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Research: Complications

The diagnostic accuracy of Neuropad® for assessing large and small fibre diabetic neuropathy

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Abstract

Aims Neuropad® is a simple visual indicator test, with moderate diagnostic performance for diabetic peripheral neuropathy. As it assesses sweating, which is a measure of cholinergic small nerve fibre function, we compared its diagnostic performance against established measures of both large and, more specifically, small fibre damage in patients with diabetes.

Methods One hundred and twenty-seven participants (89 without diabetic peripheral neuropathy and 38 with) aged 57 ± 9.7 years underwent assessment with Neuropad, large nerve fibre assessments: Neuropathy Disability Score, vibration perception threshold, peroneal motor nerve conduction velocity; small nerve fibre assessments: neuropathy symptoms (Diabetic Neuropathy Symptoms score) corneal nerve fibre length and warm perception threshold.

Results Neuropad has a high sensitivity but moderate specificity against large fibre neuropathy assessments: Neuropathy Disability Score (> 2) 70% and 50%, vibration perception threshold (> 14 V) 83% and 53%, and peroneal motor nerve conduction velocity (< 42 m/s) 81% and 54%, respectively. However, the diagnostic accuracy of Neuropad was significantly improved against corneal nerve fibre length (< 14 mm/mm^2) with a sensitivity and specificity of 83% and 80%, respectively. Furthermore, the area under the curve for corneal nerve fibre length (85%) was significantly greater than with the Neuropathy Disability Score (66%, $P = 0.01$) and peroneal motor nerve conduction velocity (70%, $P = 0.03$). For neuropathic symptoms, sensitivity was 78% and specificity was 60%.

Conclusions The data show the improved diagnostic performance of Neuropad against corneal nerve fibre length. This study underlines the importance of Neuropad as a practical diagnostic test for small fibre neuropathy in patients with diabetes.

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Introduction

Diabetic peripheral neuropathy is a major risk factor for foot ulceration and amputation [1]. Practical clinical tests such as the 10-g monofilament or Neuropathy Disability Score (NDS) have been validated as first-line screening tools for identifying people with established diabetic peripheral neuropathy and those at high risk of foot ulceration [2]. Whilst small nerve fibre damage occurs early [3,4], underlies painful neuropathy and plays a significant role in the aetiopathogenesis of foot ulceration because of loss of pain sensation, anhidrosis and deranged tissue blood flow [4], there is currently no first-line screening tool to evaluate this abnormaity.

Sudomotor dysfunction is a component of small fibre damage, which develops before sensory loss [5] and has been accepted as one of the diagnostic tests for diabetic neuropathy by the Toronto Consensus Panel [3]. Tests evaluating sudomotor function include the sympathetic skin response [5] and the quantitative sudomotor axon reflex tests (QSART) [4]. However, these tests are time-consuming and cannot be easily deployed in the clinic. The Neuropad® measures sweat production based on the colour change of a cobalt II compound from blue to pink and represents a practical clinical test for sudomotor dysfunction in diabetic peripheral neuropathy [6–11].

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What's new?

- This is the first study to compare the diagnostic performance of Neuropad® against established measures of both large and small fibre neuropathy.
- The data show the close and improved relationship between the Neuropad response and structural small fibre damage (corneal nerve fibre length).
- The findings of this study underline the importance of Neuropad as a practical diagnostic test for small nerve fibre damage with high diagnostic accuracy.

Several studies have examined the diagnostic validity of the Neuropad for diagnosing peripheral neuropathy [9–13] and have shown a high sensitivity (85–97.8%), but poor specificity (45–67.2%) [8,14]. Given that the specificity of a test denotes whether an individual is disease free, and given that diabetic peripheral neuropathy affects small fibres before large fibres, it is not surprising that previous studies have shown a low specificity for Neuropad, as they have used large fibre tests to diagnose neuropathy [8,12,14,15]. Indeed, we have previously shown that the diagnostic accuracy of Neuropad correlated better with heart rate variability, a component of small fibre damage ($r_s = -0.525$, $P < 0.001$) compared with a Neuropathy Disability Score > 5 ($r_s = -0.450$, $P < 0.001$) [9].

