Neuroimaging markers of clinical progression in chronic inflammatory demyelinating polyradiculoneuropathy

Kalliopi Pitarokoili, Dietrich Sturm, Adnan Labedi, Tineke Greiner, Lynn Eitner, Nina Kumowski, Elena K. Enax-Krumova, Anna Lena Fisse, Christoph Maier, Ralf Gold, Martin Tegenthoff, Tobias Schmidt-Wilcke and Min-Suk Yoon

Abstract

Background: One of the main goals of novel, noninvasive imaging techniques like high-resolution nerve ultrasound (HRUS) and corneal confocal microscopy (CCM) is the prediction of treatment response for patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP).

Methods: A total of 17 patients with CIDP were examined prospectively at baseline and every 9 months over a period of 18 months using CCM to quantify corneal nerve degeneration markers and immune cell infiltration as well as HRUS to detect changes of the cross-sectional area (CSA) of the peripheral nerves. Additionally, skin biopsy of the distal and proximal leg as well as quantitative sensory testing were performed at the first follow-up visit.

Results: A value of more than 30 total corneal cells/mm² in CCM at baseline identified patients with clinical progression with a sensitivity/specificity of 100% in our cohort. Corneal nerve fiber density and length remained low and stable over the study period and intra-epidermal fiber density was markedly reduced in the majority of the patients. Furthermore, an increase in Bochum ultrasound score (BUS), which summarizes the CSA of the ulnar nerve in Guyon’s canal, the ulnar nerve in the upper arm, the radial nerve in the spiral groove and the sural nerve between the gastrocnemius muscle, and a maximum BUS of 4 at study initiation identified patients with disease progression (sensitivity 80%, specificity 88%).

Conclusions: BUS and corneal total cell infiltration seem to represent early markers for clinical progression in CIDP, thus having the potential to identify at-risk patients and impact treatment decisions.

Keywords: chronic inflammatory demyelinating polyneuropathy, corneal confocal microscopy, intra-epidermal nerve fiber density, nerve conduction studies, nerve ultrasound, somatosensory profiles

Introduction

Imaging techniques such as high-resolution nerve ultrasound (HRUS) and corneal confocal microscopy (CCM) have recently provided a novel insight in our understanding of the dynamic nature of peripheral nerve morphology.

HRUS studies have confirmed the multifocal cross-sectional area (CSA) enlargement in distal and proximal segments of almost all peripheral nerves and brachial plexus in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP); additionally, some pattern analyses of the focal or diffuse swelling of peripheral nerves have been attempted.¹⁻⁹

The distribution and extent of CSA increase seem not only to differentiate acute from chronic demyelinating diseases but also to distinguish between chronic autoimmune neuropathies themselves.¹⁰⁻¹²
On the other hand, CCM, a rapidly developing, noninvasive technique, focuses on corneal imaging both in terms of inflammation and axonal loss. Its use has extended to a variety of neuropathies, mainly in diabetic neuropathy but also in uncommon neuropathies. The aspect of inflammation is depicted by a specific type of autochthonous immune cells, which had been identified as Langerhans cells. The two existing studies on CCM in CIDP have shown a reduction in corneal nerve fiber parameters and an increase in corneal immune cell infiltrates in patients with CIDP compared with healthy controls. However, the practical role of these parameters in clinical routine remains unclear. The primary objective of this prospective pilot study was to systematically investigate the potential of CCM and HRUS parameters as neuroimaging markers of disease progression in a cohort of patients with CIDP during individualized treatment based on the clinician’s decision.

Materials and methods

Study protocol: clinical assessment
A cohort of patients with CIDP were recruited during the last trimester of 2015 at the Departments of Neurology, St. Josef and Bergmannsheil University Hospital, Bochum, Germany. The diagnosis of CIDP was based on the respective criteria of the Peripheral Nerve Society/European Federation of Neurological Societies. The study was approved by the local ethics committee (Ethics Committee University of Ruhr University Bochum, Nr 4905-14). All patients gave their written informed consent prior to the inclusion into the study. The study was performed in accordance with the Declaration of Helsinki.

