauma which might affect the cornea, were excluded. From the original 80 participants, 9 were excluded due to previous refractive surgery.

Assessment of peripheral nerves

After inclusion, neurological examination was undertaken alongside the Michigan Neuropathy Screening Instrument (MNSI) [17]. Neuropathic pain severity (over the last 4 weeks) was derived from the PainDETECT questionnaire as average and peak pain intensity rated on a visual analog scale (VAS) ranging from 0-10, where 0 is no pain and 10 is the most intense pain imaginable [18]. Quantitative sensory testing and conventional nerve conduction studies were performed as previously described and reported [12–14].

Perception Threshold Tracking

Perception threshold tracking of small nerve fibres was performed following our previously published protocol using the method of limits [12,19]. In short, the participants were electrically stimulated using a custom-made pin electrode (Aalborg University, Denmark) in an area 2-3 cm proximal to the second toe on the dorsum of the right foot. The electrode consists of a concentric stainless-steel ring electrode with an area of 8.8 cm^2 serving as the anode, and a printed circuit board with 16 stainless steel pin electrodes placed in a circle serving as the cathodes. The pins were all blunted with a diameter of 0.2 mm. The electrical stimuli were delivered using a DS5 electrical stimulator (Digitimer Ltd, UK) and controlled by a protocol implemented in a custom-made program (LabBench Io, Inventors Way, Denmark) [19]. Participant-responses were captured using a custom-made handheld response button (Inventors Way, Denmark), a personal computer and a data acquisition card (LabBench Io, Inventors Way, Denmark). The perception threshold was estimated using square impulses with varying durations (0.1ms, 1 ms, 50 ms) and intensities. The rheobase (the lowest current intensity of infinite duration that results in perception of the impulse) and the chronaxie (the minimal pulse duration required to double the strength of the rheobase) (not reported) were derived from the corresponding strength-duration curve [12,20]. Due to the nature of the electrode, the electrical stimulation is almost exclusively present near the terminals of the small nerve fibres in the epidermis, without reaching the large nerve fibres in the dermis, and thus preferentially stimulate the small nerve fibres [12].

Corneal Confocal Microscopy

All participants underwent in-vivo corneal confocal microscopy (IVCM) using a Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany). One-hundred images from the corneal apex with a resolution of 400 x 400 μm were acquired using a volume scan. In participants with unilateral anterior segment trauma or refractive surgery only the non-affected eye was examined. Two authors (JR and SC) blinded for participant ID, each selected 3-4 representative images,

totalling 6-8 images per participant. Selection criteria were good contrast of the nerves compared to the background, limited motion artefacts, limited pressure lines, limited image overlapping (maximum 20%), en-face alignment and proper focus [21].

Manual morphometric analysis was conducted using CCMetrics (M.A. Dabbah, Imaging Science and Biomedical Engineering, University of Manchester, Manchester, United Kingdom) to obtain corneal nerve fibre length (CNFL), corneal nerve branch density (CNBD), corneal nerve fibre density (CNFD), and corneal nerve tortuosity [22,23]. CNFL was defined as the total length of all nerve fibres per frame (mm/mm^2). CNBD was defined as the number of primary branches from the main nerves (no./mm^2). CNFD was defined as the number of main nerve fibres taking up more than 50% of the total frame length (no./mm^2). The tortuosity coefficient was derived from the main nerve fibres [22].

Statistical Analyses

Categorical data are expressed as a percentage of participants and compared using Chi^2 or Fishers-exact tests. Continuous data are expressed as mean ± standard deviation (SD) when data were normally distributed, and as medians with interquartile ranges (IQR) when data were not normally distributed. Normally distributed data (visually defined by QQ-plots) were compared using parametric tests (Student's t tests, ANOVA), while non-normally distributed data were compared using non-parametric tests (Kruskal–Wallis H tests followed by Mann-Whitney U tests, Spearman's rank correlation). The significance level was set at α=0.05. All relevant comparisons were corrected using Bonferroni-corrections. Logistic regressions were used to generate receiver operating characteristic (ROC) curves, estimate the area under the curve (AUC), and calculate sensitivities, specificities, positive predictive values, negative predictive values for each parameter (see *figure 1* for ROC curves and *online supplementary material* for probability cut-off graphs). When using CNFL, CNBD, or CNFD as reference the cut-off points for an abnormal result was determined as the lower 5th quantile derived from the published normative values [24]. Only participants with diabetes were used for the ROC-curves.

