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REVIEW ARTICLE

Small fibre neuropathy: role in the diagnosis of diabetic sensorimotor polyneuropathy

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Summary

Small fibres constitute 70–90% of peripheral nerve fibres and regulate several key functions such as tissue blood flow, temperature and pain perception as well as sweating, all of which are highly relevant to the clinical presentation and adverse outcomes associated with foot ulcerations in patients with diabetes. Recent studies demonstrated significant abnormalities in the small fibres in subjects with impaired glucose tolerance and diabetes, despite normal electrophysiology, suggesting that the earliest nerve fibre damage is to the small fibres. Unfortunately, guidelines and consensus statements focus on large fibres and continue to advocate electrophysiology as a diagnostic modality and as a primary end point for the assessment of therapeutic benefit. (In part, this reflects the difficulties in quantifying small fibre dysfunction and damage.) We have therefore critically assessed currently available techniques that measure small fibre dysfunction in diabetic neuropathy, using quantitative sensory and sudomotor testing. We have assessed the role of identifying structural damage by quantifying intraepidermal nerve fibre density in skin biopsies and corneal nerve morphology using corneal confocal microscopy. Finally, we propose a definition for diabetic neuropathy that incorporates small fibre damage. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords small fibres; diabetic neuropathy; diagnosis; biopsy

Abbreviations: DSPN – diabetic sensori-motor polyneuropathy; IENF – intraepidermal nerve fibres; QSART – Quantitative Sudomotor Axon Reflex Testing; SFN – small fibre neuropathy; SSR – Sympathetic skin response.

Introduction and objectives

Recently it has been proposed that 'If nerve conduction is normal, a validated measure (with class 1 evidence) of small fibre neuropathy (SFN) may be used' to define and quantify the severity of diabetic sensori-motor polyneuropathy (DSPN) [1]. Nerve conduction assesses large myelinated nerve fibre function and has been used as an end point in clinical trials of human diabetic neuropathy, based on relative ease of quantification, reproducibility and reasonable sensitivity and specificity [2]. However, recent data have demonstrated minimal worsening [3] and improvements [4] in electrophysiology in placebo and epidemiological cohorts with little relation to other measures of small fibre and autonomic function in diabetic patients [5].

Small fibres constitute 79.6% [6] to 91.4% [7] of peripheral nerve fibres. Damage to this class of fibres underlies the symptoms of painful diabetic neuropathy which are typically distal, symmetrical and associated with nocturnal exacerbation. The descriptors used by patients to portray the symptoms can be variable but often include: prickling, aching, burning

pain with intermittent sharp stabbing electric shock-like pains and on examination one can elicit dysaesthesiae and allodynia. In addition to these troublesome symptoms, dysfunction and damage to this class of fibres are also key to the genesis of foot ulceration through the effect on sudomotor function [8], pressure-induced vasodilatation [9,10] and of course heat and pain perception [11]. Moreover, an increasing body of data shows that small fibre damage may precede large fibre damage in diabetic neuropathy [12–14].

Therefore it appears pertinent to address whether any definition of DSPN should include a measure of SFN. Issues that arise before we can adopt the assessment of SFN to diagnose DSPN include establishing not only the reproducibility, sensitivity, specificity and accuracy but also the practical viability of any proposed test. For the purposes of this review we will consider the available evidence for established and emerging measures of 'small fibre damage' to diagnose and stratify the severity of DSPN.

Nerve biopsy

Nerve biopsy has traditionally been used to quantify myelinated nerve fibre density which correlates with abnormalities in neurophysiology [15,16] and may also predict development of future neurophysiological deficits [17]. Unmyelinated nerve fibre damage precedes myelinated nerve fibre damage in sural nerve biopsies and therefore may be used to detect early DSPN [7]. However, nerve biopsy is an invasive and highly specialized procedure which requires electron-microscopy with considerable expertise for quantification, and therefore cannot be advocated for routine use to diagnose early DSPN [18].