The peripheral nerves were measured bilaterally at the following sites: median nerve at the entrance to carpal tunnel (flexor retinaculum), forearm (15 cm proximal to flexor retinaculum), upper arm (midpoint between medial epicondyle and axillary fossa), ulnar nerve at the Guyon canal, forearm (15 cm proximal to the Guyon canal), elbow (between medial epicondyle and olecranon), upper arm (midpoint between medial epicondyle and axillary fossa), radial nerve in the spiral groove, tibial nerve in the popliteal fossa and at the ankle, fibular nerve at the fibular head and in the popliteal fossa and sural nerve (between the lateral and medial heads of the gastrocnemius muscle).
For each of the nerves of both sides of all patients, the intra-nerve and inter-nerve CSA variabilities were calculated according to the following: ‘intra-nerve cross-sectional area variability’ (for each nerve) as maximal cross-sectional area/minimal cross-sectional area, ‘inter-nerve cross-sectional area variability’ (for each patient) as maximal intra-nerve cross-sectional area variability/minimal intra-nerve cross-sectional area variability. Furthermore, Bochum ultrasound score (BUS) was calculated for each patient and each visit, summarizing the CSA of: (1) the ulnar nerve in the Guyons’ canal, (2) the ulnar nerve in the upper arm, (3) the radial nerve in the spiral groove, and (4) the sural nerve between the gastrocnemius muscle (maximum score of 4 if CSA in every one of the four locations is increased). Bilateral CSA increase was counted only once.23 The examiner was blinded for the clinical outcome.

**Corneal confocal microscopy**

All study participants were scanned by two examiners (DS and TG) using a Heidelberg Retinal Tomograph III with a Rostock Cornea Module (HRT III RCM) (Heidelberg Engineering GmbH, Heidelberg, Germany) as previously described.13 Five high-quality images of one eye were analyzed and the mean of these results was calculated. A fully automated software was used to quantify corneal nerve fiber density (CNFD; nerves/mm²), corneal nerve branch density (CNBD; branches/mm²), and corneal nerve fiber length (CNFL; mm/mm²) (ACC Metrics version 2.0; M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK). Cell infiltrates were analyzed manually by DS in the same images that were also used to quantify corneal nerves. The total cell number of cells per mm² was calculated. These assessments were standardized for the area analyzed. Both examiners were blinded for the clinical outcome.

**Skin punch biopsy and quantitative sensory testing**

For assessment of intra-epidermal nerve fiber density skin punch biopsy was obtained during the first follow-up visit (V2) from the distal lower leg (10 cm above the lateral malleolus) and from the proximal thigh as recommended by the European Federation of Neurological Societies/Peripheral Nerve Society,25 done by two examiners (DS and EEK). Skin samples were processed as previously described.28 The intra-epidermal nerve fiber density per mm (IEFND) was quantified manually by EEK. The reference IEFND values of our department are >15 fibers/mm for the proximal thigh, and >9 fibers/mm for the distal lower leg, which were adopted from the lab of Prof. Sommer and Prof. Üceyler, Würzburg, Germany. Quan-titative sensory testing (QST) was conducted at the dorsal feet according to the standardized protocol of the German Research Network on Neuropathic Pain (DFNS) and data were analyzed as described before.29,30 QST was done by LE, data analysis was performed by NK.

**Statistics**

The analysis was performed by KP using Prism 7 (GraphPad Software, La Jolla, CA, USA). All data are presented as mean ± standard deviation (SD). D’Agostino and Pearson normality tests were applied to test the distribution of the groups and the differences were assessed using two-sample Student’s t tests. *p < 0.05 was regarded as statistically significant. The Pearson correlation coefficient r was calculated for all correlation analyses. We applied the nonlinear Spearman’s rank correlation coefficient rs for correlations with ODSS and with F-wave latency. For the correlations, the maximum F-wave latency was used for absent F-waves. Due to the large number of sonographic and electrophysiological measurements, a Bonferroni correction was performed, so that only p < 0.001 values were accepted as statistically significant.

**Results**

**Baseline clinical data for all patients**

A total of 17 patients with CIDP (mean age 62.0 years, SD ± 8.7; 7 women) underwent clinical, sonographical and electrophysiological evaluation as well as CCM at a mean of 8.8 years (SD ± 5.6 years) after disease onset (visit 1) as well as during the next 18 months in a mean time of 8.9 ± 1.2 months between visits (visits 2 and 3) (Table 1). The patients showed a mean ODSS/INCAT of 3.7 (SD ± 1.4, min–max 1–5) at visit 1. During the study period, all patients were treated with 1g/kg intravenous immunoglobulins every 4–6 weeks whereas six of them received additional oral immunosuppression (azathioprine or mycophenolate mofetil).
Baseline NCS and HRUS data for all patients
NCS at baseline (V1) showed a typical sensorimotor demyelinating polyneuropathy. A total of 6 patients showed a distal tibial compound motor action potential (CMAP) over 3 mV whereas 15 patients showed a median CMAP over 4 mV at baseline (Supplementary Table 1).