Results

Demographics and characteristics

Participants with complete data were divided as follows: T1DM+PDPN (n=19), T1DM+DPN (n=14), T1DM-DPN (n=19), and HCs (n=19). There was a significant difference between the groups for haemoglobin A1c, sural nerve conduction velocity and amplitude, cold and heat detection thresholds, pain scores, MNSI and DN4-scores (all $p<0.001$) (Table 1).

(Table 1)

Perception threshold tracking and corneal confocal microscopy

There was a significant difference in the rheobase, CNFD, CNBD, and CNFL (all $p<0.001$) with no difference in tortuosity ($p=0.221$) between all four groups (Table 2).

(Table 2)

Diagnostic performance of perception threshold tracking

The sensitivity, specificity, positive predictive value, and negative predictive value of the rheobase (perception threshold tracking) to detect small fibre neuropathy (as defined by CNFD, CNBD and CNFL) was generally very good with the highest performance against CNFL with an AUC of 0.93, sensitivity of 94%, specificity of 94%, positive predictive value of 97% and negative predictive value of 89% at an optimal cut-off of 0.29 mA (Table 3, Figure 1, Supplementary figure 1).

(Table 3)

(Figure 1)

Relationship between perception threshold tracking and corneal confocal microscopy

There was a significant correlation between the rheobase with CNFD, CNBD, CNFL and MNSI (all $p<0.001$) (Table 4).

(Table 4)

Discussion

The perception threshold tracking technique is a novel small nerve fibre test with a unique ability to selectively stimulate small sensory nerve fibres in the skin. We now demonstrate a close relationship and high diagnostic agreement between perception threshold tracking and CCM, an acknowledged structural marker for small fibre damage[25]. Due to the novelty of perception threshold tracking no studies have previously made a similar comparison. In participants with type 1 diabetes a significant correlation was demonstrated between conventional threshold tracking of motor nerves, a measure of peripheral nerve excitability and axonal ion channel dysfunction and CCM parameters [26,27]. In another study of participants with type 2 diabetes there was a significant relationship between conventional threshold tracking and CCM, with a corresponding decrease in the recovery cycle, suggesting an abnormality in voltage-gated potassium channels [28]. Thus, this sensory perception threshold tracking technique may provide important insights into voltage-gated ion channel function on sensory nerves.

Functional tests of small fibre damage have been a corner stone for the diagnoses of small fibre neuropathy. Indeed, the German Research Network on Neuropathic Pain (DFNS) developed a comprehensive standardized protocol to enable deep-phenotyping and subtyping of neuropathic pain [29,30]. However, quantitative sensory testing has repeatedly failed as a clinical endpoint in clinical trials [31,32]. Thus, it has been proposed that objective measures of corneal nerve and intraepidermal nerve fibre pathology may have diagnostic utility for small fibre neuropathy[33]. However, the invasive nature and technical requirements for skin biopsies and limited availability and extensive image analysis of CCM have limited their wider adoption [6]. The sensory perception threshold tracking technique presented in this paper, is a simple, rapid (< 5 min) measure of peripheral sensory nerve fibre function, feasible for large scale screening.

There is no consensus as to whether there are functional or structural measures of small fibre damage which differentiate painful from painless neuropathy [34,35]. Several recent studies with large cohorts have shown greater corneal nerve fibre loss in people with diabetes and painful compared to painless diabetic neuropathy and a relationship with the severity of pain, whilst others have shown increased nerve branching or have failed to establish such a relationship [36–38]. Similarly, some studies using conventional threshold tracking have associated altered axonal excitability with painful diabetic peripheral neuropathy, while others have reported altered sodium conductance and ion-channel function, alteration in Na+/K+ pump function, and membrane depolarization in diabetic peripheral neuropathy irrespective of the presence of neuropathic pain [39–41]. Recently, a large multicentre study reported no difference in axonal excitability when comparing those with painless to those with painful diabetic peripheral neuropathy, concluding that electrophysiological measures targeting the small nerves are needed to rule out axonal excitability changes in painful diabetic peripheral neuropathy [42]. One such method is microneurography, which is capable of assessing the function of small cutaneous C-fibres, and have previously associated increased spontaneous activity of cutaneous C-fibres with painful diabetic peripheral neuropathy [43,44]. Microneurography is however limited by invasiveness and vast technical requirements and time-consumption, while simultaneously being unable to assess small cutaneous Aδ-fibres[45].

