Received: 28 August 2023 | Revised: 5 July 2024 | Accepted: 14 July 2024

DOI: 10.1002/mus.28213

CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

Differences in corneal nerve fiber density and fiber length in patients with painful chronic idiopathic axonal polyneuropathy and diabetic polyneuropathy

Mariska D. Nieuwenhoff MD[1,2] | Hoang-Ton Nguyen MD[3] | Sjoerd P. Niehof PhD[1] | Frank J. P. M. Huygen MD, PhD[1] | Ajay Verma PhD[4] | Erica S. Klaassen MSc[2] | Malik Bechakra PhD[1] | Wouter J. Geelhoed PhD[2] | Joost L. M. Jongen MD, PhD[1,5] | Annette C. Moll MD, PhD[3] | Alexander F. J. E. Vrancken MD, PhD[6] | Axel Petzold MD, PhD[3,7,8] | Geert Jan Groeneveld MD, PhD[2,9]

[1]Department of Anesthesiology and Pain Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Centre for Human Drug Research, Leiden, The Netherlands

[3]Department of Ophthalmology, Amsterdam UMC location VU University Medical Center, Amsterdam, The Netherlands

[4]Biogen, Cambridge, Massachusetts, USA

[5]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[6]Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands

[7]Department of Neurology, Amsterdam UMC location VU University Medical Center, Amsterdam, The Netherlands

[8]Moorfields Eye Hospital, The National Hospital for Neurology and Neurosurgery and the Queen Square Institute of Neurology, UCL, London, UK

[9]Department of Anesthesiology, Leiden University Medical Center, Leiden, The Netherlands

Correspondence
Mariska D. Nieuwenhoff, Department of Anesthesiology and Pain Medicine, Erasmus MC University Medical Center, P.O. Box 2040, 3000CA, Rotterdam, The Netherlands.
Email: m.nieuwenhoff@erasmusmc.nl

Present address
Ajay Verma, Formation Venture Engineering Foundry, Boston, Massachusetts, USA.

Funding information
Dutch Technology Foundation STW, Grant/Award Number: 10730; Biogen

Abstract

Introduction/Aims: Corneal confocal microscopy (CCM) detects small nerve fiber loss and correlates with skin biopsy findings in diabetic neuropathy. In chronic idiopathic axonal polyneuropathy (CIAP) this correlation is unknown. Therefore, we compared CCM and skin biopsy in patients with CIAP to healthy controls, patients with painful diabetic neuropathy (PDN) and diabetics without overt neuropathy (DM).

Methods: Participants with CIAP and suspected small fiber neuropathy ($n = 15$), PDN ($n = 16$), DM ($n = 15$), and healthy controls ($n = 16$) underwent skin biopsy and CCM testing. Inter-center intraclass correlation coefficients (ICC) were calculated for CCM parameters.

Abbreviations: BMI, body mass index; CCM, corneal confocal microscopy; CHDR, Centre for Human Drug Research; CIAP, chronic idiopathic axonal polyneuropathy; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; COMPASS, Composite Autonomic Symptom Score; DM, diabetic patients without overt neuropathy; ICC, intraclass correlation coefficient; IENFD, intraepidermal nerve fiber density; Mean ± SD, mean with standard deviation; Median [IQR], median and interquartile range; MNSI, Michigan Neuropathy Screening Instrument; NA, not applicable; NIS-LL, Neuropathy Impairment Score-Lower Limbs; NRS, numerical rating score; PDN, painful diabetic neuropathy; r_s, Spearman rank correlation coefficient; SFN, small fiber neuropathy; VUmc, VU University Medical Center (now Amsterdam UMC, location VUmc).

Mariska D. Nieuwenhoff and Hoang-Ton Nguyen contributed equally to this study.

Results: Compared with healthy controls, patients with CIAP and PDN had significantly fewer nerve fibers in the skin (IENFD: 5.7 ± 2.3, 3.0 ± 1.8, 3.9 ± 1.5 fibers/mm, all $p < .05$). Corneal nerve parameters in CIAP (fiber density 23.8 ± 4.9 no./mm^2, branch density 16.0 ± 8.8 no./mm^2, fiber length 13.1 ± 2.6 mm/mm^2) were not different from healthy controls (24.0 ± 6.8 no./mm^2, 22.1 ± 9.7 no./mm^2, 13.5 ± 3.5 mm/mm^2, all $p > .05$). In patients with PDN, corneal nerve fiber density (17.8 ± 5.7 no./mm^2) and fiber length (10.5 ± 2.7 mm/mm^2) were reduced compared with healthy controls ($p < .05$). CCM results did not correlate with IENFD in CIAP patients. Inter-center ICC was 0.77 for fiber density and 0.87 for fiber length.

Discussion: In contrast to patients with PDN, corneal nerve parameters were not decreased in patients with CIAP and small nerve fiber damage. Therefore, CCM is not a good biomarker for small nerve fiber loss in CIAP patients.

KEYWORDS

chronic idiopathic axonal polyneuropathy, corneal confocal microscopy, diabetic neuropathy, intra epidermal nerve fiber density, pain

1 | INTRODUCTION

Chronic idiopathic axonal polyneuropathy (CIAP) ranks as the second most prevalent polyneuropathy following diabetic neuropathy.[1] While primarily impacting large nerve fibers, a substantial number of CIAP patients also exhibit symptoms of small nerve fiber involvement.[2] Neuropathic pain is experienced by up to 68% of CIAP patients,[3] which is likely associated with degeneration or dysfunction of small nerve fibers. Notably, reduced nerve fiber counts have been observed in skin biopsies of CIAP patients.[4] Although skin biopsy serves as the current gold standard for small nerve fiber assessments,[5] its invasiveness and labor-intensive nature call for a noninvasive, rapid, and readily available alternative. One such alternative is corneal confocal microscopy (CCM).[6]

CCM is a noninvasive technique employed for detecting small nerve fiber loss in peripheral neuropathy. Automated analysis software facilitates repeated assessments with same-day results, making CCM an appealing test for patients and physicians alike. Normative reference values have been established, allowing for a standardized cut-off point for small fiber neuropathy (SFN).[7] Prior research has demonstrated high reproducibility, sensitivity, and specificity in diagnosing early nerve fiber damage in diabetic neuropathy[8–10] and various other neuropathies.[11–14] In addition, Quattrini et al.[15] found CCM parameters correlate with skin biopsy results. However, no information currently exists on CCM in CIAP patients. We hypothesize that, in line with other neuropathies, CCM parameters will be decreased in CIAP patients. Should CCM results in CIAP correlate with skin biopsy results, it could prove valuable for longitudinal follow-up of CIAP patients with small nerve fiber involvement and for evaluating the impact of future therapies aimed at enhancing nerve regrowth.