In the present study, we have evaluated the diagnostic accuracy of Neuropad in a large cohort of people with diabetes and specifically compared the sensitivity and specificity for the diagnosis of peripheral neuropathy using different measures of small and large fibre neuropathy.

Research design and methods

The participants in the study were recruited from the Manchester Diabetes Centre, Manchester Royal Infirmary in Manchester, UK. The study was performed at the Wellcome Trust Clinical Research Facility from 3 January 2011 until 30 March 2012, involving 127 people with diabetes mellitus (68 with Type 1 diabetes and 59 with Type 2 diabetes) with an average age of 57 ± 10 years. We determined by means of an unpaired *t*-test that the minimum sample required was 46 participants with a power of 95%. Exclusion criteria included history of neuropathy as a result of a non-diabetic cause and corneal trauma or surgery. This study was approved by the Local Research Ethics committee and all patients gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

Demographic measures

All study participants underwent assessment of their HbA_{1c}, BMI, systolic and diastolic blood pressure, cholesterol and triglycerides.

Neuropathy assessments

All patients underwent a detailed assessment of neuropathy based on a standard protocol including: a Neuropathy Disability Score (NDS) to classify participants into normal (NDS 0–2) and abnormal (NDS 3–10) [16,17]. Quantitative sensory testing included an assessment of vibration perception threshold, measured using a Neurothesiometer (Horwell Scientific Laboratory Supplies, Nottingham, UK), and warm perception thresholds (C fibres) using the method of limits with the Medoc TSA II (Medoc Ltd, Ramat Yishai, Israel) on the dorsum of the left foot. The Diabetic Neuropathy Symptom (DNS) score was employed to assess symptoms of neuropathy [18] and to classify participants into normal (0–1) and abnormal (2–4). Electro-diagnostic studies were undertaken using a Dantec 'Keypoint' system (Dantec Dynamics Ltd, Bristol, UK) equipped with a DISA temperature regulator to keep limb temperature constantly between 32 and 35 °C. Sural nerve conduction velocity, sural nerve amplitude, peroneal motor nerve conduction velocity and peroneal motor nerve amplitude were assessed in the right lower limb by a consultant neurophysiologist. The motor study was performed using silver–silver chloride surface electrodes at standardized sites defined by anatomical landmarks, and recordings for the sural nerve were taken using anti-dromic stimulation over a distance of 140 mm.

Assessment of sudomotor function

The Neuropad (miro Verbandstoffe, Wiehl-Drabenderhöhe, Germany) was applied to the plantar aspect of the first metatarsal head after callus removal and removed after 10 min. Immediately after removal, the percentage colour change of the Neuropad in pink was blindly estimated by three independent clinicians. An established series of scanned Neuropads ($n = 50$) ranging from 0% to patchy to 100% pink were estimated by the clinicians on two occasions to establish consistency in colour interpretation.

Corneal confocal microscopy

Patients underwent examination with the Heidelberg Retina Tomograph (HRT III RCM) *in vivo* corneal confocal microscope (IVCCM) (Heidelberg Engineering GmbH, Heidelberg, Germany) [19]. The subject's eyes were anaesthetized using a drop of 0.4% Benoxinate hydrochloride and Viscotears were applied on the front of the eye for lubrication. A drop of viscoelastic gel was placed on the tip of the objective lens and a sterile disposable TomoCap (Heidelberg Engineering GmbH, Dossenheim, Germany) was placed over the lens, allowing optical coupling of the objective lens to the cornea. The patient was instructed to fixate on a target with the eye not being examined. Several scans of the entire depth of the cornea were recorded by turning the fine focus of the objective lens backwards and forwards for approximately 2 min using the

section mode which enables manual acquisition and storage of single images from the central cornea, focusing on the sub-basal layer. This provides face-to-face two-dimensional images with a lateral resolution of approximately 2 μm/pixel and final image size of 400 × 400 pixels of the sub-basal nerve plexus of the cornea. Corneal nerve fibre length—the total length of all nerve fibres and branches (mm/mm^2)—and corneal nerve fibre density—the total number of major nerves (n/mm^2) within the area of cornea captured by the image—was quantified from approximately five adjacent images/subject, using purpose-built manual image analysis software [20].

