

Nervecheck for the detection of early sensory loss and neuropathic pain in diabetes

Running head: NerveCheck for diabetic neuropathy

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Abstract

Aim: Detection of early sensory loss in patients with diabetes may allow identification of patients with symptoms as having painful diabetic neuropathy and also allow timely identification and treatment of patients to prevent foot ulceration. Widely deployed methods such as the monofilament only detect moderate to severe neuropathy. This study was undertaken using NerveCheck, a portable quantitative sensory testing (QST) device to determine the prevalence of early sensory loss in relation to neuropathic symptoms and to determine its diagnostic performance in relation to objective end points for small and large fibre damage.

Methods: 144 subjects (74 with and 70 without diabetes) underwent QST; nerve conduction study (NCS); corneal nerve fibre density (CNFD) and intra epidermal nerve fibre density (IENFD) assessment. Diabetic peripheral neuropathy (DPN) was defined using the neuropathy disability score (NDS).

Results: Of the 74 subjects with diabetes 41 had DPN. The incidence of sensory deficits was high in subjects without symptoms of numbness (43 %) and was significantly higher in those with positive neuropathic symptoms (65 %) ($P \leq 0.0001$). The prevalence of sensory deficits in subjects with painful symptoms was 78 %. NerveCheck performed best for vibration testing against NCS (AUC 82 %) and with the cold testing for CNFD (AUC 78 %) and for IENFD (AUC 70 %), respectively.

Conclusions: This study shows the clinical utility of NerveCheck, an inexpensive (\$500) QST device for identifying large and small fibre deficits in relation to symptoms

and deficits. The diagnostic performance of NerveCheck is high in relation to established end points for large and small fibre damage.

Keywords:

NerveCheck, quantitative sensory testing, neuropathy, diagnostic device, diabetes

Introduction

The prevalence of diabetic peripheral neuropathy (DPN) has been reported to be up to 50% [1, 2]. About half of the people with DPN are affected by asymptomatic neuropathy which is sensory loss without symptoms [3]. An absence of symptoms does not mean an absence of DPN. Neuropathy is central to the development of painful neuropathy and foot ulceration in patients with diabetes [4, 5]. Hence, it is important to detect early sensory deficits in diabetes in order to predict and prevent progression of DPN. Both sensory deficits and threshold points to sensory stimuli can be measured reliably by quantitative sensory testing (QST), through standardised stimuli and a quantified response [6].

NerveCheck is an inexpensive (\$500), portable QST device that measures the vibration (VPT), cold (CPT), warm (WPT) perception thresholds and heat pain threshold (HPT) of the patient. It uses the method of limits where a series of predefined stimuli over a broad range of intensities are applied to produce a categorical output to define the severity of neuropathy. We have recently shown that it has good reproducibility and comparable diagnostic accuracy to established QST equipment for the diagnosis of DPN (Add our ref.).

QST assessment has been endorsed for use in the quantification of sensory deficits by the NeuPSIG consensus [7]. The diagnosis of neuropathic pain is based on a combination of positive neuropathic symptoms and sensory deficits as well as evoked pain [8]. In this respect, NerveCheck, can accurately identify sensory deficits and allodynia to a heat stimulus to help identify patients with painful diabetic neuropathy [9].

A loss of vibration sensation is related to large fibre neuropathy [7], whilst loss of thermal sensation is related to small fibre neuropathy. Structural loss of small nerve fibres can be detected by corneal confocal microscopy (CCM) in the eye and intra-epidermal nerve fibre density (IENFD) in skin biopsies taken from the foot [10-12]. NerveCheck detects functional deficits of both large and small fibres [7].

In the present study we have established the prevalence and association of sensory deficits in relation to numbness and painful neuropathic symptoms using the McGill questionnaire. As NerveCheck detects both large and small fibre neuropathy, we compared its diagnostic performance to objective end-points for large fibre dysfunction by comparing with nerve conduction studies (NCS), and small fibre dysfunction by comparing with corneal confocal microscopy (CCM) and intra-epidermal nerve fibre density (IENFD).

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 NIHR Wellcome Trust Clinical Research Facility between 7 January 2013 and 19 September 2014. Exclusion criteria included subjects with communication disorders, cognitive deficits, severe anxiety, severe depression or history of neuropathy due to a non-diabetic cause. Control subjects suffering from any acute or chronic pain condition were excluded. All subjects were without any pain medication for at least 24 hours before the investigation. 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 glycated haemoglobin (HbA1c), body mass index (BMI), blood pressure and cholesterol.