This study's aim was to assess CCM and skin biopsies in patients with CIAP and compare the results with those of healthy controls. In addition, we included patients with painful diabetic neuropathy (PDN) and diabetic patients without neuropathy (DM) for a comprehensive comparison. We chose a cohort of CIAP patients because it is a common polyneuropathy and also shares similarities with diabetic polyneuropathy. The selection of CIAP and diabetic neuropathy patients with pain was to maximize the likelihood of small fiber involvement. Furthermore, we explored the inter- and intra-center reproducibility of CCM to assess its reliability for clinical use.

2 | METHODS

This study was approved by the Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands (NL46921.058.13) and was conducted in accordance with the Declaration of Helsinki. We obtained written informed consent from all participants before enrolment in the study. The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden and at the VU University Medical Center (VUmc) in Amsterdam (now Amsterdam UMC, location VUmc). This study was part of a larger study aimed at validating noninvasive small fiber tests in patients with polyneuropathy.

2.1 | Study participants

Participants were divided into four groups: chronic idiopathic axonal polyneuropathy and suspected small nerve fiber involvement (CIAP), PDN, diabetes without overt neuropathy (DM), and healthy controls. To have a similar age and gender distribution between groups, healthy controls of similar age (±5 years), and same-sex were included for included PDN patients. Patients with CIAP were recruited via our affiliated hospitals (Erasmus MC, UMC Utrecht, Amsterdam UMC location VUmc) and through advertisement at the national patient society. In addition, patients with CIAP, PDN, and DM were recruited

via advertisements in free newspapers and via the CHDR website. Healthy controls were recruited via the CHDR website.

General inclusion criteria were age between 18 and 80 years and a body mass index (BMI) of 18–32 kg/m^2. Patients in the CIAP group had the diagnosis of CIAP confirmed by a neurologist based on signs and symptoms, clinical examination, abnormal nerve conduction studies confirming axonal polyneuropathy, and the absence of an identifiable cause.[16] Small nerve fiber involvement was suspected in patients experiencing neuropathic pain, sensory or autonomic signs and symptoms. Patients in the PDN group had a clinical diagnosis of diabetes mellitus, and the diagnosis of polyneuropathy was made by a neurologist based on medical history, signs and symptoms, and findings on clinical examination. Patients with CIAP and PDN were required to have a mean pain score ≥4 at medical screening (numerical rating score of 0–10) and a pain duration of 6 months to 6 years to be eligible for inclusion. This inclusion criterion served to maximize the likelihood of small fiber involvement and to increase homogeneity within and between both neuropathy groups. Patients on neuropathic pain medication had to have a stable analgesic regimen for at least 14 days before inclusion. Patients in the DM group had a clinical diagnosis of diabetes mellitus without overt neuropathy, defined as a questionnaire score <4 and a clinical exam score <3 on the Michigan Neuropathy Screening Instrument.[17] Healthy controls were free from conditions associated with polyneuropathy.

Following a screening visit to assess eligibility, all enrolled participants underwent skin biopsy and CCM testing. Nerve conduction studies were performed and sensory nerve action potential amplitude of the sural nerve (measured baseline-to-peak in μV) was selected as a measure of large fiber function. Neuropathy impairment in motor, sensory, and reflex activity was quantified using the NIS-LL (Neuropathy Impairment Score-Lower Limbs).[18] Autonomic dysfunction was assessed with the COMPASS-31 questionnaire (Composite Autonomic Symptom Score).[19] Autonomic function is assessed in six domains; orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor domains.[19]

2.2 | Skin biopsy

A 3-mm circular skin biopsy was performed ±10 cm above the lateral malleolus under local anesthesia (2% lidocaine/adrenalin). Biopsies were placed in 2% paraformaldehyde-lysine-periodate and fixed overnight at 4°C. Sections of 50 μm thick were immunostained with anti-PGP 9.5 (Enzo Life Sciences, Farmingdale, NY, USA), according to a published bright-field immunohistochemistry protocol.[20] Slides were digitized (Hamamatsu NanoZoomer 2.0-HT, Hamamatsu Photonics, Hamamatsu City, Japan) and analyzed on a computer at ×40 magnification (Leica Aperio ImageScope software, available at http://www.leicabiosystems.com/pathology-imaging/aperio-epathology/integrate/imagescope/). Intraepidermal nerve fiber density (IENFD) was quantified as the number of intraepidermal nerve fibers/mm epidermal length. The slides were counted in a blind fashion according to international standards.[21]

2.3 | Corneal confocal microscopy

To examine small nerve fibers at the sub-basal nerve plexus of the cornea, a Heidelberg Retina Tomograph (HRT III, Heidelberg, Germany) corneal confocal microscope with Rostock Cornea module was used. The eyes of participants were instilled with anesthetic drops (oxybuprocaine hydrochloride drops 0.4%; Bausch & Lomb) and ophthalmic gel (Vidisic Carbogel; Bausch & Lomb) was applied for lubrication. Ophthalmic gel was also applied to the microscope lens, and a disposable sterile cap (Tomocap) was used for optical coupling. From all captured images, six high-quality images from the sub-basal plexus of the central cornea were selected for analysis (three of each eye) as previously published.[22] The images were selected by the examiner on the day of the examination and had a maximum of 20% overlap. The examiner was not blinded to the clinical status (i.e., assigned group) of the participant. Image analysis was performed using ACCMetrics V.2 software (courtesy of R.A. Malik, University of Manchester). At the end of the study, all selected images were run through ACCMetrics in a single run. The software quantified the following parameters: (1) corneal nerve fiber density (CNFD), the number of nerve trunks/mm^2 of corneal tissue; (2) corneal nerve branch density (CNBD), the number of branches originating from nerve trunks/mm^2 of corneal tissue; and (3) corneal nerve fiber length (CNFL), the total length of all nerve fibers and branches in mm/mm^2.