The BUS was $\geq 2$ for 12 patients at baseline (mean CSA values and intra-nerve/inter-nerve variability values are presented in Supplementary Table 2). The HRUS values of any of the nerves at baseline did not correlate with disease duration or INCAT/ODSS disability score.

Baseline CCM data for all patients
CCM showed a mean CNFD $\pm$ SD of 27.4 $\pm$ 8.8/mm$^2$, a mean CNBD of 39.3 $\pm$ 26.4/mm$^2$, a mean CNFL of 15.9 $\pm$ 5.1 mm/mm$^2$ and a mean number of 49 $\pm$ 59.6 total cells/mm$^2$. We found no correlation of the CCM parameters for disease duration or INCAT/ODSS (Figure 1) but the two patients with the highest number of total cells at baseline belonged to the groups with lowest disease duration (2 and 3 years) and the highest ODSS (ODSS 5 for both of them). There was no correlation between total cell number and corneal nerve fiber length or density.

QST and IEFND data for all patients
QST was performed at the first follow-up visit (V2) in 15 patients with CIDP and detected abnormally decreased z-values for cold detection threshold ($-2.3 \pm 1.1$), for mechanical detection threshold ($-2.1 \pm 2.1$) and vibration detection threshold ($-3 \pm 2$), indicating a mixed sensory loss of detection. Hypoesthesia to cold stimuli, vibration or mechanical stimuli was evident in 67%, 73% and 53%, respectively. 53% of the patients reported paradoxical heat sensation as a sign for central disinhibition within the corresponding central pathways of the small fibers. All assessed pain thresholds (cold, heat, mechanical stimuli and pressure pain thresholds) were within the normal range.

Skin biopsy was assessed in 13 patients with CIDP (V2) and IEFND was reduced for all of them as an indication of small fiber nerve affection (IEFND, mean $\pm$ SD, lower leg: 2.9 $\pm$ 3 fibers/mm, upper leg 4.7 $\pm$ 4.8 fibers/mm). IEFND and CCM parameters did not correlate.

Of the 13 patients with signs of small fiber nerve affection, 10 received membrane stabilizing substances in the context of neuropathic pain.

Longitudinal studies: markers of clinical progression
Epidemiological data on stable ($n = 7$) and progressive ($n = 10$) patients with CIDP are presented in Table 1. Age, sex and ODSS/INCAT did not differ significantly between both groups, whereas patients with a progressive disease showed a slightly increased disease duration (stable $7.2 \pm 4.6$ versus progressive $9.9 \pm 6.2$ years from first disease.
manifestation, n.s.). Furthermore, from the six patients receiving oral immunosuppression two remained clinically stable, whereas four patients presented with a disease progression. The mean values of the NCS did not differ significantly between patients with stable disease and disease progression (see Supplementary Tables 3 and 4).

Figure 1. Corneal confocal microscopy data at baseline (visit 1): Total corneal cells infiltrates, CNFD, CNBD and CNFL values are depicted in relation to disease duration and ODSS/INCAT score at baseline. No statistical significant correlation of these parameters was found. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; INCAT, inflammatory neuropathy cause and treatment; ODSS, overall disability score scale.

Regarding the clinical phenotype and previous disease course of the stable patients (n = 7) one of them was characterized by an acute sensorimotor disease onset but improved and remained stable until the beginning of our current study, all other stable patients were characterized by a typical sensory onset of the symptoms and a further
sensorimotor progression, two of them still received immunosuppression due to course of the disease in the previous years. In the group of progressive patients, four of them received immunosuppression due to aggressive disease course (Table 1).

Patients with clinical progression included the following ODSS/INCAT increase (raw data, number of patients: ODSS at the beginning of the study → at the end of the study): 2 patients: 4 → 6, 1 patient: 2 → 4, 3 patients: 5 → 6, 1 patient: 1 → 2, 1 patient: 2 → 3, 1 patient: 3 → 4, 1 patient: 4 → 5.

Longitudinal HRUS data: CSA and CSA variability measures
The mean CSA values for the patients with stable and progressive disease are presented in Supplementary Tables 5 and 6.