Quantitative sensory testing

Subject were familiarised with the procedure and allowed to acclimatize for 10 minutes in the examination room (Add our ref.). NerveCheck (Phi Med Europe S.L. Barcelona, Spain) `applies the method of levels where a series of predefined stimuli (in terms of vibration intensity, heat waveform and ramped stimuli (1 °C/s)) are applied to the skin and, for each stimulus, the subject has to report whether the stimulus is perceived or not for vibration (VPT), cold (CPT) and warm perception threshold (WPT) or whether it is painful or not for the heat painful threshold (HPT). The output is categorical in terms of

degree of abnormality. The normal and abnormal range for VPT is (12-8 & 7-0) and for CPT and WPT is (6-3 & 2-0). More information about the NerveCheck can be found online (<http://www.phimedeuropa.com/>).

Nerve conduction studies

Electrodiagnostic 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 (SNCV), sural sensory nerve action potential (SNAP) and peroneal motor nerve conduction velocity (PMNCV) were assessed in the right lower limb by a consultant neurophysiologist.

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) using our established methodology [13]. Corneal Nerve Fibre Density (CNFD), the total number of nerve fibres (no./mm²), Corneal Nerve Branch Density (CNBD), the total number of nerve branches (no./mm²), and Corneal Nerve Fibre Length (CNFL), the total length of all nerve fibres and branches (mm/mm²) captured within the area of cornea were quantified from ~5 adjacent images/subject, using the ACCMetrics, an automated image analysis software [13]. ACCMetrics is available to all potential collaborators solely for research purposes (non-for-profit/non-commercial) protected by the University of Manchester in the form of license agreement which can be requested online (

development.manchester.ac.uk/ena/ACCMetricsuserinstructions#Researchlicenceagreement).

Intraepidermal Nerve Fibre Density

A 3 mm punch skin biopsy was taken from the dorsum of the foot under 1 % lidocaine local anaesthesia. Skin samples were immediately fixed in 4 % (wt/vol.) paraformaldehyde for 24 hours and then cryoprotected in sucrose for 18 hours and cut into 50 μm sections. Immunohistochemistry was performed as previously described [14]. An image analysis camera AxioCam MRc (Zeiss, Germany) and Leica QWin Standard V2.4 (Leica Microsystem Imaging, Cambridge, UK) were used to quantify intra epidermal nerve fibre density (IENFD), which is the total number of nerve fibres per millimeter length of epidermis (no./mm).

Study definition of Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy (DPN) was defined according to the neuropathy disability score (NDS). The maximum score for the NDS is 10, indicating a complete loss of all sensory responses and absent reflexes. Scores > 2 are abnormal [5, 15]. The NDS includes the following clinical tests:

Vibration perception threshold – Using a 128-Hz tuning fork, we scored 1 for each foot if the individual can distinguish between with and without vibration when the tuning fork was applied to the apex of the big toe.

Temperature perception – Using a cold and warm metallic rode, we scored 1 for each foot if the individual distinguished cold and warm on the dorsum of the foot.

Pin prick testing – Using a sharp pin applied proximally to the big toe nail, with just enough pressure to deform the skin, we scored 1 for each foot if the individual can distinguish between sharp and not sharp.

Achilles tendon reflex – we scored 0,1 or 2 if the angle reflex present, present with reinforcement or absent, respectively.

Statistical analysis

We performed an unpaired t- test to assess quantitative variables between groups using GraphPad Prism, version 6.05. All data were expressed as mean \pm standard error of mean. A P value <0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curve analysis was used to compare the diagnostic performance of NerveCheck against objective end-points for large and small fibre neuropathy using GraphPad Prism, version 6.05. ROC curve analysis established the area under the curve (AUC) to determine the optimal sensitivity and specificity of the NerveCheck test.

Results

Clinical data

Of the 144 subjects 41 were diagnosed with and 33 without diabetic peripheral neuropathy (DPN) based on the neuropathy disability score (NDS) and 70 were control subjects. The demographic and clinical characteristics of the subjects with and without DPN and controls are presented in Table 1. BMI and HbA1c levels did not differ between the groups with and without DPN, but age ($P \leq 0.0001$), duration of diabetes ($P \leq 0.0001$) and systolic blood pressure ($P \leq 0.001$) were significantly greater in those with DPN. NerveCheck showed that the group with DPN had significantly lower scores for vibration (VPT) ($P \leq 0.0001$), cold (CPT) ($P \leq 0.0001$) and warm perception threshold (WPT) ($P \leq 0.0001$). The group with DPN also had a significantly lower peroneal motor nerve conduction velocity (PMNCV), sural nerve action potential (SNAP) and conduction velocity (SNCV) ($P \leq 0.0001$ for all comparisons), corneal nerve fibre density (CNFD) ($P \leq 0.0001$) and intra epidermal nerve fibre density (IENFD) ($P \leq 0.001$).