Skin biopsy

Skin biopsy, a minimally invasive procedure, allows morphometric quantification of intraepidermal nerve fibres (IENF) most commonly expressed as the number of IENF per length of section (IENF/mm) [19,20] (Figure 1). Intra- and inter-observer variability for the assessment of IENF density demonstrates good agreement [20,21], declines with age and does not appear to be influenced by weight or by height [22]. An international consortium of investigators has recently compiled a normative data base for intra-epidermal nerve fibre density (IENFD) in 550 participants and has shown an effect of age, but no influence of height, weight or body mass index [23]. The blister technique is an alternative less invasive procedure which assesses innervation of the epidermis alone and shows good agreement with punch biopsy [24].

Diagnostic yield of IENF quantification

No study assessing the sensitivity and specificity of IENF in DSPN is available. However, several studies in SFN have included patients with DSPN. In 58 patients with pure SFN, a cut-off IENF density of $\leq$8.8/ mm at the ankle was associated with a sensitivity of 77.2% and a specificity of 79.6% [25]. Similarly, in 67 patients with pure SFN a sensitivity of 88% and a specificity of 88.8% have been reported [26]. In a study of 210 patients with SFN, which included 65 diabetic patients, the *Z*-scores and fifth percentile provided the highest specificity (98 and 95%, respectively) but a very low sensitivity (31 and 35%, respectively) compared with the receiver operating characteristic analysis (specificity 64%, sensitivity 78%) [27]. These findings suggest that the diagnostic yield of skin biopsy may depend on the reference and cut-off values selected and the definition of SFN adopted. IENF density correlates inversely with thermal thresholds. Whilst some have reported a closer correlation with warm and heat-pain thresholds [25,28–30] compared to cooling thresholds [31,32] others have reported the opposite, with a closer correlation with cold rather than heat detection thresholds [33,34]. A recent study has demonstrated no correlation between IENFD and the neuropathy symptom score, but interestingly an inverse correlation was demonstrated with the severity of pain assessed using the VAS-max [35]. The correlation between Quantitative Sensory Testing and IENF density therefore remains controversial.

The American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation have concluded however that skin biopsy may be considered for the diagnosis of DSPN, particularly SFN, with a level C recommendation [36]. More recently, under the auspices of the European Federation of the Neurological Societies and the Peripheral Nerve Society,

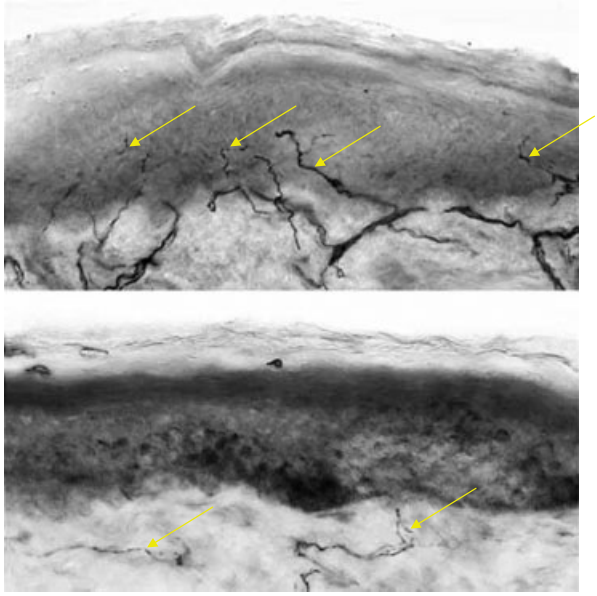

Figure 1. Skin biopsy with PGP 9.5 immunostaining for intraepidermal nerve fibres showing normal intraepidermal nerve fibres (→) in a control subject (top) and absence of intraepidermal nerve fibres with only dermal nerve fibres (→) in a diabetic patient with severe neuropathy (bottom)

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revised guidelines on the use of skin biopsy concluded that IENF density is a reliable and efficient technique to confirm the clinical diagnosis of SFN with level A recommendation [37].

Additional morphological features of IENFs include the branch density, length and mean dendritic length; all show an early reduction which progresses with neuropathic severity [13,38]. Several studies with serial skin biopsies in patients with SFN have shown that axonal swellings predict a decline in IENF density [39–41]. However, they occur not only in patients with SFN [42] but also in healthy individuals [43] and isolated swellings with normal IENF densities have been observed in a variety of other neuropathies [43–46].