In the present study, there was no difference in CCM measures or perception threshold tracking between people with painful and painless diabetic neuropathy, possibly due to the small cohort size. Also, the selectivity of perception threshold tracking declines as the required current intensity rises in those with more severe neuropathy, ultimately reaching a point where the acquired threshold is likely the threshold of the large (Aβ) fibres rather than the threshold for the small (primarily Aδ) fibres [12]. Threshold tracking may be more useful in differentiating people with PDPN grouped into irritable and non-irritable sub types, which may provide insights into peripheral ion-channel composition utilizing computational modelling alongside perception threshold tracking [46,47]. Such analyses would however require more extensive and time-consuming perception threshold tracking protocols including pulses relevant for assessing i.e., threshold-electrotonus[27].

Based on the present study, it would appear diabetic peripheral neuropathy is associated with comparable changes to both nerve fibre structure and function, although further studies are needed to confirm these findings.

In the present study the diagnostic agreement between perception threshold tracking and CCM were highest when comparing the rheobase of perception threshold tracking with CNFL from CCM. CNFL is the most established and well-examined of the corneal parameters and based on our results it might also be the most reliable. In fact, perception threshold tracking correctly classified all participants with clinically established neuropathy (T1DM+PDPN or T1DM-DPN) when compared to CNFL.

We acknowledge that the small numbers of participants studied may limit our ability to differentiate different subtypes of DPN, especially painful DPN. The perception threshold tracking technique also loses its selectivity at very high current intensities, which may impact on the correlations in this paper, but does not impact on sensitivity, specificity, positive and negative predictive value. The CCM values in the controls were slightly lower than previously published normative values, which may have impacted on the identification of abnormal values [24]. The very high diagnostic agreement between the two different methods may reflect the highly selected population used in the present study and it remains unknown if the diagnostic agreement will remain this high in other populations.

Conclusion

Perception threshold tracking is a rapid measure of small nerve fibre function and appears to have clinical utility as a method for neuropathy screening given that CCM identifies early small nerve fibre damage. Further validation of the method is required against skin biopsies at the site of threshold testing in a larger, randomly selected, cohort.

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JR wrote the manuscript, conducted most examinations, researched data, and contributed to the idea and study design. NE, CM, JF and TH contributed to the idea and study design, reviewed the manuscript, and conducted critical editing of written text. SC assisted conducting some of the examinations, contributed to the idea and study design, reviewed the manuscript, and conducted critical editing of written text. BS, CS and EN conducted the CCM examinations and conducted critical editing of written text. RM and IP assisted CCM image selection and analysis, contributed with expert knowledge and insight, and conducted critical editing of the written text. Each author is accountable for his own contribution, disclosure of potential interests and approved the final version of the manuscript. NE is the guarantor and is responsible for all aspects of the manuscript. CM is part of the Center for Neuroplasticity and Pain (CNAP), supported by the Danish National Research Foundation (DNRF121). No specific grant was received to undertake this study.