Detection of sensory deficits and painful neuropathy

The prevalence of asymptomatic and painful neuropathy detected by the NerveCheck is shown in Table 2. Subjects with diabetes had significantly lower NerveCheck scores than controls ($P \leq 0.05 - 0.0001$). The NerveCheck detected sensory loss in 12 out of 28 (43%) subjects without numbness. Of the 45 subjects who reported numbness 30 (65%) had sensory loss. An abnormal vibration or thermal response was significantly more

likely to occur in subjects with numbness compared to those without symptoms ($P \leq 0.0001$ for all comparisons).

In subjects with painful symptoms the prevalence of painful diabetic peripheral neuropathy (PDPN) (signs and symptoms) was 78%. The NerveCheck detected sensory loss in 14 out of 18 subjects with painful symptoms. Sensory deficits were significantly more likely to occur in subjects with pain compared to those without pain (VPT and WPT, $P \leq 0.05$).

NerveCheck diagnostic performance against large and small fibre neuropathy and neuropathic painful symptoms

The diagnostic performance of NerveCheck was compared to large fibre dysfunction, small fibre loss and neuropathic pain symptoms using nerve conduction studies (NCS), corneal confocal microscopy (CCM) and intra-epidermal nerve fibre density (IENFD), and McGill questionnaire, respectively, as reference methods. The diagnostic performance expressed in AUC % using ROC analysis is shown in Table 3. Vibration perception using NerveCheck had an AUC of 82 % (95% CI 0.72 - 0.93), $P < 0.0001$ for SNAP and 84 % (95% CI 0.75 - 0.94), $P < 0.0001$ for SNCV. The sensitivity and specificity of VPT against SNCV was 88 % (95% CI 67.64% to 97.34%) and 82 % (95% CI 71.11% to 90.02%), respectively. For cold perception testing the AUC was 78% (95% CI 0.66 - 0.91), $P < 0.0001$ for CNFD and 70% (95% CI 0.54 - 0.87), $P = 0.01$ for IENFD. The sensitivity and specificity of CPT against CNFD was 67 % (95% CI 44.68% to 84.37%) and 85 % (95% CI 75.27% to 92.44%), respectively and against IENFD it was 53 % (95% CI 27.81% to 77.02%) and 82 % (95% CI 67.98% to 91.24%), respectively. For neuropathic pain symptoms, NerveCheck performed best with

vibration perception testing, with an AUC of 70% (95% CI 0.57 - 0.83), $P = 0.006$ and a sensitivity and specificity of 70 % (95% CI 45.72% to 88.11%) and 68 % (95% CI 56.42% to 78.07%), respectively.

Conclusions

In the present study, we have tested the clinical utility of NerveCheck a quantitative sensory testing (QST) device, which provides a composite of measures for the diagnosis of sensory deficits. It is an inexpensive (\$500), portable (9.5 cm X 6.1 cm X 23.6 cm) device that performs both vibration and thermal testing. We show NerveCheck identifies a greater prevalence of sensory deficits in patients with negative or positive neuropathic symptoms. NerveCheck is capable of detecting both large and small fibre neuropathy. Vibration perception threshold (VPT) testing using NerveCheck is the most efficient method for diagnosis of large fibre dysfunction and neuropathic pain symptoms measured by nerve conduction study (NCS) and McGill questionnaire, respectively. With respect to diagnosis of small fibre neuropathy, NerveCheck showed the best performance between cold perception threshold (CPT) testing and corneal nerve fibre density (CNFD) and intra epidermal nerve fibre density (IENFD).

DPN is an insidious condition defined by the presence of both symptoms and signs of neuropathy. The diagnosis of DPN is confirmed with a history of neuropathic symptoms and a careful assessment of neurological deficits. There is no treatment that can reverse nerve damage in patients with DPN. However, treatment can prevent or slow the progression of neuropathy and may improve symptoms, especially if commenced early. Identifying early neuropathy is key to preventing the long-term sequelae of foot ulceration and amputation as well as helping to identify those with neuropathic as opposed to nociceptive pain. Currently widely deployed tests include neurological examination and monofilament testing, which both identify advanced rather than early neuropathy [15].