Diabetic neuropathy

In patients with diabetic neuropathy, the prevalence of abnormal nerve conduction, Quantitative Sensory Testing and IENF was comparable [35]. However, IENF density was significantly reduced in patients with normal nerve conduction, suggesting early damage to small nerve fibres [12,14]. A recent study has shown comparable abnormalities in electrophysiology thermal thresholds and loss of IENF in diabetic patients with mild neuropathy [35]. There is an inverse correlation between IENF density and the severity of DSPN, defined by the Neurological Disability Score [13,29,47] and the Neuropathy Impairment Score [14]. Additionally, IENF density appears to be lower in diabetic patients with painful neuropathy compared with painless neuropathy [13,29,48]. A 1-year diet and exercise intervention program in patients with SFN and impaired glucose tolerance led to increased IENF density [49]. However, no change was observed in 18 diabetic patients after pancreas/kidney transplantation [50]. This may reflect the marked IENF loss at baseline [51], particularly in diabetic patients undergoing pancreas/kidney transplantation and the slower regeneration rate of IENF [52]. These data suggest that IENF loss is an early feature of diabetes, progresses with increasing neuropathic severity and may improve with appropriate intervention.

Sudomotor innervation

Recently, a novel stereologic technique has been applied in skin biopsies and showed a correlation between sweat gland nerve fibre density, neuropathic symptoms, neurological deficits and sweat production [53]. However, morphometric data in patients with diabetic SFN are limited and further studies are warranted.

Quantitative Sensory Testing

Thermal thresholds

Abnormalities in heat-pain thresholds reflect small fibre dysfunction and a number of instruments including CASE IV, thermoaesthesiometer and Medoc have been used to quantify this parameter. In 498 type 2 diabetic patients and 434 control subjects an elevated warm threshold was the most frequent abnormality (60.2%) compared with an abnormal cold threshold (39.6%) and abnormal sural nerve conduction velocity (12.9%), and it was related to both symptoms and glycaemic control [54]. However, a careful study of 59 diabetic patients showed that unlike cold perception thresholds and IENFD, warm perception thresholds did not differentiate diabetic patients with and without symptoms [14]. Similarly, in a study of 191 diabetic patients there was no difference in heat-pain thresholds between those with and without painful neuropathy [33].

Pain-related evoked potentials

In a study of 57 diabetic patients with entirely normal electrophysiology, the latency was increased and amplitude was reduced for pain-related evoked potentials, elicited by nociceptive electrical stimulation of the skin [55].

Nerve axon reflex/flare response

Stimulation of the nociceptive C fibre results in both orthodromic conduction to the spinal cord and antidromic conduction to other axon branches, i.e. the axon reflex (Figure 2) which can stimulate the release of peptides, such as substance P and calcitonin gene-related peptide, resulting in vasodilation and increased permeability. Studies have shown that this neurovascular response mediated by the nerve axon reflex is reduced in diabetic neuropathic patients, correlates with other nerve function measurements and has reasonable sensitivity and specificity in identifying patients with diabetic neuropathy [56,57]. The LDIflare test evaluates 44 °C heat-induced vasodilation [58] and is reduced in subjects with impaired glucose tolerance [59], and in type 2 diabetic patients with and

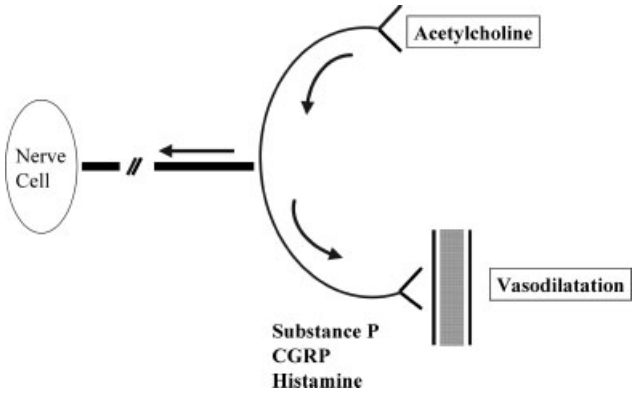

Figure 2. Nerve axon reflex: Stimulation of the C nociceptive nerve fibres leads to antidromic stimulation of the adjacent C fibres, which secrete various vasomodulators such as substance P, calcitonin gene-related peptide and histamine that cause vasodilatation and increased blood flow