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Figure 1

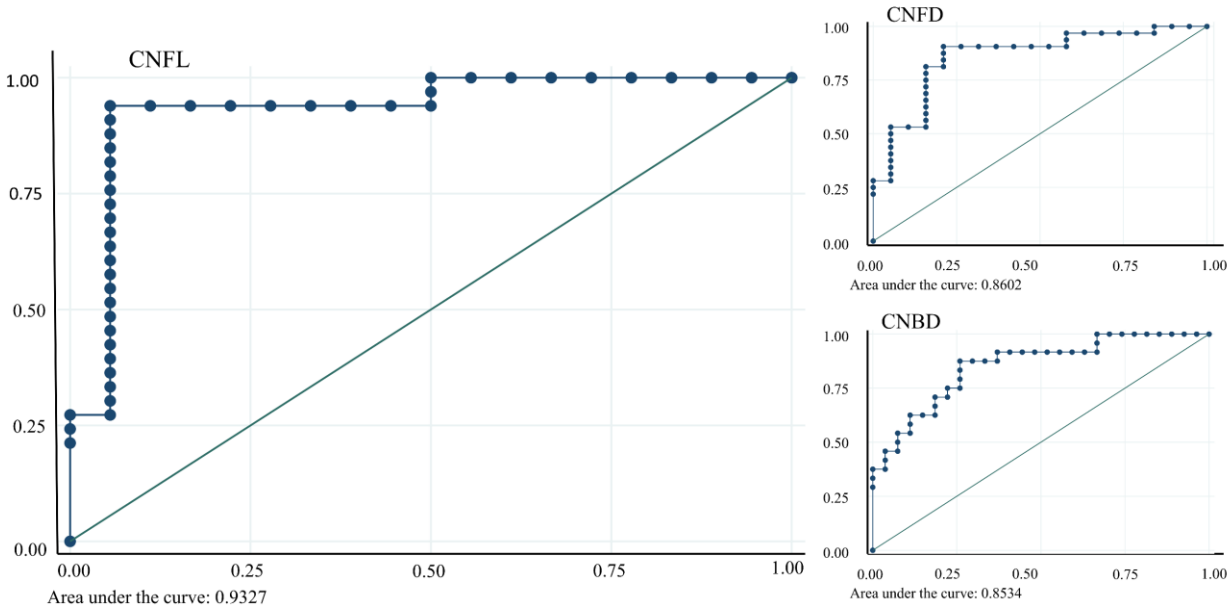

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Table 1

	T1DM+PDPN n=19	**T1DM+DPN n=14**	**T1DM-DPN n=19**	**Healthy controls n=19**	***P*-value**
Age (years)	51 [43;57] [a]	51 [44;60] [a]	52 [44;58] [a]	46 [44;53] [a]	ns
Sex (% male)	53% [a]	53% [a]	53% [a]	53% [a]	ns
BMI (Kg/m^2)	27.1 [24.5;31.0] [a]	27.8 [25.6;34.5] [a]	27.0 [24.1;30.3] [a]	24.3 [23.2;27.5] [a]	ns
HbA1c (mmol/mol)	71 [60;80] [a]	73 [67;84] [a]	65 [59;73] [a]	34 [32;35] [b]	p< 0.001*
Diabetes Duration (years)	34 [23;42] [a]	35 [30;41] [a]	26 [14;31] [a]	-	ns
NCV (m/sec)	19.0 [0.0;39.0] [a]	22.0 [0.0;41.0] [a]	48.0 [45.0;50.0] [b]	55.0 [51.0;58.0] [c]	p< 0.001*
NCA (μV)	2.4 [0.0;3.6] [a]	2.1 [0.0;4.3] [a]	5.4 [2.9;7.9] [b]	10.0 [7.3;12.4] [c]	p< 0.001*
CDT (°Celsius)	20.5 [7.4;26.5] [a]	17.8 [2.1;23.9] [a]	28.2 [27.0;30.4] [b]	30.4 [26.1;30.7] [b]	p< 0.001*
HDT (°Celsius)	45.3 [42.2;48.5] [a]	42.1 [39.5;48.5] [a]	39.8 [36.0;41.7] [b]	36.9 [35.1;41.2] [b]	p< 0.001*
Pain Score (Peak Intensity)	8.0 [6.0;9.0] [a]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	p< 0.001*
Pain Score (Average Intensity)	5.0 [4.0;8.0] [a]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	p< 0.001*
MNSI	4.0 [1.5;6.0] [a]	4.0 [4.0;5.0] [a]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	p< 0.001*
DN4 Score	5.0 [4.0;6.0] [a]	0.0 [0.0;2.0] [b]	0.0 [0.0;0.0] [b]	0.0 [0.0;0.0] [b]	p< 0.001*

Table 1. Demographics and test results for participants in T1DM+PDPN, T1DM+DPN, T1DM-DPN, and healthy controls. Data are displayed as medians with interquartile ranges. Pairwise statistically significant differences are denoted by symbols a-c. Integers denoted by the same letter are not statistically different from each other but are statistically different from groups denoted with a different letter. Statistical differences between the groups are tested using Mann-Whitney U tests. Average and peak pain intensity are reported as average or peak over the last four weeks on a scale from 0-10, where 0 is no pain and 10 is the worst imaginable pain. In cases where the sural nerve response could not be elicited the values were set to 0.0.