Patients with diabetes for more than 10 years of diabetes and loss of sensation in the foot are at risk of developing neuropathic symptoms and foot ulcers [16]. In a study of 6487 patients with diabetes, the prevalence of large fibre neuropathy defined by VPT (≥ 15 V) was reported to be 20.8 % (19.1 - 22.5 %) in patients with diabetes duration < 5 years and 36.8 % (34.9 – 38.7 %) in patients with diabetes duration > 10 years [2]. In the United Kingdom Prospective Diabetes Study (UKPDS), the prevalence of severe large fibre neuropathy defined by VPT (> 25 V) was reported to be 11.5 % in patients with newly diagnosed T2 diabetes [17]. We have shown that assessment of VPT using NerveCheck has high sensitivity 84% (95% CI 63.92% to 95.46%) and specificity 81% (95% CI 72.07% to 87.66%) compared to the Neurothesiometer, an established method for VPT testing (Add our ref.). NerveCheck provides a simple categorical output which can easily be interpreted in relation to the severity of neuropathy and hence allows ready risk stratification.

The diagnosis of painful diabetic peripheral neuropathy (PDPN) is based on a combination of painful neuropathic symptoms and eliciting sensory deficits and evoked pain [8] which occurs in 20-30% of patients with diabetes [18, 19]. Painful symptoms include burning, tingling, electric shock or stabbing sensations, which are generally worse at night and disturb sleep. We have shown that the prevalence of painful symptoms assessed using the neuropathy symptom score (NSS) (> 6) and PDPN assessed using the NSS (> 6) and NDS (> 2) was 34 % and 21 %, respectively in 15,692 community-based patients with diabetes from Northwest England [19]. Furthermore, PDPN is significantly more likely to occur in patients with moderate or severe DPN compared to mild DPN ($P < 0.0001$) [20, 21]. NerveCheck, by detecting

sensory deficits allows the confirmation of PDPN and is significantly more likely to be abnormal in subjects with painful symptoms compared to those without pain.

Since QST detects both signs of large and small fibre neuropathy, its diagnostic performance for sensory loss has been compared to that of large fibre dysfunction and small fibre loss using NCS and intra-epidermal nerve fibre density (IENFD), respectively. A study comparing QST of mechanical testing and nerve conduction studies reported 75% sensitivity and 89% specificity for the detection of neuropathy [22]. In the present study, the sensitivity and specificity of the VPT using NerveCheck against sural nerve conduction velocity (SNCV) was 88 % and 82 %, respectively. Thermal testing compared to IENFD has previously reported a sensitivity ranging from 36–100% [23]. Our data shows that the sensitivity and specificity of the CPT using NerveCheck against IENFD was 53 % and 82 %, and 67% and 85% for CNFD, respectively.

One of the inherent limitations of this study as for all QST devices is that testing depends on patient participation, which could be affected by lack of attention or motivation. Age, duration of diabetes and systolic blood pressure influence DPN and therefore could have influenced the NerveCheck measures.

In conclusion, the current study shows that NerveCheck, an inexpensive, portable QST device has considerable clinical utility to accurately detect deficits in thermal and vibration perception with high sensitivity and specificity compared to established measures of neuropathy.

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Author contributions

G.P. researched data and wrote the manuscript. A.O, M.N.O, S.O, A.M, and M.B.O. designed and created the NerveCheck. I.N.P, S.A, H.F, U.A, O.A, A.M, A.K and A.A researched data. R.A.M. and A.O. sponsored and lead the study, reviewed and edited the manuscript. R.A.M. is the guarantor of this work.

Conflicts of Interest

Phi Med Europe S.L. provided the Nervecheck device, funded half of the study and covered some of the travel expenses and conference registration fees for presenting the results of this study.