Patients with progressive disease during the study period had the following sonographical characteristics:

CSA mean values of the median nerve at forearm, of the radial nerve at the spiral groove, of the fibular nerve at fibular head and of the sural nerve between the lateral and medial head of gastrocnemius muscle were above reference values at V1 and remained abnormal during the whole study period. This was not the case for patients with stable disease as these mean values improved over this period.

These results were confirmed in the evaluation of the different nerve segments. A total of 260 segments (bilaterally) were evaluated in each visit for the progressive and 182 for the stable CIDP group. For the progressive group at visit 1, visit 2 and visit 3 44%, 47% and 55% of these segments had increased CSA values mostly at the above-mentioned locations. For the stable CIDP group these values were lower and decreased over time (40%, 47% and 34% for visit 1, 2 and 3 respectively).

As some of these segments are included in the BUS, we evaluated its use in the longitudinal CIDP evaluation for the first time.

BUS increased or remained stable at a maximum of 4 points for 8 (true positive – 2 false negative) of the 10 patients in the progressive CIDP group and for 1 patient (false positive – 7 true negative) in the stable disease group. Therefore, a BUS increase from V1 to V3 or BUS of 4 points at V1 predicted with a sensitivity of 80% (8/2 + 8), specificity 87.5% (7/1 + 7), a positive predictive value (PPV) of 88% (8/1 + 8) and a negative predictive value (NPV) of 77% (7/2 + 7) a disease progression in our cohort. Representative HRUS pictures of the BUS for a stable and a progressive patient are presented in Figure 2.

Furthermore, all mean values of intra-nerve CSA variability as well as the mean inter-nerve CSA variability increased over time. Stable patients did not show this homogenous increase of variability measures but fluctuations with an improvement for the majority of the values at V3 (Supplementary Tables 3 and 4). These results were confirmed after evaluating the individual nerves with increased CSA variability. For the stable group, 56 nerves were evaluated and among them 32% had increased intra-nerve variability at V1 and 35% at V3 (increase of 3%). The progressive CIDP group showed 26% of the nerves with an increased CSA variability at V1 and 41% at V3 (increase of 15%).

Longitudinal CCM parameters of inflammation and degeneration
Total cell count as a marker of inflammation revealed significantly increased corneal cell infiltrates for patients in the progressive group compared with patients in the group with stable disease (mean total cell count stable CIDP 13 ± 11, progressive CIDP 74.8 ± 63.7, *p = 0.02). All patients with progressive disease over the next 18 months presented with more than 30 total cells/mm² in the cornea at baseline (sensitivity, specificity, PPV, NPV for clinical progression of 100% in our cohort; Figures 3 and 4). Total corneal cell values correlated neither with ODSS score nor with NCS or HRUS parameters at any of the visits. During the study period, inflammatory cell infiltrates in the cornea remained high for progressive patients and low for patients with stable CIDP disease (Table 2).

On the other hand, corneal nerve parameters did not differ significantly between the two groups and remained unchanged during 18 months (Table 2). Furthermore, the subgroups of stable (n = 6) and progressive CIDP (n = 9) displayed...
similar somatosensory profiles at baseline, with the exception of less sensory loss in the progressive group in the assessment of cold and vibration detection threshold and heat pain threshold (QST data, Supplementary Table 7).

Discussion
To summarize, in our CIDP cohort corneal cell infiltration at baseline assessed by CCM was related to further clinical progression, and increase in BUS assessed using HRUS correlated positively with disease activity.

In terms of HRUS, the majority of patients with a BUS increase over the period of 18 months or a high BUS score at baseline were identified as patients with a disease progression. BUS has been previously proposed from our group as a marker to distinguish between CIDP and acute inflammatory neuropathies (AIDP) with a sensitivity and specificity of 90%.6,9,22 Its main advantages are easy applicability in daily routine by clinical neurologists as it includes only four nerve segments bilaterally.

Figure 2. Representative ultrasound pictures of the nerves evaluated for the BUS for a stable and a progressive patient at the beginning of the study (ulnar nerve in Guyon’s canal and upper arm, radial and sural nerve). The stable patient has a BUS of 1 (only ulnar nerve in Guyon’s canal shows an increased CSA of 9 mm² [normal values of our laboratory < 7.22 mm²]) whereas the progressive patient has a BUS of 3 (increased CSA of the ulnar nerve in upper arm (< 10.17 mm²), of the radial nerve (< 6.2 mm²) and of the sural nerve (< 3.01 mm²)).

BUS, Bochum ultrasound score; CSA, cross-sectional area.