Subjects' eyes were examined twice at one research center (CHDR), or at two different centers by different examiners (CHDR and VUmc) to determine intra-center and inter-center reproducibility, respectively, for CCM. The examinations were performed a maximum of 14 days apart. The decision regarding which subjects were examined at two different centers was based on the logistical and operational feasibility of CCM at the VUmc. The remaining subjects were measured twice at the same center. The CCM measurement during the first study visit at CHDR was termed the *initial measurement* because it was the common denominator among all subjects. The other measurement was called the *repeat measurement*. Since no intervention was performed, no changes in corneal innervation were expected within this short time period. The examination and image selection at the VUmc were performed by HTN and at the CHDR by MN.

2.4 | Statistical analysis

An a priori power calculation was performed. Assuming a correlation coefficient of $r_s = .35$ between IENFD and CCM in patients with painful CIAP, to detect a correlation with 80% power and a two-sided α of .05, 62 subjects are required. Therefore, we included 64 subjects in total, 16 subjects per group.

Statistical analysis was performed using SAS software (version 9.4 for Windows, SAS Institute Inc., Cary, NC, USA), and graphs were drawn using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). Data on normality were evaluated to decide on appropriate statistical tests for each parameter. Results are presented

as mean with standard deviation (mean ± SD) or median and interquartile range (median [IQR]) where appropriate. The chi-square test was used to analyze gender and smoking status. ANOVA with control and patient groups as fixed factors were used to calculate differences between groups for participant characteristics, sural nerve amplitude, NIS-LL, and COMPASS-31. For skin biopsy and CCM measurements, a mixed model ANOVA with group, measurement, and group by measurement as fixed factors and participants as random factors was used to calculate differences compared to healthy controls. Spearman rank correlation coefficient (r_s) was calculated to investigate correlations between skin biopsy and corneal nerve fiber parameters between groups. A $p < .05$ was considered statistically significant.

Intra- and inter-center reproducibility was assessed using the intraclass correlation coefficient (ICC). An ICC of 0.80 to 1 was considered as excellent and very good if 0.60 to 0.79.[23] In addition, Bland–Altman plots were used to illustrate agreement between the measurements.[24] A $p < .05$ was considered statistically significant. Two-tailed tests were used throughout.

3 | RESULTS

Sixty-four participants were included for measurements. Data on CCM measurements were available for 62 participants. In two, measurements were unsuccessful because the optical coupling of the microscope with the cornea was not tolerated despite topical anesthesia of the eye. Subject characteristics are summarized in Table 1. Diabetic patients without overt neuropathy were younger than patients in the CIAP and PDN groups. Pain duration and pain intensity at study enrolment were similar in patients with CIAP and PDN. Ten patients used neuropathic pain medication at the time of the study. In addition, five patients reported using neuropathic pain medication in the past but discontinued use due to side effects and/or limited efficacy.

Table 2 shows neurologic function and autonomic dysfunction scores. Sural nerve amplitude was decreased in both neuropathy groups. Large fiber function assessed with the NIS-LL was more severely affected in patients with CIAP compared to PDN patients. Autonomic small fiber function was more severely affected in the PDN group compared to the CIAP group (COMPASS-31 questionnaire), with scores for the secretomotor (sweating, dry eyes/mouth) and bladder domains significantly higher in the PDN group. Six CIAP and seven PDN patients reported dry eyes.

Representative skin biopsy and CCM images are shown in Figure 1. Cutaneous nerve fibers were lowest in both neuropathy groups (Figures 1 and 2). Patients with CIAP (3.0 ± 1.8 fibers/mm) and patients with PDN (3.9 ± 1.5 fibers/mm) had significantly lower IENFD than healthy controls (5.7 ± 2.3 fibers/mm). Nerve fiber density in the DM group (5.2 ± 2.1 fibers/mm) was not significantly different from healthy controls. In 53% of CIAP and 44% of PDN patients, IENFD was abnormal (below the 5th percentile of Lauria et al.[21]) and marginally above the 5th percentile in ∼20% (three CIAP and three PDN patients). All CIAP patients had an IENFD well below the normative median reported by Lauria et al.[21]

Figure 3 and Table 3 show the results of CCM. All corneal nerve parameters were significantly reduced in PDN patients compared with healthy controls; however, no significant differences in CNFD, CNBD, or CNFL could be observed between CIAP patients and healthy controls. The correlations between IENFD and CCM for all patients ($n = 62$) were: CNFD ($r_s = .27$, $p = .03$), CNBD ($r_s = .41$, $p < .01$), and CNFL ($r_s = .23$, $p = .07$). Correlations increased, but were generally moderate, when the CIAP patients were excluded ($n = 47$): IENFD and CNFD ($r_s = .43$, $p < 0.01$), CNBD ($r_s = .40$, $p < 0.01$) and CNFL ($r_s = 0.39$, $p < 0.01$).

TABLE 1 Participant characteristics.