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	Control subjects	No neuropathy	Neuropathy
n	70	41	33
Age	41.8 ± 1.63 [≡]	44.3 ± 2.19	64.1 ± 1.79 [≡]
Duration of diabetes	-	23.3 ± 2.03	37.6 ± 3.2 [≡]
BP-sys (mm Hg)	122.8 ± 2.87 [≡]	129.9 ± 3.1	147.9 ± 4.78 [≡]
BMI (Kg/m ²)	25.8 ± 0.79 [˘]	27.6 ± 0.87	28.4 ± 0.8
HbA1c (%)	5.29 ± 0.12 [≡]	7.5 ± 0.18 [≡]	7.9 ± 0.26
HbA1c (mmol/mol)	34.3 ± 1.35 [≡]	59.1 ± 1.97 [≡]	62.6 ± 2.82 [≡]
Nervecheck VPT	8.6 ± 0.37 [≡]	6.4 ± 0.75 [≡]	2.1 ± 0.6 [≡]
Nervecheck CPT	5.9 ± 0.07 [≡]	5.5 ± 0.22 [˘]	3.2 ± 0.46 [≡]
Nervecheck WPT	4.7 ± 0.25 [≡]	5.7 ± 0.15 [≡]	2.5 ± 0.4 [≡]
PMNCV (m/s)	49.6 ± 0.66 [≡]	42.9 ± 0.88 [≡]	36.6 ± 1.6 [≡]
SNCV (uV)	50.9 ± 0.77 [≡]	44.2 ± 0.92 [≡]	35.9 ± 1.4 [≡]
SNAP (m/s)	20.5 ± 1.7 [≡]	12.3 ± 1.02 [≡]	5.1 ± 1.12 [≡]
CNFD (no./mm ²)	28.8 ± 0.92 [≡]	22.9 ± 1.3 [˘]	14.2 ± 1.6 [≡]
IENFD (no./mm)	10.9 ± 0.8 [≡]	8.05 ± 0.7 [˘]	3.8 ± 0.75 [≡]

Table 1. Comparison of clinical data of subjects with diabetes with and without diabetic peripheral neuropathy (DPN) defined by the neuropathy disability score (NDS) and control subjects. Data are mean ± standard error of the mean, P values are derived from unpaired t-test: P≤0.05 (˘), P≤0.001 (˘˘), P≤0.0001 (≡). The P values for with vs without DPN are in the left column, no DPN vs controls in the middle column and DPN vs controls in the right column.

Deficits	Controls	No numbness	Numbness	No pain	Pain
n	69	28	45	56	18
Signs / total no. subjects (%)	4/69 (6 %)	12/28 (43 %)	30/45 (67 %)	28/56 (50 %)	14/18 (78 %)
Nervecheck VPT	8.98 ± 0.37 ^{≡≡}	5.4 ± 1.02 [˘]	3.75 ± 0.68 ^{≡≡}	5 ± 0.65 [≡]	2.67 ± 0.9 [˘]
Nervecheck CPT	5.89 ± 0.07 ^{≡≡}	5.3 ± 0.28	4.11 ± 0.38 ^{≡≡}	4.78 ± 0.28 [≡]	3.61 ± 0.66
Nervecheck WPT	4.74 ± 0.25 ^{≡˘}	5.15 ± 0.31 [≡]	3.88 ± 0.4 [≡]	4.72 ± 0.29	3.17 ± 0.63 [˘]

Table 2. Comparison of the Nervecheck results of controls, subjects with diabetes with and without numbness defined by the neuropathy symptom profile (NSP) and with and without painful symptoms. Data are mean ± SEM, P values are derived by unpaired t-test: P≤0.05 (˘), P≤0.001 (˘˘), P≤0.0001 (˘˘˘). The P values for controls vs numbness and painful symptoms are in the control column, respectively, controls vs no symptoms are in the no symptom columns and present vs absent symptoms are in the symptom columns.

	VPT	CPT	WPT
	AUC % (95% CI), P value	AUC % (95% CI), P value	AUC % (95% CI), P value
SNAP	82% (0.72 - 0.93), < 0.0001	70% (0.56 - 0.84), 0.005	78% (0.65 - 0.9), 0.0002
SNCV	84% (0.75 - 0.94), < 0.0001	71% (0.58 - 0.84), 0.002	75% (0.63 - 0.87), 0.0003
CNFD	76% (0.66 - 0.87), 0.0002	78% (0.66 - 0.91), < 0.0001	71% (0.59 - 0.84), 0.002
IENFD	60% (0.42 - 0.79), 0.2	70% (0.54 - 0.87), 0.01	68% (0.52 - 0.85), 0.03
McGill (pain)	70% (0.57 - 0.83), 0.006	63% (0.48 - 0.78), 0.07	65% (0.5 - 0.8), 0.04

Table 3. ROC curve analysis was used to evaluate the diagnostic performance of Nervecheck for sensory loss against sural nerve action potential (SNAP), sural nerve conduction velocity (SNCV), corneal nerve fibre density (no./mm²), intra-epidermal nerve fibre density (IENFD) (no./mm) and McGill questionnaire for symptoms of painful neuropathy.