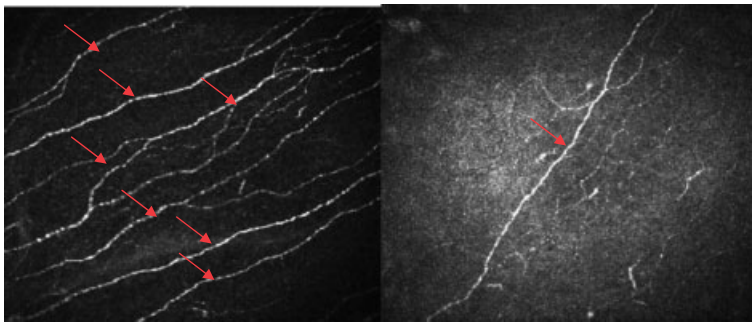

Figure 3. Corneal confocal microscopy image of a control subject (right panel) with normal corneal nerve (→) density compared to an image from a diabetic patient with severe neuropathy and marked loss of corneal nerve fibres (left panel)

without neuropathy [60,61] but interestingly is normal in patients with type 1 diabetes of long duration [59].

More longitudinal data and perhaps assessment after interventions when compared with established tests are necessary before these techniques can be recommended for clinical use.

Sudomotor dysfunction

Sympathetic skin response

Sympathetic skin response (SSR) assesses sudomotor and hence small fibre dysfunction. In an early study it failed to differentiate the presence or absence of neuropathy in a series of 337 diabetic patients [62]. However, it has recently been shown to predict the risk of foot ulceration comparable with abnormalities in neuropathy deficit score (NDS) and elevated vibration perception [63]. It has also been shown to have a sensitivity of 87.5% and a specificity of 88.2% for detecting diabetic autonomic neuropathy [64].

Quantitative Sudomotor Axon Reflex Testing

Quantitative Sudomotor Axon Reflex Testing (QSART) evaluates sudomotor function by assessing the local sweat response to iontophoresis of acetylcholine [65] and has been shown to be highly sensitive in the detection of distal SFN [66]. QSART evaluates postganglionic axon function as opposed to the polysynaptic pathways assessed using SSR. In a series of 31 diabetic patients with early neuropathy it appeared to be better at detecting early neuropathy than SSR [67].

Neuropad

The neuropad test is a simple visual indicator test which uses a colour change to define the integrity of skin sympathetic cholinergic innervation. Neuropad responses have been shown to correlate with modified NDS, Quantitative Sensory Testing, cardiac autonomic neuropathy and IENF loss with relatively high sensitivity but lower specificity for detecting DSPN [68,69]. A recent study has shown that an abnormal result of Neuropad test in those with a normal NDS may predict the development of diabetic neuropathy after 5 years [70]. This appears to reflect early small fibre involvement which is missed using NDS as a measure of neuropathy.

Corneal confocal microscopy

Corneal confocal microscopy is a non-invasive ophthalmic technique that has been shown to detect small sensory corneal nerve fibre loss in diabetic neuropathy (Figure 3) [71], idiopathic SFN [72] and Fabry disease [73]. Corneal nerve fibre damage correlates with IENF loss and severity of neuropathy in diabetic patients [13,74] and is more marked in patients with painful diabetic neuropathy [13]. Corneal nerve fibre density also improves 6 months after combined pancreas/kidney transplantation [75]. It has been shown to have high reproducibility [76], sensitivity and specificity [77]. To enhance the practical application of this technique an automated image analysis system has also been developed recently [78].

Definition of SFN

In diabetic patients, we propose to grade SFN as follows: (1) Possible: presence of distal symmetrical symptoms and/or clinical signs of small fibre damage; (2) Probable: presence of distal symmetrical symptoms, clinical signs of small fibre damage, and normal or abnormal sural nerve conduction study; (3) Definite: presence of length-dependent symptoms, clinical signs of small fibre damage, normal or abnormal sural nerve conduction study and/or abnormal Quantitative Sensory Testing thermal thresholds at the foot and reduced IENF density at the ankle.

At present it is not possible to suggest criteria to define the severity of SFN in diabetic polyneuropathy. However, as normative ranges are established for the different tests of small fibre dysfunction and damage, it may be possible to devise a measure of severity using different percentiles or quartiles as cut-offs.

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Conflict of interest

None declared.

Appendix

The Toronto Consensus Panel on Diabetic Neuropathy

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