	CIAP ($n = 15$)	PDN ($n = 16$)	DM ($n = 15$)	Healthy controls ($n = 16$)
Men/women	11/4	9/7	9/6	9/7
Age (years)	64.2 ± 8.0**	63.2 ± 12.4**	52.7 ± 20.4	61.8 ± 12.4
BMI (kg/m^2)	26.6 ± 3.3***	28.3 ± 3.3***	27.5 ± 2.6***	23.9 ± 2.3
HbA_{1c} (%)	5.5 [5.2–5.9]*,**	7.2 [6.5–9.3]***	7.4 [6.2–8.2]***	5.4 [5.3–5.5]
Smoker (n)	3	3	4	4
Duration of diabetes mellitus (years)	NA	12.9 ± 8.5	12.5 ± 8.5	NA
Duration of neuropathy (years)	4.9 ± 3.1	3.6 ± 1.4[a]	NA	NA
Duration of pain (years)	3.1 ± 1.6	2.9 ± 1.4	NA	NA
MNSI total	NA	10.5 [7.25–13.5]**	1 [0–2]	NA
NRS average pain	5 [3–6]	5 [4–6.75]	NA	NA
NRS worst pain	8 [5–8]	7 [5.25–7]	NA	NA

Note: Values are presented as mean ± SD or as median [interquartile range].
Abbreviations: BMI, body mass index; MNSI, michigan neuropathy screening instrument; NA, not applicable; NRS, numerical rating score.
[a]Four patients did not know the duration of polyneuropathy, data presented for n = 12.
*$p < .05$ compared to PDN; **$p < .05$ compared to DM; ***$p < .05$ compared to healthy controls.

TABLE 2 Neurological function and autonomic dysfunction.

	CIAP ($n = 15$)	PDN ($n = 16$)	DM ($n = 15$)	Healthy controls ($n = 16$)
NIS-LL motor	0 [0–8]*,**,***	0 [0–1]	0 [0–0]	0 [0–0]
NIS-LL sensory	8 [4–10]**,***	6 [4–8]**,***	0 [0–2]	0 [0–0]
NIS-LL reflex	6 [4–6]*,**,***	2 [0–4]**,***	0 [0–0]	0 [0–0]
NIS-LL total	14 [10–26]*,**,***	8 [6.5–14]**,***	0 [0–2]	0 [0–0.75]
COMPASS-31	16.4 ± 13.7*,***	26.0 ± 16.0**,***	11.6 ± 8.0	3.5 ± 5.0
Sural nerve amplitude (μV)	2 [0.5–5.3]**,***	3.7 [0.5–5.5]***	7.1 [2–9.5]	8.9 [6.4–10.7]

Note: Values are presented as median [interquartile range] or mean ± SD.
Abbreviations: COMPASS, Composite Autonomic Symptom Score; NIS-LL, Neuropathy Impairment Score-Lower Limbs.
*$p < .05$ compared to PDN; **$p < .05$ compared to MN; ***$p < .05$ compared to healthy controls.

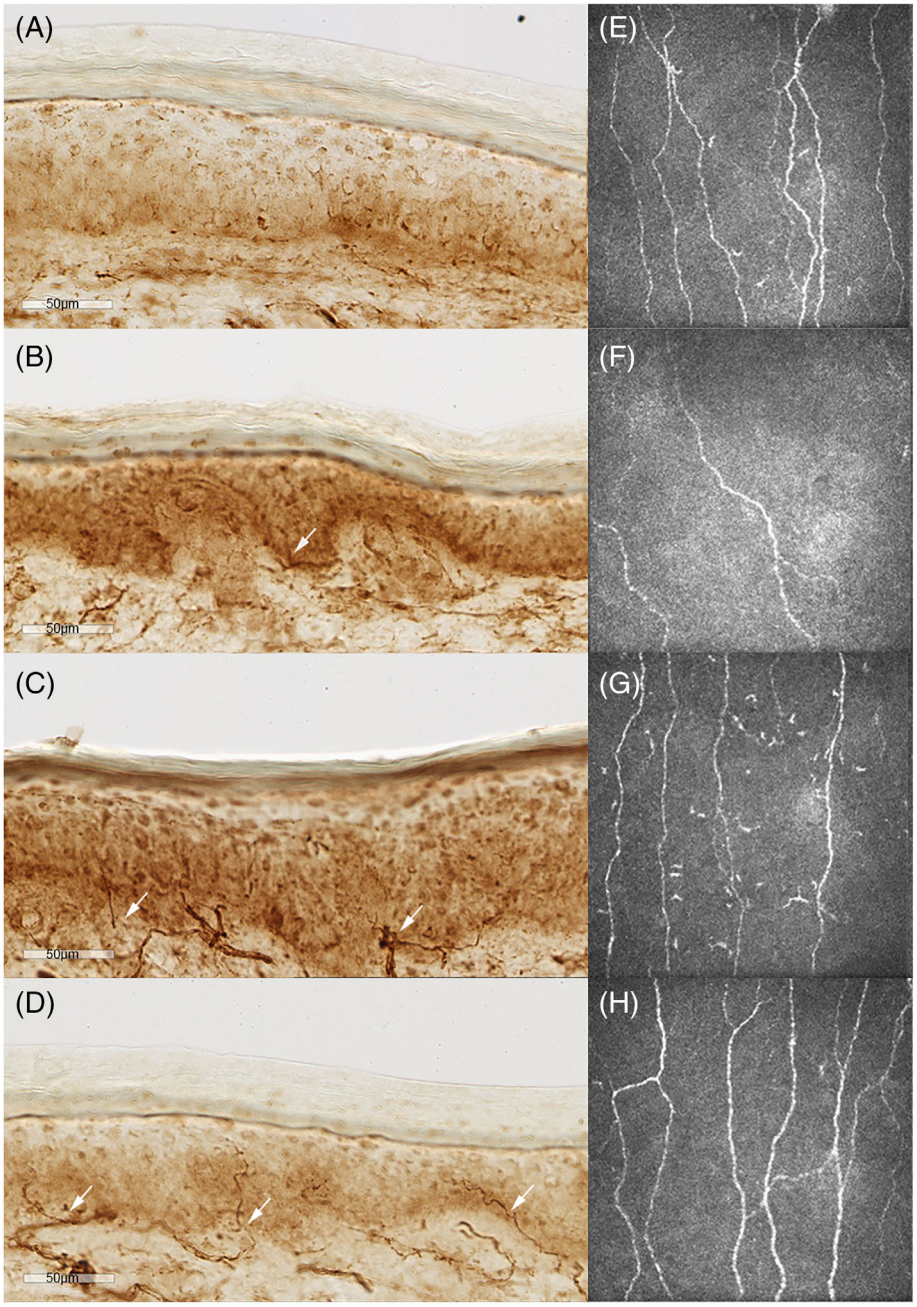

FIGURE 1 Skin biopsy image and corresponding corneal nerve plexus image in a patient with CIAP (A, E), PDN (B, F), DM (C, G) and a healthy control (D, H). White bar in the skin biopsy image indicates 50 μm, intraepidermal nerve fibers are marked with white arrows.

