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Structural and functional characterization of nerve fibres in polyneuropathy and healthy subjects

Páll Karlsson[a,d,*,1], Simon Haroutounian[a,b,1], Michael Polydefkis[c], Jens R. Nyengaard[d], Troels S. Jensen[a,e]

[a] Danish Pain Research Center, Aarhus University Hospital, Aarhus, Denmark
[b] Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO, USA
[c] Department of Neurology, The Johns Hopkins Hospital, Baltimore, MD, USA
[d] Stereology and Electron Microscopy Laboratory and Centre for Stochastic Geometry and Advanced Bioimaging, Aarhus University Hospital, Aarhus, Denmark
[e] Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

HIGHLIGHTS

- We present a rigorous comparison between functional and morphological parameters.
- There was a less relationship between nerve fibre structure and function in patients.
- Combining small fibre parameters may improve the diagnostic accuracy of DSP.

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ABSTRACT

Objectives: Quantification of intraepidermal nerve fibre density (IENFD) is an important small fibre measure in distal symmetric polyneuropathies (DSP), but quantitative evaluation of additional structural and functional factors may help in elucidating the underlying mechanisms, and in improving the diagnostic accuracy in DSP. The literature reports a weak or moderate relationship between IENFD and spontaneous and evoked pain in neuropathies, but the relationship between functional and structural small fibre parameters in patients with DSP is unclear. The objectives of the current study, therefore, were to determine morphological and functional parameters related to small nerve fibres in subjects with distal symmetric polyneuropathy (DSP) and healthy controls, and to characterize the interplay among these parameters in these two groups.
Materials and Methods: 17 patients with painful DSP (≥4 on 0–10 numerical rating scale) and with symptoms and signs of small fibre abnormality (with or without large fibre involvement) and 19 healthy control subjects underwent comprehensive functional and structural small fibre assessments that included quantitative sensory testing, response to 30 min topical application of 10% capsaicin and analysis of skin biopsy samples taken from the distal leg (IENFD, epidermal and dermal nerve fibre length densities (eNFLD, dNFLD) using global spatial sampling and axonal swelling ratios (swellings/IENFD and swellings/NFLD)).
Results: DSP patients had reduced sensitivity to cold (median −11.07 °C vs. −2.60, $P \leq 0.001$) and heat (median 46.7 vs. 37.70, $P \leq 0.001$), diminished neurovascular (median 184 vs. 278 mean flux on laser Doppler, $P = 0.0003$) and pain response to topical capsaicin (median 10 vs. 35 on 0–100 VAS, $P = 0.0002$), and lower IENFD, eNFLD and dNFLD values combined with increased swelling ratios (all $P < 0.001$) compared to healthy controls. The correlation between structural and functional parameters was poor in DSP patients, compared with healthy controls. In healthy controls eNFLD and dNFLD, IENFD and eNFLD, IENFD and dNFLD all correlated well with each other ($r = 0.81$; $P \leq 0.001$, $r = 0.58$; $P = 0.009$, $r = 0.60$; $P = 0.007$, respectively). In DSP, on the other hand, only eNFLD and dNFLD showed significant correlation ($r = 0.53$, $P = 0.03$). A diagnostic approach of combined IENFD and eNFLD utilization increased DSP diagnostic sensitivity from 82.0% to 100% and specificity from 84.0% to 89.5%.

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* Corresponding author at: Danish Pain Research Center, Aarhus University Hospital, Norrebrogade 44, Building 1A, 8000 Aarhus C, Denmark. Tel.: +45 78463380; fax: +45 78463269.
E-mail address: pkar@svf.au.dk (P. Karlsson).
[1] These authors contributed equally to this manuscript.

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Conclusions: This study presents a rigorous comparison between functional and morphological parameters, including parameters such as eNFDL and dNFLD that have not been previously evaluated in this context. The correlation pattern between functional and structural small fibre parameters is different in patients with DSP when compared to healthy controls. The findings suggest a more direct relationship between structure and function of nerve fibres in healthy controls compared to DSP. Furthermore, the findings suggest that combining IENFD with measurement of NFLD improves the diagnostic sensitivity and specificity of DSP.

Implications: Combining small fibre parameters may improve the diagnostic accuracy of DSP.

1. Introduction

Painful distal sensory polyneuropathies (DSP), with exclusive or preferential involvement of small nerve fibres of the A-delta and C types are very common in clinical practice [1].