Figure 3. Total corneal cell infiltrates at baseline (Visit 1, V1): Patients with progressive disease (ODSS/INCAT increase of ≥ 1) showed already at baseline higher inflammatory infiltrates compared with patients with stable CIDP, *p < 0.05. Using a cut-off value of 30 total cells/mm² (dotted line) all patients with progressive disease show higher values [sensitivity, specificity for clinical progression 100%].

CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; INCAT, inflammatory neuropathy cause and treatment; ODSS, overall disability score scale.
In a previous pilot study with 11 patients with CIDP, the CSA variability increased parallel to clinical deterioration. However, the patients in that study were evaluated on average 8.5 ± 3.2 days (range: 2–45 days) after symptom onset, in contrast to the present cohort of chronic disease stages (on average 8.8 ± 5.6 years after disease onset). Intra-nerve CSA variability of all nerves seems to increase parallel to ODSS/INCAT deterioration at this later stage. The echogenicity of peripheral nerves was not evaluated in the present study. This aspect represents a challenge for future HRUS studies.

An increase of total corneal cell infiltrates has been described in two cross-sectional CIDP studies. In the current study total cell infiltrates with more than 30/mm² at baseline identified all patients with a clinical progression in the next 18 months, showing the potential of this parameter to predict disease progression for the first time. These findings suggest residual inflammatory infiltrates in patients...
with progressive disease, which are visible in CCM, but probably also present in peripheral nerves thus leading to the clinical deterioration. Interestingly, the inflammatory infiltrates in the cornea remained increased until the end of the study, which poses the question whether they would improve after further treatment escalation.

In contrast with previous CCM studies, we could not confirm the correlation of corneal cell infiltrates and degeneration markers with disease duration and ODSS/INCAT at baseline. However, our cohort was smaller than the one reported before \( n = 88 \). Still, the previously reported increased dendritic cells in contact with axons for patients with higher INCAT scores points to the same direction as our current study.\(^{19}\)

Including two patients with diabetes may have influenced the results of the NCSs. However, it has been shown that there is no relevant CSA increase in the nerves composing the BUS.\(^{32}\) Furthermore, a corneal cell infiltration in context to diabetes mellitus has only been reported in a rodent model (but not in humans) with a different methodical approach.\(^{33}\)

Compared with published normative data, corneal nerve parameters did not achieve abnormal values in our study (with one exception) and showed only a slight decrease within the studied period.\(^{34}\) This may be not only related to a progression of a disease. Other factors, like age, also influence the corneal nerves and may explain the changes.\(^{35}\) Furthermore, the corneal sub-basal plexus has been evaluated longer than 12 months only in a few cases and mainly in diabetic neuropathy.\(^{36}\)

In contrast with that, we found a severe histological affection of small fibers in the skin with reduced IENFD and sensory impairment, similar to previous studies.\(^{37-39}\) The mismatch between the CCM nerve parameters and the sensory and morphological findings in the skin might reflect different pathophysiological mechanisms of small fiber damage in different organs (cornea, skin) similarly to findings in diabetic neuropathy.\(^{40}\)

To our knowledge, this is the first study to characterize the somatosensory profile in CIDP. In our cohort with longer lasting CIDP duration the sensory abnormalities corresponded to the cluster of deafferentation, which also dominated a previously published larger cohort of polyneuropathy of various origin, and might reflect the advanced stage of the disease.\(^{41,42}\) In contrast with corneal cells, neither intra-epidermal nerve fiber density nor nerve parameters in CCM or QST parameters were suitable as a marker of disease progression in our group.

Surely, the present pilot study has some major limitations. We performed a single-center, prospective data analysis with a small number of patients representing every day clinical practice using a variety of imaging studies. CIDP treatment was heterogenous as it was adapted by the treating neurologist individually, based on the clinical course of the disease and NCS and therefore intravenous immunoglobulins did not correspond to the concentration of 1 g/kg every 3 weeks reported by the IGIV-C CIDP Efficacy study as an optimal treatment protocol.\(^{42,43}\) However, as concluded from Dalakas and colleagues, protocols of immunoglobulin treatment vary in every day practice as the clinical presentation and treatment response differ between patients.\(^{44}\) The purpose of the present study was indeed to prove whether novel HRUS and CCM markers are able to predict clinical stability in the context of this heterogenous disease. Further larger multicenter studies, including patients at earlier disease stages, are needed to confirm our results.

In conclusion, the reported novel neuroimaging biomarkers (corneal cell infiltrates in CCM and BUS in HRUS) have the potential to predict clinical disease course and aid the clinical decision towards treatment escalation or de-escalation for patients with CIDP.