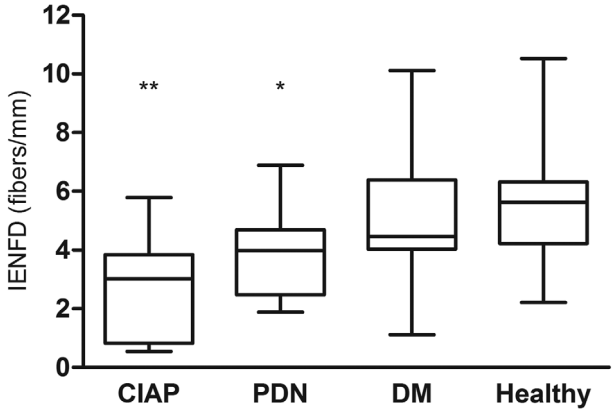

FIGURE 2 Nerve fiber density in the skin. Boxplots of IENFD in patients and healthy controls, whiskers represent 5th–95th percentile, $^{*}p < .05$; $^{**}p < .01$ compared to healthy controls. IENFD, intra epidermal nerve fiber density.

The two CCM measurements were performed a median of 7 days (range 2–14 days) apart. Thirty-five subjects were assessed for intra-center reproducibility. The remaining subjects were assessed at two centers for inter-center reproducibility ($n = 27$). Intra-center reproducibility of CNFD, CNFL, and CNBD was assessed from 420 captured images. The intra-center ICC was 0.89 for CNFD, 0.61 for CNBD, and 0.91 for CNFL. Inter-center reproducibility was assessed from 324 CCM images; inter-center ICCs were as follows: CNFD, 0.77; CNBD, 0.63; CNFL, 0.87. A Bland–Altman analysis was performed to calculate agreement (Supplement S1). Intra-center bias ± SD for CNFD was −0.9 ± 3.2, 0.8 ± 7.0 for CNBD, and −0.2 ± 1.2 for CNFL (Supplement S1A,C,E). The bias ± SD values for inter-center agreement were −2.1 ± 3.9 for CNFD, −12.3 ± 11.5 for CNBD, and −1.7 ± 2.0 for CNFL (Supplement S1B,D,F).

4 | DISCUSSION

Our findings revealed that IENFD was reduced in both CIAP and PDN patients when compared to healthy controls. CCM values were decreased in PDN but not in CIAP, indicating a discrepancy between the two neuropathies in terms of corneal nerve fiber changes.

In our patients with CIAP and suspected small fiber involvement, IENFD was abnormal in 53% and marginally above the lower cut-off score in 20% (normative data from Lauria et al.[21]). Nebuchennykh et al.[25] found that IENFD was abnormal in 43% of idiopathic neuropathy patients. The slightly higher incidence that we found may be due to differences in case definition and the presence of pain and, therefore, a suspicion of small fiber involvement in our group. Although CIAP predominantly affects large nerve fibers, small nerve fibers may also be affected, especially in patients with CIAP and neuropathic pain.[2] In the current study, we limited the duration of pain to 6 years in an attempt to have a more or less homogenous population, although it is unknown if longer disease duration or longer duration of pain impacts the outcome measures.

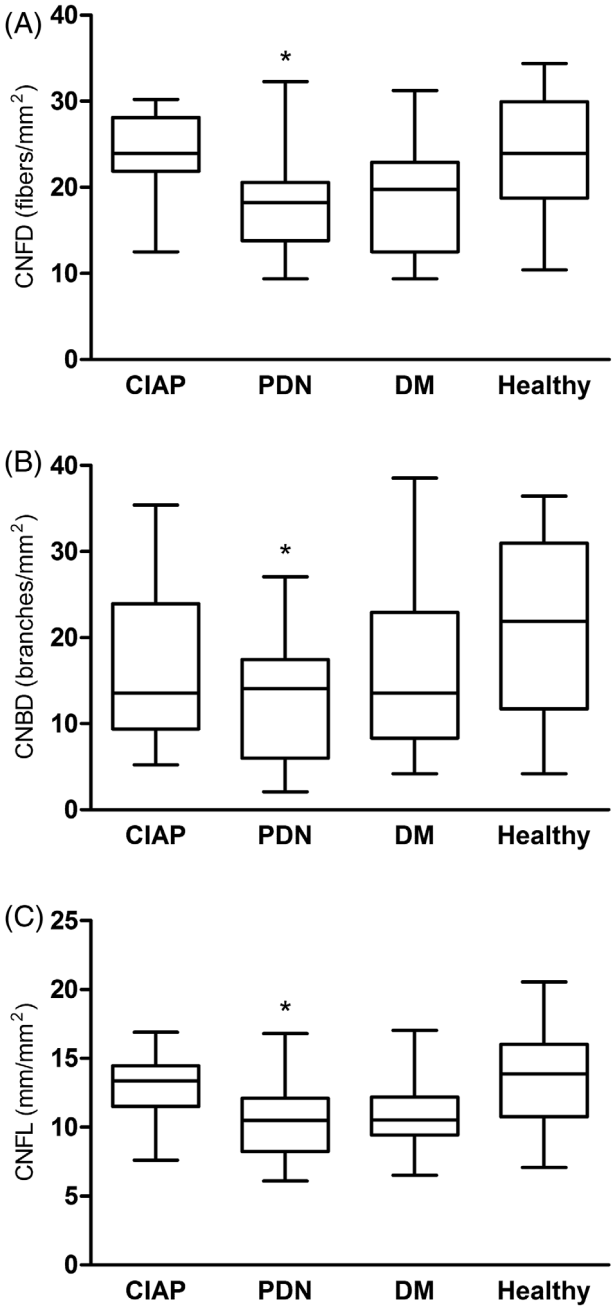

FIGURE 3 Boxplots of CCM parameters CNFD (A), CNBD (B), and CNFL (C) in patients and healthy controls. Whiskers represent 5th–95th percentile, $^{*}p < .05$ compared to healthy controls. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length.