The introduction of skin biopsies to examine small nerve fibre morphology together with functional measures such as quantitative sensory testing (QST) has led to an improvement in diagnosing patients with small fibre neuropathy [2–10]. A correlation between loss of epidermal nerve fibres and reduction in sensitivity to thermal stimuli has been documented for a variety of conditions such as diabetic neuropathy [5], HIV neuropathy [11], Fabry's disease [12] and other inherited and genetic conditions [13]. Several studies have indicated a weak or moderate relationship between intraepidermal nerve fibre density (IENFD) and spontaneous, as well as evoked pain in neuropathies [14–18], but the correlations between sensory thresholds and loss of skin innervation are not straightforward and are currently debated. It is especially unclear how this relationship between functional and structural small fibre parameters in patients with DSP compares to healthy subjects.

Previous studies have primarily relied on one morphological measure, the IENFD, to determine nerve fibre structural pathology. More recently, additional morphological parameters have been introduced and reported to be abnormal in polyneuropathies. These additional measures include small fibre axonal swellings and the epidermal and dermal nerve length density (eNFLD and dNFDL, respectively) [19–24]. It is currently unclear if the combination of two or more morphological or functional measures may yield higher sensitivity and/or specificity rates in diagnosing painful DSP with diseased small fibres.

Here we report the results of a study designed at determining whether patients with DSP and healthy controls have differential patterns of correlations between structural and functional nerve measurements. Additionally, we investigated if combinations of structural and functional measurements can improve the diagnostic accuracy of DSP with small fibre involvement.

2. Materials and methods

This study was approved by the regional ethics committee (No. 20090234), and all participants gave written consent to participate. We enrolled patients aged 18–80 years with a confirmed diagnosis of painful DSP with symptoms and signs of small fibre abnormality (with or without large fibre involvement) based on clinical characteristics and assessment by a trained neurologist [4], and "probable" or "definite" neuropathic pain [25,26] with intensity ≥4 on 0–10 numerical rating scale (NRS). The patients underwent a thorough clinical neurological examination and a routine lab screening including endocrine, renal, and hepatic functions, IgG, IgM, IgA and M-component, vitamin B12, and methylmalonate determination. Patients were excluded from the study if they had any previous application of capsaicin to or near the skin biopsy area (distal leg). Healthy subjects aged 18–80 with no prior history of chronic pain, neurological diseases, diabetes (or other metabolic disorders), alcohol- or substance misuse served as controls. Healthy subjects were recruited using flyers posted at Aarhus University and Aarhus University Hospital, and advertising on the social media.

2.1. Quantitative sensory testing (QST)

Quantitative sensory testing was performed on the dorsal foot to evaluate sensory nerve fibre function. Mechanical detection threshold (MDT), cold and warm detection thresholds (CDT and WDT, respectively) and cold and heat pain thresholds (CPT and HPT, respectively) were determined. Von Frey filaments graded from 0.25 mN to 512 mN applied force were used to assess tactile detection threshold to mechanical stimuli using method of limits [27]. Thermal threshold parameters, i.e. CDT, WDT, CPT, and HPT were determined using a Thermal Sensory Analyser device (Medoc, Israel) to evaluate thermal function of C and A-delta nerve fibres as previously described [28,29]. The thresholds were determined by the method of limits, by continuous ramping of temperature by 1 °C/s from a baseline temperature of 32 °C up until first detection of change to warm (WDT) or first sensation of painful heat (HPT); or ramping down until first detection of change to cold (CTD) or first sensation of painful cold (CPT), as described in detail elsewhere [30]. Cut-off temperatures were 0 °C for cold and 50 °C for heat to avoid thermal skin damage. An average threshold was calculated from three measurements in each area. Cold detection threshold was determined as change from baseline (CDT temperature [°C] – Baseline temperature [32 °C]). For each patient, we calculated the warm sensibility index (WSI), which represents the range in which non-noxious heat is perceived [31], calculated with the following formula: WSI = 1 – (WDT-BL)/(HPT-BL), where BL is the baseline temperature (32 °C in the current study).

2.2. Topical capsaicin response

Capsaicin, which causes a painful response and local vasodilation due to activation of TRPV1 (Transient Receptor Potential Vanilloid 1) channels on nociceptors [32], was used as an additional measure of small fibre function. Pain and local vasodilation induced by topical application of capsaicin were assessed by the following procedure at the same site where QST was performed. The skin in the painful area on the dorsal foot was heated to 34 °C using a feedback lamp [33,34], and baseline spontaneous pain intensity was assessed on 0–100 numerical rating scale (NRS) (0 = no pain, 100 = worst pain imaginable). On both dorsal feet, measurement of cutaneous blood flow was performed on with laser Doppler, followed by application of 100 μL of 10% capsaicin cream on a circular area of 2 cm in diameter for 30 min. Participants' ratings of spontaneous pain intensity on the 0–100 NRS were recorded at baseline (0 min), with 5-min intervals up to 30 min, and again 10 min after capsaicin had been removed (40 min). The skin temperature was kept constant at 34 °C during the 30-min period,