Acknowledgements
Kalliopi Pitarokoili and Dietrich Sturm contributed equally to this work. The following are the author contributions:
Kalliopi Pitarokoili study design, acquisition of data, analysis and interpretation of data, drafting/revising the manuscript for content.
Dietrich Sturm study design, acquisition of data, analysis and interpretation of data, drafting/revising the manuscript for content.
Adnan Labedi acquisition of data, analysis and interpretation of data, revising the manuscript for content.
Tineke Greiner acquisition of data, analysis and interpretation of data, revising the manuscript for content.
Lynn Eitner acquisition of data, analysis and interpretation of data, drafting/revising the manuscript for content.
Nina Kumowski acquisition of data, analysis and interpretation of data, drafting and revising the manuscript for content.
Elena K. Enax Krumowa acquisition of data, analysis and interpretation of data, drafting and revising the manuscript for content.
Anna Lena Fisse acquisition of data, analysis and interpretation of data, drafting and revising the manuscript for content.
Ralf Gold study design, drafting/revising the manuscript for content.
Martin Tegenthoff study design, study supervision, drafting/revising the manuscript for content.
Tobias Schmidt-Wilcke study design, study supervision, drafting/revising the manuscript for content.
Min-Suk Yoon study design, analysis and interpretation of data revising the manuscript for content.

The study was approved by the local ethics committee (Ethics Committee University of Ruhr University Bochum, Nr 4905-14). All patients gave their written informed consent prior to the inclusion into the study. The study was performed in accordance with the Declaration of Helsinki.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts of interest
Nina Kumowski, Lynn Eitner, Tineke Greiner, Anna Lena Fisse and Adnan Labedi report no disclosures. Dietrich Sturm received funding from the Ruhr University, Bochum (FoRUM-Program). Kalliopi Pitarokoili received travel funding and speaker honoraria from Biogen Idec, Novartis and Bayer Schering Pharma, and funding from the Ruhr University, Bochum (FoRUM-Program). R. Gold has received consultation fees and speaker honoraria from Bayer Schering, Biogen Idec, Merck Serono, Novartis, Sanofi-Aventis and TEVA. He also acknowledges grant support from Bayer Schering, Biogen Idec, Merck Serono, Sanofi-Aventis and TEVA, none related to this manuscript.
Elena K. Enax-Krumova is member of the German Research Network on Neuropathic pain e.V. (BMBF, grants 01EM0107 & 01EM0502), members of the European Collaboration, which has received support from the Innovative Medicines Initiative Joint Undertaking, under grant agreement no 11507, resources of which are composed of financial contribution from the European Union’s Seventh Framework Programme (FP7/2007-2013) and EFPIA companies (AstraZeneca, Pfizer, Esteve, UCB-Pharma, Sanofi-Aventis, Grünenthal, Eli Lilly und Boehringer Ingelheim) inkid contribution, was supported by intramural funding of the Ruhr University Bochum (FoRUM: grant number K046-10, Heinemann Award 2013) and has received a travel grant from Mundipharma GmbH and Bayer Vital GmbH, speaker fees from Grünenthal GmbH and Pfizer GmbH.
Christoph Maier has received honoraria for speaking and advisory board membership from Grünenthal, MSD, Köhler Chemie, Mundipharma, Pfizer, and Wyeth. Martin Tegenthoff has received speaker honoraria from Pfizer, Novartis and Mundipharma. He also acknowledges a grant support from Genzyme, not related to this manuscript. Tobias Schmidt-Wilcke received consulting fees from Daiichi Sankyo.
Min-Suk Yoon has received speaker honoraria from CSL Behring and Grifols, a scientific grant from CSL Behring, none related to this manuscript.

Supplemental material
Supplemental material for this article is available online.

ORCID iDs
Kalliopi Pitarokoili https://orcid.org/0000-0002-2483-4929
Anna Lena Fisse  https://orcid.org/0000-0003-0493-8656

References
1. Kerasnoudis A, Pitarokoili K, Behrendt V, et al. Cross-sectional area reference values for sonography of peripheral nerves and brachial plexus. Clin Neurophysiol 2013; 124: 1881–1888.
2. Pitarokoili K, Schlammann M, Kerasnoudis A, et al. Comparison of clinical, electrophysiological, sonographic and MRI features in CIDP. J Neurol Sci 2015; 357: 198–203.
3. Zaidman CM and Pestronk A. Nerve size in chronic inflammatory demyelinating neuropathy varies with disease activity and therapy response
over time: a retrospective ultrasound study. *Muscle Nerve* 2014; 50: 733–738.