TABLE 3 CNFD, CNBD, and CNFL.

	CIAP	PDN	DM	Healthy
CNFD	23.8 ± 4.9	17.8 ± 5.7*	19.2 ± 6.7	24.0 ± 6.8
CNBD	16.0 ± 8.8	12.7 ± 6.7*	16.1 ± 9.2	22.1 ± 9.7
CNFL	13.1 ± 2.6	10.5 ± 2.7*	10.7 ± 2.7	13.5 ± 3.5

Note: Values are presented as mean ± SD.
Abbreviations: CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length.
$^{*}p < .05$ compared to healthy controls.

With respect to CCM, CNFD, and CNFL values in patients with PDN, DM, and healthy controls were similar to those reported by others.[26–29] CCM images were analyzed with automated software. This software correlates well with manual analysis performed by an expert, although it slightly underestimates CNFD and CNFL counts compared to the expert.[30] This should be taken into account when comparing different studies. No normative reference values for automated CCM analysis in a Western population are available. Correlations between CCM parameters and IENFD, and between CCM parameters and small fiber functional tests in diabetes, have been reported by some,[15,31] whereas others could not identify significant correlations. Gylfadottir et al.[32] found CCM and IENFD correlation was very poor. The difference in correlation might partially be attributed to shorter diabetes duration and lower HbA1c levels compared to our population. In our study correlation between CNFD, CNFL, and IENFD was poor and greatly affected by the absence of a reduction in CCM parameters in CIAP patients. Correlations markedly improved when the CIAP patients were excluded. Due to the small sample size, correlations for distinct patient groups were not significant.

Surprisingly, CNFD, CNBD, and CNFL in CIAP patients did not differ from healthy controls. This is in contrast to our findings in diabetic neuropathy patients. Although CCM has been shown to be a useful biomarker for the detection of diabetic neuropathy,[8,10] non-length-dependent SFN,[11] and in various other neuropathies,[13,14] it was normal in our patients with CIAP. The difference is probably related to different underlying mechanisms leading to neuropathy in CIAP and PDN. CIAP is a length-dependent polyneuropathy without an identifiable cause. Diabetes mellitus is a systemic disease where multiple organ systems are affected, including the peripheral nerves. It has been suggested that corneal small fiber changes in diabetes mellitus may result from global metabolic processes that are not length-dependent.[33,34] Nevertheless, reduced corneal small fibers have been found in many other neuropathies without underlying metabolic disturbances.[35]

Others report that corneal nerve fibers may mainly consist of autonomic fibers.[36,37] Tavakoli et al.[36] found that CCM parameters were substantially lower in diabetics with diabetic autonomic neuropathy compared with diabetic patients without autonomic impairment. In the present study, COMPASS-31 scores were higher in PDN patients compared with CIAP patients, which may contribute to the observed difference. However, the difference was modest and had a large variability, and we therefore think it is unlikely that differences in autonomic impairment can fully explain the observed differences.

Thaisetthawatkul et al.[38] found that obesity, high triglycerides, and low HDL cholesterol affect small fiber structure more than function. Moreover, metabolic syndrome was found to accelerate the progression of neuropathy in patients with diabetes.[39] Microvascular function may be impaired in diabetes mellitus[40] and the degree of endoneurial microangiopathic changes has been shown to correlate with nerve fiber loss.[41] However, there is also evidence that hyperglycemia, dyslipidemia and inflammation lead to impaired vasodilation which has been found to correlate with polyneuropathy in patients with diabetes.[42] This is consistent with our report that endothelium-dependent vasodilation of the skin was diminished in Type 2 diabetes mellitus patients with and without polyneuropathy, whereas microvascular function was normal in patients with CIAP.[43] Unfortunately, there are no studies describing the effect of (micro)vascular dysfunction on corneal nerve fiber morphology.

The CNFD and CNFL measurements were highly reproducible even when performed at different sites by different examiners, using automated software, with CNFL yielding the highest reproducibility. This is consistent with previous small and large studies.[44–46] CNBD varied to a greater extent than CNFD and CNFL. This study confirmed the reported poorer reproducibility of CNBD.[45] We found an ICC of 0.61–0.63 for CNBD, which is slightly higher than the 0.54 as reported for inter-observer variability of CNBD by Petropoulos et al.[45]

In addition, we investigated the multicenter reproducibility of CNFD, CNBD, and CNFL. CCM yielded high inter-center reproducibility in a healthy and neuropathic population using automated software. This is important for the successful implementation of CCM in multicenter trials. The intra-center variability was smaller than the inter-center variability. Similar intra-center bias and ICC for CNFL were found by Pacaud et al.,[47] our inter-center CNFL bias of −1.7 was smaller than the −2.58 reported by Pacaud.[47] Variability in CCM parameters can be attributed to multiple factors such as slight variation in measurement plane and measurement location for repeat measurements and differences in individual image selection preferences among examiners. Also, differences in examiner experience level may play a role,[22] which could have been a contributing factor to the negative inter-center bias in our study. A limitation of the current study was that the examiners were not blinded. Variability may also differ for different pathologies. Furthermore, sample size limitations call for a cautious interpretation of Bland–Altman analyses for each group.