Study definition of diabetic peripheral neuropathy

The Toronto Diabetic Neuropathy Expert group recommendation was followed to define diabetic peripheral neuropathy: (1) abnormal nerve conduction studies (NCS) [peroneal motor nerve conduction velocity (< 42 m/s)] and (2) abnormal symptoms or signs of neuropathy [NDS (> 2)] [3].

To define an abnormal result for the Neuropathy Disability Score (NDS), vibration perception threshold (VPT), sensory nerve action potential (SNAP), sensory nerve conduction velocity (SNCV), peroneal motor nerve action potential (PMNAP), peroneal motor nerve conduction velocity (PMNCV), warm perception threshold (WPT), corneal nerve fibre density (CNFD) and corneal nerve fibre length (CNFL), we have used a mean ± 2 SD cut-off based on our control population (n = 104).

Statistical analysis

Statistical analysis was performed using StatsDirect statistical software, version 2.7.9 (StatsDirect Ltd, Altrincham, UK). We examined the distribution of the data by means of relevant histograms and statistical test (Shapiro–Wilk test). All data were expressed as median (5th percentile, 95th percentile). The Mann–Whitney U-test was performed to analyse differences between the medians. A P-value < 0.05 was considered statistically significant.

Receiver operating characteristic curve analysis was used to compare the diagnostic accuracy of Neuropad against measures of large and small nerve fibre damage and neuropathy symptoms. Receiver operating characteristic curve analysis established the area under the curve to determine the optimal sensitivity and specificity of the Neuropad test. Statistical difference between two receiver operating characteristic curves were expressed in P-values, as described by Hanley and McNeil [21].

Results

Clinical data and reproducibility of Neuropad estimation

In 127 people with diabetes aged 57.0 ± 9.7 years, 38 were diagnosed with peripheral neuropathy. The demographic and clinical characteristics of the participants with (n = 38) and without (n = 89) peripheral neuropathy are presented in Table 1. HbA_{1c}, BMI, cholesterol and triglyceride levels did not differ between the two groups, but age (P = 0.01), duration of diabetes (P = 0.01) and blood pressure (systolic: P = 0.0004; diastolic: P = 0.05) were significantly greater in those with peripheral neuropathy. There were significant differences for the large fibre tests, comparing the group without diabetic peripheral neuropathy to the group with: Neuropathy Disability Score (NDS) (P < 0.0001), vibration perception threshold (VPT) (P < 0.0001), sural nerve action potential (SNAP) (P < 0.0001), sural nerve conduction velocity (SNCV) (P = 0.0004), peroneal motor nerve action potential (PMNAP) (P < 0.0001) and peroneal motor nerve conduction velocity (PMNCV) (P < 0.0001). Similarly, there were significant differences for the small fibre tests: warm perception threshold (WPT) (P < 0.0001), corneal nerve fibre density (CNFD) (P = 0.0002), corneal nerve fibre length (CNFL) (P < 0.0001) and Neuropad (P < 0.0001). Neuropathic symptoms were also significantly different between the groups. Neuropad response was significantly lower in the group with diabetic peripheral neuropathy (50%, 0–100) compared with the group without (90%, 8–100). The evaluation of Neuropad's percentage colour change was subjective, but the reproducibility was good. The coefficient of repeatability for intra- and inter-observer variability was 0.3 and 0.4, respectively.