*Abbreviations: **BMI:** Body Mass Index, **CDT:** Cold detection threshold, **DPN**: Diabetic peripheral neuropathy, **HbA1c:** Glycated haemoglobin A1c, **HDT:** Heat detection threshold, **MNSI:** Michigan Neuropathy Screening Instrument **NCA**: Nerve conduction amplitude (sural nerve), **NCV**: Nerve conduction velocity (sural nerve), **PDPN**: Painful diabetic peripheral neuropathy, **T1DM**: Type 1 diabetes.*

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Table 2

	T1DM+PDPN n=19	**T1DM+DPN n=14**	**T1DM-DPN n=19**	**Healthy controls n=19**	***P*-value**
Rheobase, mA	1.13 [0.44;25.0] [a]	0.59 [0.16;1.03] [a]	0.27 [0.17;0.45] [b]	0.14 [0.09;0.23] [c]	< 0.001*
CNFD, no./mm^2	10.0 [6.9;11.3] [a]	10.0 [6.3;12.5] [a]	15.6 [11.3;16.7] [b]	19.3 [18.8;21.9] [c]	< 0.001*
CNBD, no./mm^2	14.8 [10.7;22.3] [a]	21.1 [15.0;21.9] [a]	37.5 [31.3;48.8] [b]	70.6 [56.3;76.3] [c]	< 0.001*
CNFL, mm/mm^2	9.0 [7.0;13.5] [a]	10.3 [8.0;12.2] [a]	15.2 [14.4;18.8] [b]	19.7 [18.0;21.7] [c]	< 0.001*
Tortuosity†	0.25 ± 0.06 [a]	0.23 ± 0.04 [a]	0.21 ± 0.04 [a]	0.22 ± 0.04 [a]	0.221

*Table 2. Results from corneal confocal microscopy and perception threshold tracking of the small nerve fibres. Data are presented as medians with interquartile ranges unless otherwise stated. Pairwise statistically significant differences are denoted by symbols a-c. Integers denoted by the same letter are not statistically different from each other but are statistically different from groups denoted with a different letter. Statistical differences between the groups are tested using Mann-Whitney U tests * Marks statistical significance between the groups calculated by Kruskal-Wallis H Tests. † Indicates the data are normally distributed and is thus presented as a mean ± standard deviation and differences between groups are calculated one-way analysis of variance (ANOVA).*

*Abbreviations: **CNBD**: Corneal Nerve Branch Density, **CNFD**: Corneal Nerve Fibre Density, **CNFL**: Corneal Nerve Fibre Length, **DPN**: Diabetic Peripheral Neuropathy, **PDPN**: Painful Diabetic Peripheral Neuropathy, **T1DM**: Type 1 Diabetes Mellitus.*

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Table 3

	PTT vs CNFD	**PTT vs CNBD**	**PTT vs CNFL**
Sensitivity	81.3%	75.0%	93.9%
Specificity	84.2%	74.1%	94.4%
PPV	89.7%	72.0%	96.9%
NPV	72.7%	77.0%	89.5%
AUC	0.86	0.85	0.93
Optimal rheobase cut-off	0.25 mA	0.36 mA	0.29 mA

Table 3. Comparison of the rheobase derived from perception threshold tracking and corneal confocal microscopy measurements. The results are obtained utilizing logistic regression with Receiver Operating Characteristic (ROC) curves and estimation of the Area Under the Curve (AUC). The optimal cut-off value for the rheobase is determined as the value with the least differences between the sensitivity and specificity of each measurement.

*Abbreviations: **AUC**: Area Under the Curve, **CNBD**: Corneal Nerve Branch Density, **CNFD**: Corneal Nerve Fibre Density, **CNFL**: Corneal Nerve Fibre Length, **mA**: Milli Ampere, **NPV**: Negative Predictive Value, **PPV**: Positive Predictive Value, **PTT**: Perception Threshold Tracking.*

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Table 4

	Rheobase	CNFD	CNBD	CNFL	MNSI
Rheobase	ρ= 1.00				
CNFD	ρ= -0.50*	ρ= 1.00			
CNBD	ρ= -0.52*	ρ= 0.82*	ρ= 1.00		
CNFL	ρ= -0.57*	ρ= 0.91*	ρ= 0.89*	ρ= 1.00	
MNSI	ρ= 0.54*	ρ= -0.55*	ρ=-0.66*	ρ=-0.67*	ρ= 1.00

*Table 4. Correlations between the rheobase measured by perception threshold tracking and corneal confocal microscopy measures and neuropathy severity score. All analyses were performed using Spearman's rank-order correlation and presented using Spearman's rho. * Marks statistical significance with a p-value <0.001.*

*Abbreviations: **CNBD**: Corneal Nerve Branch Density, **CNFD**: Corneal Nerve Fibre Density, **CNFL**: Corneal Nerve Fibre Length, **MNSI**: Michigan Neuropathy Screening Instrument.*