4. Fisse AL, Pitarokoili K, Trampe N, Motte J, Kerasnoudis A, Gold R, Yoon MS. Clinical, Sonographic, and Electrophysiologic Longitudinal Features of Chronic Inflammatory Demyelinating Polyneuropathy. *J Neuroimaging*. 2019 Mar;29(2):223–232.

5. Grimm A, Rattay TW, Winter N, et al. Peripheral nerve ultrasound scoring systems: benchmarking and comparative analysis. *J Neurol* 2017; 264: 243–253.

6. Grimm A, Winter N, Rattay TW, et al. A look inside the nerve: morphology of nerve fascicles in healthy controls and patients with polyneuropathy. *Clin Neurophysiol* 2017; 128: 2521–2526.

7. Di Pasquale A, Morino S, Loreti S, et al. Peripheral nerve ultrasound changes in CIDP and correlations with nerve conduction velocity. *Neurology* 2015; 84: 803–809.

8. Dionne A, Nicolle MW and Hahn AF. Clinical and electrophysiological parameters distinguishing acute-onset chronic inflammatory demyelinating polyneuropathy from acute inflammatory demyelinating polyneuropathy. *Muscle Nerve* 2010; 41: 202–207.

9. Kerasnoudis A, Pitarokoili K, Behrendt V, et al. Nerve ultrasound score in distinguishing chronic from acute inflammatory demyelinating polyneuropathy. *Clin Neurophysiol* 2014; 125: 635–641.

10. Grimm A, Décard BF, Axer H, et al. The ultrasound pattern sum score - UPSS. A new method to differentiate acute and subacute neuropathies using ultrasound of the peripheral nerves. *Clin Neurophysiol* 2015; 126: 2216–2225.

11. Kerasnoudis A, Pitarokoili K, Haghiikia A, et al. Nerve ultrasound protocol in differentiating chronic immune-mediated neuropathies. *Muscle Nerve* 2016; 54: 864–871.

12. Goedee HS, van der Pol WL, van Asseldonk JH, et al. Diagnostic value of sonography in treatment-naïve chronic inflammatory neuropathies. *Neurology* 2017; 88: 143–151. DOI: 10.1212/WNL.0000000000003483.

13. Jiang MS, Yuan Y, Gu ZX, et al. Corneal confocal microscopy for assessment of diabetic peripheral neuropathy: a meta-analysis. *Br J Ophthalmol*. 2016; 100: 9–14. DOI: 10.1136/bjophthalmol-2014-306038.

14. Sturm D, Schmidt-Wilcke T, Greiner T, et al. Confocal cornea microscopy detects involvement of corneal nerve fibers in a patient with light-chain amyloid neuropathy caused by multiple myeloma: a case report. *Case Rep Neurol* 2016; 8: 134–139.

15. Kemp HI, Petropoulos IN, Rice ASC, et al. Use of corneal confocal microscopy to evaluate small nerve fibers in patients with human immunodeficiency virus. *JAMA Ophthalmol* 2017; 135: 795–800.

16. Culver DA, Dahan A, Bajorunas D, et al. Cibinetide improves corneal nerve fiber abundance in patients with sarcoidosis-associated small nerve fiber loss and neuropathic pain. *Invest Ophthalmol Vis Sci* 2017; 58: BIO52–BIO60.

17. Zhivov A, Stave J, Vollmar B, et al. In vivo confocal microscopic evaluation of Langerhans cell density and distribution in the normal human corneal epithelium. *Graefes Arch Clin Exp Ophthalmol* 2005; 243: 1056–1061.

18. Schneider C, Bucher F, Cursiefen C, et al. Corneal confocal microscopy detects small fiber damage in chronic inflammatory demyelinating polyneuropathy (CIDP). *J Peripher Nerv Syst* 2014; 19: 322–327.

19. Stettner M, Hinrichs L, Guthoff R, et al. Corneal confocal microscopy in chronic inflammatory demyelinating polyneuropathy. *Ann Clin Transl Neurol* 2015; 3: 88–100.

20. Van den Bergh PY, Hadden RD, Bouche P, et al.; European Federation of Neurological Societies; Peripheral Nerve Society. European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society - first revision. *Eur J Neurol* 2010; 17: 356–363. Erratum in: *Eur J Neurol* 2011; 18: 796.