Neuropad diagnostic performance for diabetic peripheral neuropathy

The diagnostic performance of Neuropad evaluated against measures for large and small fibre neuropathy and neuropathic symptoms is shown in Table 2. The sensitivity and specificity of Neuropad for detecting large nerve fibre damage based on the Neuropathy Disability Score (NDS) was 70% and 50%, for the vibration perception threshold (VPT) 83% and 53%, for sural nerve action potential (SNAP) 70% and 64%, for sural nerve conduction velocity (SNCV) 64% and 54%, for peroneal motor nerve action potential (PMNAP) 82% and 50% and for peroneal motor nerve conduction velocity (PMNCV) 81% and 54%, respectively. For small nerve fibre assessment using the warm perception threshold as a reference method, the sensitivity and specificity were 68% and 49%, respectively. However, they were significantly improved against corneal nerve fibre length, with a sensitivity of 83% and specificity of 80%. Whilst the sensitivity of Neuropad in detecting diabetic peripheral neuropathy was high for measures of both large and small fibre damage, it showed high specificity only for small fibre neuropathy identified using corneal nerve fibre length as a reference method (Fig. 1). The area under the curve for corneal nerve fibre length (85%) was significantly larger than for the Neuropathy Disability Score (NDS) (66%, P = 0.01), sural nerve conduction velocity (SNCV) (63%,

Table 1 Comparison of clinical data of people with Type 1 and Type 2 diabetes according to presence or absence of neuropathy defined by the Toronto criteria

Variables	Diabetes without neuropathy*	Diabetes with neuropathy*	*P*-value†
Demographic measures			
n	89	38	
Age (years)	56 (42–73)	61 (29–73)	0.01
Diabetes duration (years)	19 (1–46)	36 (2–54)	0.01
Gender (male/female)	65/24	25/13	
Type of diabetes (Type 1/Type 2)	44/45	24/14	
HbA_{1c} (mmol/mol) (%)	61 (40–84)	62 (48–110)	0.15
	7.7 (5.8–9.8)	7.8 (6.5–12)	
BMI (kg/m^2)	27.5 (21–38)	27.3 (21–42)	0.73
Systolic blood pressure (mmHg)	129 (106–168)	150 (114–179)	0.0004
Diastolic blood pressure (mmHg)	71 (58–85)	75 (64–96)	0.05
Cholesterol (mmol/l)	3.9 (2.9–6)	4.2 (2.8–6.2)	0.35
Triglyceride (mmol/l)	1 (1–3)	1.4 (0.5–4.1)	0.5
Large fibre assessments			
Neuropathy Disability Score	2 (0–6)	6 (3–10)	<0.0001
Vibration perception threshold (volts)	9 (4–24)	22.3 (10–48)	<0.0001
Sural nerve action potential (μV)	10 (3–23)	5.2 (1–13)	<0.0001
Sural nerve conduction velocity (m/s)	45.2 (39–54)	41.2 (29–52)	0.0004
Peroneal motor nerve action potential (mV)	3.7 (1–8)	1.35 (0.2–5)	<0.0001
Peroneal motor nerve conduction velocity (m/s)	44.2 (38–50)	37.5 (22–41)	<0.0001
Small fibre assessments			
Warm perception threshold (°C)	39.6 (36–46)	43.4 (38–50)	<0.0001
Corneal nerve fibre density (*n*/mm^2)	28 (14–46)	20.94 (4.2–38)	0.0002
Corneal nerve fibre length (mm/mm^2)	25 (10–32)	13.8 (3.6–29.8)	<0.0001
Neuropad (%)	90 (8–100)	50 (0–100)	<0.0001
Neuropathy symptoms			
Diabetic Neuropathy Symptoms (DNS) score	0 (0–1)	2 (1–3)	<0.0001

*Data are medians (5th percentile, 95th percentile).
†*P*-values are derived from a Mann–Whitney *U*-test.

Table 2 The diagnostic performance of the Neuropad test evaluated against large (Neuropathy Disability Score, vibration perception threshold, sensory nerve action potential, sensory nerve conduction velocity, peroneal motor nerve action potential and peroneal motor nerve conduction velocity) and small (warm perception threshold, corneal nerve fibre density and corneal nerve fibre length) nerve fibre assessments as reference methods using receiver operating characteristic curve analysis