Despite its utility as a biomarker for diabetic neuropathy[8,10] and other neuropathies,[13,14] CCM did not demonstrate decreased values in CIAP patients with small fiber damage. The absence of corneal nerve fiber changes in CIAP patients might be indicative of different underlying neuropathic mechanisms compared to PDN patients. Further research is needed to elucidate the distinct pathophysiological processes in CIAP and PDN, shedding light on the differential impact on small nerve fiber involvement.

AUTHOR CONTRIBUTIONS

Mariska D. Nieuwenhoff: Conceptualization; investigation; writing – original draft; writing – review and editing; project administration; formal analysis. **Hoang-Ton Nguyen:** Investigation; writing – original draft; writing – review and editing; formal analysis. **Sjoerd P. Niehof:** Conceptualization; funding acquisition; writing – review and editing; methodology. **Frank J. P. M. Huygen:** Conceptualization; writing – review and editing; funding acquisition; methodology. **Ajay Verma:** Conceptualization; writing – review and editing; methodology; funding acquisition. **Erica S. Klaassen:** Conceptualization; methodology; writing – review and editing; formal analysis. **Malik Bechakra:** Formal analysis; writing – review and editing; methodology; writing – original draft. **Wouter J. Geelhoed:**

Investigation; writing – review and editing; formal analysis; writing – original draft. **Joost L. M. Jongen:** Conceptualization; methodology; writing – review and editing. **Annette C. Moll:** Writing – review and editing; conceptualization; methodology. **Alexander F. J. E. Vrancken:** Conceptualization; writing – review and editing; methodology. **Axel Petzold:** Methodology; writing – review and editing; conceptualization. **Geert Jan Groeneveld:** Conceptualization; funding acquisition; writing – review and editing; methodology; writing – original draft.

ACKNOWLEDGMENTS

We would like to thank Dr. J. Dorrestijn and A. Krijnen (Rotterdam Ophthalmic Institute) for their technical assistance in CCM. We would also like to thank Prof. R.A. Malik (University of Manchester) and his department for the CCM training course and analysis software. This study was supported by the Dutch Technology Foundation STW (Grant 10730), which is part of the Netherlands Organization for Scientific Research (NWO) and partly funded by the Ministry of Economic Affairs. This study was also supported by Biogen.

CONFLICT OF INTEREST STATEMENT

A.V. was an employee of Biogen at the time of study conduct. The remaining authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ETHICS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Mariska D. Nieuwenhoff https://orcid.org/0009-0007-9496-9726
Axel Petzold https://orcid.org/0000-0002-0344-9749

REFERENCES

1. Visser NA, Notermans NC, Linssen RS, van den Berg LH, Vrancken AF. Incidence of polyneuropathy in Utrecht, the Netherlands. *Neurology*. 2015;84(3):259-264.
2. de Schryver EL, van Schelven LJ, Notermans NC, de Valk HW, Oey PL. Small-fibre neuropathy can be detected in patients with chronic idiopathic axonal polyneuropathy. *Eur J Neurol*. 2011;18(7):1003-1005.
3. Erdmann PG, van Genderen FR, Teunissen LL, et al. Pain in patients with chronic idiopathic axonal polyneuropathy. *Eur Neurol*. 2010;64(1):58-64.
4. Bechakra M, Nieuwenhoff MD, van Rosmalen J, et al. Pain-related changes in cutaneous innervation of patients suffering from bortezomib-induced, diabetic or chronic idiopathic axonal polyneuropathy. *Brain Res*. 2020;1730:146621.
5. Lauria G, Hsieh ST, Johansson O, et al. 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*. 2010;17(7):903-912.
6. Argyriou AA, Park SB, Islam B, et al. Neurophysiological, nerve imaging and other techniques to assess chemotherapy-induced peripheral neurotoxicity in the clinical and research settings. *J Neurol Neurosurg Psychiatry*. 2019;90(12):1361-1369.
7. Tavakoli M, Ferdousi M, Petropoulos IN, et al. Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. *Diabetes Care*. 2015;38(5):838-843.
8. Tavakoli M, Quattrini C, Abbott C, et al. Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. *Diabetes Care*. 2010;33(8):1792-1797.
9. Ahmed A, Bril V, Orszag A, et al. Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. *Diabetes Care*. 2012;35(4):821-828.
10. Ziegler D, Papanas N, Zhivov A, et al. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. *Diabetes*. 2014;63(7):2454-2463.
11. Gemignani F, Ferrari G, Vitetta F, Giovanelli M, Macaluso C, Marbini A. Non-length-dependent small fibre neuropathy. Confocal microscopy study of the corneal innervation. *J Neurol Neurosurg Psychiatry*. 2010;81(7):731-733.
12. Tavakoli M, Marshall A, Banka S, et al. Corneal confocal microscopy detects small-fiber neuropathy in Charcot–Marie–Tooth disease type 1A patients. *Muscle Nerve*. 2012;46(5):698-704.
13. Petropoulos IN, Al-Mohammedi A, Chen X, et al. The utility of corneal nerve fractal dimension analysis in peripheral neuropathies of different etiology. *Transl Vis Sci Technol*. 2020;9(9):43.
14. Merkies IS, Faber CG, Lauria G. Advances in diagnostics and outcome measures in peripheral neuropathies. *Neurosci Lett*. 2015;596:3-13.
15. Quattrini C, Tavakoli M, Jeziorska M, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes*. 2007;56(8):2148-2154.
16. Visser NA, Vrancken AF, van der Schouw YT, van den Berg LH, Notermans NC. Chronic idiopathic axonal polyneuropathy is associated with the metabolic syndrome. *Diabetes Care*. 2013;36(4):817-822.
17. Herman WH, Pop-Busui R, Braffett BH, et al. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabet Med*. 2012;29(7):937-944.
18. Bril V. NIS-LL: the primary measurement scale for clinical trial endpoints in diabetic peripheral neuropathy. *Eur Neurol*. 1999;41(suppl 1):8-13.
19. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. *Mayo Clin Proc*. 2012;87(12):1196-1201.
20. McCarthy BG, Hsieh ST, Stocks A, et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. *Neurology*. 1995;45(10):1848-1855.
21. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst*. 2010;15(3):202-207.
22. Kalteniece A, Ferdousi M, Adam S, et al. Corneal confocal microscopy is a rapid reproducible ophthalmic technique for quantifying corneal nerve abnormalities. *PLoS One*. 2017;12(8):e0183040.
23. Bland JM, Altman DG. A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Comput Biol Med*. 1990;20(5):337-340.
24. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.
25. Nebuchennykh M, Loseth S, Jorde R, Mellgren SI. Idiopathic polyneuropathy and impaired glucose metabolism in a Norwegian patient series. *Eur J Neurol*. 2008;15(8):810-816.