21. Merkies IS, Schmitz PI, Van Der Meché FG, et al. Comparison between impairment and disability scales in immune-mediated polyneuropathies. *Muscle Nerve* 2003; 28: 93–100.

22. Kerasnoudis A, Pitarokoili K, Behrendt V, et al. Bochum ultrasound score versus clinical and electrophysiological parameters in distinguishing acute-onset chronic from acute inflammatory demyelinating polyneuropathy. *Muscle Nerve* 2015; 51: 846–852.

23. Kerasnoudis A, Pitarokoili K, Behrendt V, et al. Correlation of nerve ultrasound,
electrophysiological and clinical findings in chronic inflammatory demyelinating polyneuropathy. *J Neuroimaging* 2015; 25: 207–216.

24. Stöhr et al. Clinical Electromyography and Neurology, 6th Edition, Kohlhammer, p. 374.

25. Kerasnoudis A, Pitarokoili K, Gold R, et al. Bochum ultrasound score allows distinction of chronic inflammatory from multifocal acquired demyelinating polyneuropathies. *J Neurol Sci* 2015; 348: 211–215.

26. Kerasnoudis A, Pitarokoili K, Gold R, et al. Nerve ultrasound and electrophysiology for therapy monitoring in chronic inflammatory demyelinating polyneuropathy. *J Neuroimaging* 2015; 25: 931–939.

27. 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: 903–912, e44–e49.

28. Üçeyler N, Kafke W, Riediger N, et al. Elevated proinflammatory cytokine expression in affected skin in small fiber neuropathy. *Neurology* 2010; 74: 1806–1813.

29. Rolke R, Baron R, Maier C, et al. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. *Pain* 2006; 123: 231–243.

30. Magerl W, Krumova EK, Baron R, et al. Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. *Pain* 2010; 151: 598–605.

31. Härtig F, Ross M, Dammeier NM, et al. Nerve ultrasound predicts treatment response in chronic inflammatory demyelinating polyradiculoneuropathy—a prospective follow-up. *Neurotherapeutics* 2018; 15(2): 439–451.

32. Pitarokoili K, Kerasnoudis A, Behrendt V, et al. Facing the diagnostic challenge: nerve ultrasound in diabetic patients with neuropathic symptoms. *Muscle Nerve* 2016; 54: 18–24.

33. Leppin K, Behrendt AK, Reichard M, et al. Diabetes mellitus leads to accumulation of dendritic cells and nerve fiber damage of the subbasal nerve plexus in the cornea. *Invest Ophthalmol Vis Sci* 2014; 55: 3603–3615.

34. 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: 838–843.

35. Dehghani C, Pritchard N, Edwards K, et al. Morphometric stability of the corneal subbasal nerve plexus in healthy individuals: a 3-year longitudinal study using corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2014; 55: 3195–3199.

36. Dehghani C, Pritchard N, Edwards K, et al. Natural history of corneal nerve morphology in mild neuropathy associated with type 1 diabetes: development of a potential measure of diabetic peripheral neuropathy. *Invest Ophthalmol Vis Sci* 2014; 55: 7982–7990.

37. Chiang MC, Lin YH, Pan CL, et al. Cutaneous innervation in chronic inflammatory demyelinating polyneuropathy. *Neurology* 2002; 59: 1094–1098.

38. Wasner G, Schattschneider J, Binder A, et al. Topical menthol – a human model for cold pain by activation and sensitization of C nociceptors. *Brain* 2004; 127: 1159–1171.

39. Backonja MM, Attal N, Baron R, et al. Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. *Pain* 2013; 154: 1807–1819.

40. Ziegler D, Papanas N, Zhivov A, et al.; German Diabetes Study (GDS) Group. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. *Diabetes* 2014; 63: 2454–2463.

41. Maier C, Baron R, Tolle TR, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. *Pain* 2010; 150: 439–450.

42. Baron R, Maier C, Attal N, et al. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. *Pain* 2017; 158: 261–272.

43. Hughes RA, Donofrio P, Bril V, et al.; ICE Study Group. Intravenous immune globulin (10% caprylate-chromatography purified) for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (ICE study): a randomised placebo-controlled trial. *Lancet Neurol* 2008; 7: 136–144. Erratum in: *Lancet Neurol* 2008; 7: 771.

44. Dalakas MC; Medscape. Advances in the diagnosis, pathogenesis and treatment of CIDP. *Nat Rev Neurol* 2011; 7: 507.