Variables	Area under the curve (%)	Sensitivity % (ratio)	Specificity % (ratio)	Positive and negative predictive value (%)	Positive and negative likelihood ratio
Large fibre assessments					
Neuropathy Disability Score (>2)	66	70 (49/70)	50 (28/57)	63, 57	1.4, 0.6
Vibration perception threshold (>14 volts)	73	83 (45/54)	53 (39/73)	45, 39	1.77, 0.32
Sural nerve action potential (<3 μV)	70	70 (14/20)	64 (68/107)	26, 92	1.94, 0.47
Sural nerve conduction velocity (<43 m/s)	63	64 (30/47)	54 (43/80)	45, 72	1.39, 0.67
Peroneal motor nerve action potential (<2 mV)	69	82 (22/27)	50 (50/100)	31, 91	1.64, 0.36
Peroneal motor nerve conduction velocity (<42 m/s)	70	81 (46/57)	54 (38/70)	59, 78	1.76, 0.35
Small fibre assessments					
Warm perception threshold (>43°C)	60	68 (26/38)	49 (44/89)	26, 44	1.33, 0.65
Corneal nerve fibre density (<24 *n*/mm^2)	67	74 (37/50)	60 (46/77)	54, 78	1.85, 0.43
Corneal nerve fibre length (<14 mm/mm^2)	85	83 (20/24)	80 (82/103)	49, 95	4.15, 0.21
Neuropathy symptoms					
DNS score	68	78 (21/27)	60 (60/100)	34, 91	1.95, 0.37

$P = 0.006$), peroneal motor nerve action potential (PMNAP) (69%, $P = 0.04$), peroneal motor nerve conduction velocity (PMNCV) (70%, $P = 0.03$) and was borderline significant against vibration perception threshold (73%, $P = 0.06$) and sensory nerve action potential (SNAP) (70%, $P = 0.07$). However, this high sensitivity and specificity could not be replicated against corneal nerve fibre density, which showed good sensitivity (74%) but relatively low specificity (60%). With neuropathic symptoms (DNS), the sensitivity was 78% and specificity 60%.