26. Raasing LRM, Vogels OJM, Datema M, Tannemaat MR, Veltkamp M, Grutters JC. Fully automatic, semiautomatic, and manual corneal nerve fiber analysis in patients with sarcoidosis. *Transl Vis Sci Technol*. 2023;12(12):3.
27. Giannaccare G, Pellegrini M, Sebastiani S, Moscardelli F, Versura P, Campos EC. In vivo confocal microscopy morphometric analysis of corneal subbasal nerve plexus in dry eye disease using newly developed fully automated system. *Graefes Arch Clin Exp Ophthalmol*. 2019;257(3):583-589.
28. Perkins BA, Lovblom LE, Bril V, et al. Corneal confocal microscopy for identification of diabetic sensorimotor polyneuropathy: a pooled multinational consortium study. *Diabetologia*. 2018;61(8):1856-1861.
29. Dabbah MA, Graham J, Petropoulos IN, Tavakoli M, Malik RA. Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging. *Med Image Anal*. 2011;15(5):738-747.
30. Petropoulos IN, Alam U, Fadavi H, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. *Invest Ophthalmol Vis Sci*. 2014;55(4):2071-2078.
31. Sivaskandarajah GA, Halpern EM, Lovblom LE, et al. Structure-function relationship between corneal nerves and conventional small-fiber tests in type 1 diabetes. *Diabetes Care*. 2013;36(9):2748-2755.
32. Gylfadottir SS, Itani M, Kristensen AG, et al. Assessing corneal confocal microscopy and other small fiber measures in diabetic polyneuropathy. *Neurology*. 2023;100(16):e1680-e1690.
33. Kass-Iliyya L, Javed S, Gosal D, et al. Small fiber neuropathy in Parkinson's disease: a clinical, pathological and corneal confocal microscopy study. *Parkinsonism Relat Disord*. 2015;21(12):1454-1460.
34. Chen DK, Frizzi KE, Guernsey LS, Ladt K, Mizisin AP, Calcutt NA. Repeated monitoring of corneal nerves by confocal microscopy as an index of peripheral neuropathy in type-1 diabetic rodents and the effects of topical insulin. *J Peripher Nerv Syst*. 2013;18(4):306-315.
35. Gemignani F, Bellanova MF, Saccani E, Pavesi G. Non-length-dependent small fiber neuropathy: not a matter of stockings and gloves. *Muscle Nerve*. 2022;65(1):10-28.
36. Tavakoli M, Begum P, McLaughlin J, Malik RA. Corneal confocal microscopy for the diagnosis of diabetic autonomic neuropathy. *Muscle Nerve*. 2015;52(3):363-370.
37. Maddaloni E, Sabatino F, Del Toro R, et al. In vivo corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in type 1 diabetes. *Diabet Med*. 2015;32(2):262-266.
38. Thaisetthawatkul P, Lyden E, Americo Fernandes J Jr, Herrmann DN. Prediabetes, diabetes, metabolic syndrome, and small fiber neuropathy. *Muscle Nerve*. 2020;61(4):475-479.
39. Kazamel M, Stino AM, Smith AG. Metabolic syndrome and peripheral neuropathy. *Muscle Nerve*. 2021;63(3):285-293.
40. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33(10):2285-2293.
41. Malik RA, Tesfaye S, Newrick PG, et al. Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. *Diabetologia*. 2005;48(3):578-585.
42. Cosmo E, Midena G, Frizziero L, Bruno M, Cecere M, Midena E. Corneal confocal microscopy as a quantitative imaging biomarker of diabetic peripheral neuropathy: a review. *J Clin Med*. 2022;11(17):5130.
43. Emanuel AL, Nieuwenhoff MD, Klaassen ES, et al. Relationships between type 2 diabetes, neuropathy, and microvascular dysfunction: evidence from patients with cryptogenic axonal polyneuropathy. *Diabetes Care*. 2017;40(4):583-590.
44. Ostrovski I, Lovblom LE, Farooqi MA, et al. Reproducibility of in vivo corneal confocal microscopy using an automated analysis program for detection of diabetic sensorimotor polyneuropathy. *PLoS One*. 2015;10(11):e0142309.
45. Petropoulos IN, Manzoor T, Morgan P, et al. Repeatability of in vivo corneal confocal microscopy to quantify corneal nerve morphology. *Cornea*. 2013;32(5):e83-e89.
46. Hertz P, Bril V, Orszag A, et al. Reproducibility of in vivo corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. *Diabet Med*. 2011;28(10):1253-1260.
47. Pacaud D, Romanchuk KG, Tavakoli M, et al. The reliability and reproducibility of corneal confocal microscopy in children. *Invest Ophthalmol Vis Sci*. 2015;56(9):5636-5640.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Nieuwenhoff MD, Nguyen H-T, Niehof SP, et al. Differences in corneal nerve fiber density and fiber length in patients with painful chronic idiopathic axonal polyneuropathy and diabetic polyneuropathy. *Muscle & Nerve*. 2024;1-9. doi:10.1002/mus.28213