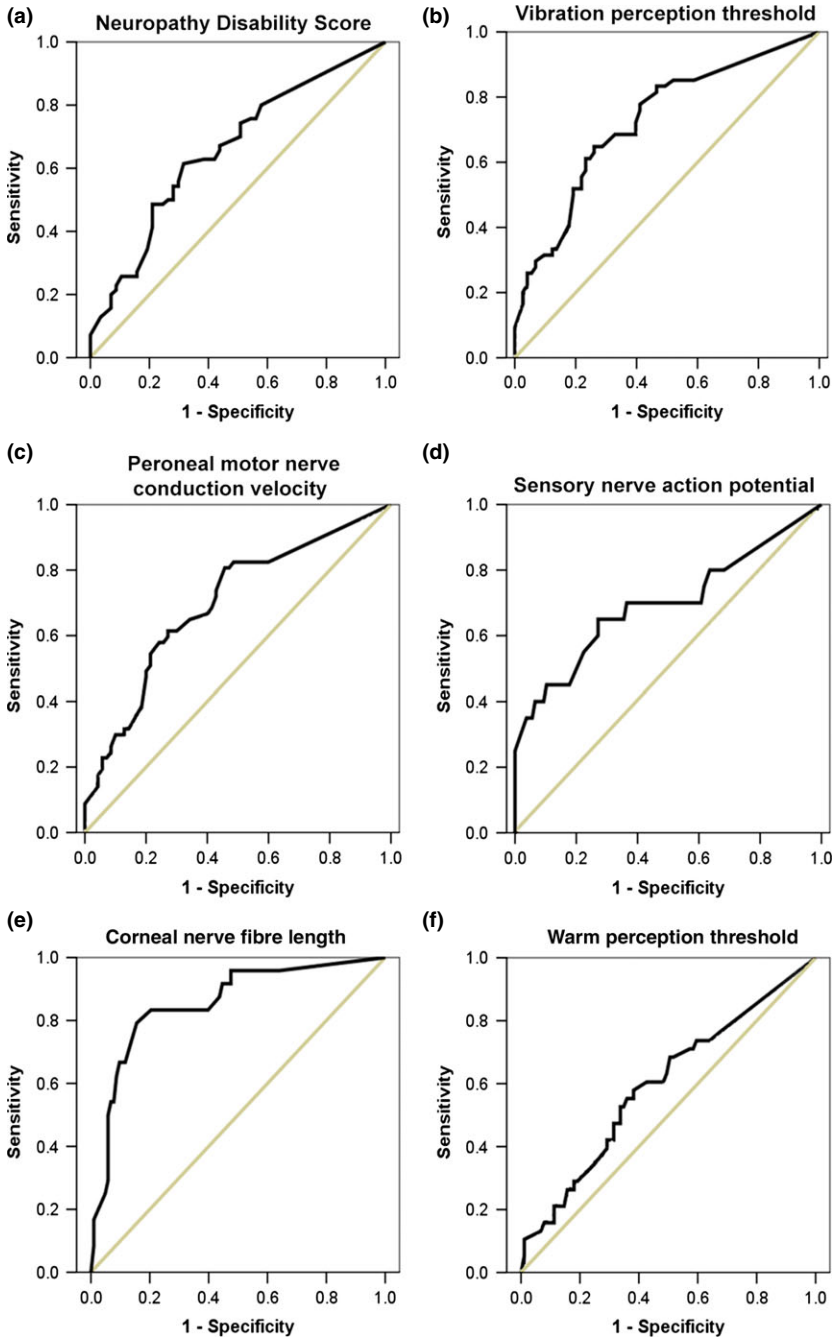

FIGURE 1 Receiver operating characteristic curve analysis was used to compare the diagnostic accuracy of Neuropad in detecting large with small nerve fibre damage (black line). The grey line is the null value of the receiver operating characteristic curve. The area under the curve for large nerve fibre damage was (a) 66% for Neuropathy Disability Score (95% CI 0.51–0.8), (b) 73% for vibration perception threshold (95% CI 0.54–0.91), (c) 70% for peroneal motor nerve conduction velocity (95% CI 0.53–0.87) and (d) 70% for sensory nerve action potential (95% CI 0.45–0.94). The area under the curve for small nerve fibre damage was and (e) 85% for corneal nerve fibre length (95% CI 0.5–1). (f) 60% for warm perception threshold (95% CI 0.46–0.73)

Discussion

The Neuropad provides a visual diagnostic screening test for sudomotor and hence small fibre dysfunction in diabetic peripheral neuropathy, therefore it is not surprising that it has been shown to have good sensitivity but modest specificity in detecting peripheral neuropathy evaluated using measures of large fibre neuropathy [8,9,12]. Hence, we have compared the diagnostic ability of Neuropad against established measures of large fibre neuropathy, but also

specifically against small fibre dysfunction (warm perception threshold) and damage (corneal nerve fibre density and corneal nerve fibre length) [19,22].

The findings in the literature are consistent on Neuropad, where approximately one third of people with diabetes with normal clinical examination have an abnormal Neuropad response because of the low specificity. Papanas *et al.* [12] examined 154 patients with Type 2 diabetes and reported that the sensitivity and specificity of Neuropad for the Neuropathy Disability Score (NDS) (> 5) were 97.8% and 67.2%, respectively. Quattrini *et al.* [9] examined 57 people with diabetes and reported that the sensitivity and specificity of Neuropad for the Neuropathy Disability Score (NDS) (≥ 5) were 85% and 45%, respectively. Similarly, Papanas *et al.* reported that the sensitivity and specificity of Neuropad for a vibration perception threshold > 25 volts were 98.8% and 59.5%, respectively [8].

Small fibre neuropathy is an early manifestation of diabetic peripheral neuropathy [4]. Using large fibre tests such as the Neuropathy Disability Score (NDS), vibration perception threshold and nerve conduction studies to define 'neuropathy' will clearly miss patients with early neuropathy, providing an explanation for the low specificity of the Neuropad in previous studies. In the present study, we have demonstrated good sensitivity (83%) but a markedly increased specificity (80%) when using corneal nerve fibre length (a marker of small fibre damage) as a reference method to define peripheral neuropathy. Thus, whilst the sensitivity for corneal nerve fibre length (83%) was comparable with that for the Neuropathy Disability Score (NDS) (70%), vibration perception threshold (83%), peroneal motor nerve action potential (82%) and peroneal motor nerve conduction velocity (81%), the specificity for corneal nerve fibre length (CNFL) (80%) was higher than that observed for the Neuropathy Disability Score (NDS) (50%), vibration perception threshold (53%), sensory nerve conduction velocity (50%) and peroneal motor nerve conduction velocity (54%). However, the specificity of corneal nerve fibre density was lower, indicating that corneal nerve fibre density may not be as robust as corneal nerve fibre length. Furthermore, a prospective study, which examined the predictive validity of Neuropad, suggested that early small fibre dysfunction pre-dates the development of large fibre dysfunction [14]. In patients with Type 2 diabetes who initially had an abnormal Neuropad response, 5 years later 25.64% developed significant neuropathy (NDS > 6) compared with only 2.86% of those with a normal Neuropad response.

The diagnostic performance of the Neuropad is better with structural small fibre damage (corneal nerve fibre length), as opposed to small fibre dysfunction (warm perception threshold). Recently, corneal nerve fibre length has been shown to correlate highly significantly with three independent measures of small fibre neuropathy, including cold thresholds, heart rate variability and the laser Doppler imaging flare technique (LDIflare) [23]. Sweat glands are innervated by the sympathetic nervous system, primarily by cholinergic fibres, but also by adrenergic fibres [24]. Warm perception threshold is mediated by the small myelinated (Aδ) fibres and unmyelinated C-fibres [25]. It is conceivable that the severity of small fibre neuropathy may vary between the different types of small fibres. Of relevance, the Neuropad has been compared with intra-epidermal nerve fibre density (IENFD), which is widely accepted as a gold standard measure of small C-fibre damage, and patients with diabetes with a normal Neuropad result already had a reduction in intra-epidermal nerve fibre density (7.37 ± 0.93 fibres/mm) compared with control subjects (11.06 ± 0.82 fibres/mm), which was further reduced in patients with a patchy (5.01 ± 0.93 fibres/mm) and absent response (5.02 ± 0.77 fibres/mm) [9].

In the current study, we have also evaluated the relationship of Neuropad with neuropathic symptoms using the four-item Diabetic Neuropathy Symptoms (DNS) score and show a high sensitivity (78%) but relatively low specificity (60%). Although the Diabetic Neuropathy Symptoms (DNS) score may have moderate reliability because of its subjectivity, the low specificity may be attributable to the fact that small fibre dysfunction pre-dates the development of neuropathic symptoms.

This study has several strengths and limitations. The strengths of this study are the inclusion of a wide range of gold standard clinical techniques to assess large and small nerve fibre dysfunction and damage on a larger number of participants with diabetes than previously reported work evaluating the diagnostic performance of the Neuropad. The cut-off points for the diagnosis of diabetic peripheral neuropathy by corneal nerve fibre length (< 14 mm/mm^2) and peroneal motor nerve conduction velocity (< 42 m/s) are comparable with previous studies of Petropoulos *et al.* [26], Ahmed *et al.* [27] and Malik *et al.* [28]. In previous studies, Neuropad colour change was estimated in a categorical manner as normal or abnormal after 10 min [10]. We have assessed the diagnostic ability of Neuropad using a continuous output as a percentage. The cut-off value with the highest accuracy was defined for each reference method. Even though the continuous output is subjective and more liable to error, we have shown good reproducibility for three independent graders. Nevertheless, this argues for the development of software or an image analysis system for rapidly and consistently grading the colour change to a percentage output, enabling a continuous and more accurate assessment of sudomotor, and hence small fibre, dysfunction.

In conclusion, by employing a continuous output for Neuropad, this study has shown that the specificity of Neuropad is significantly improved when its diagnostic ability is assessed against corneal nerve fibre length, a sensitive measure of small fibre damage. The findings in this study also suggest that the Neuropad test is particularly suitable for the diagnosis of small fibre diabetic peripheral neuropathy. Furthermore, its assessment using a less

subjective, continuous output, perhaps via image analysis, merits investigation in a longitudinal study of diabetic peripheral neuropathy.

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Competing interests

None declared.

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