

## Original Article: Complications

# Neuropad as a diagnostic tool for diabetic autonomic and sensorimotor neuropathy

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### Abstract

**Aims** The aim of the present study was to determine the diagnostic accuracy of the Neuropad sudomotor test for diabetic cardiovascular autonomic neuropathy (CAN) and diabetic polyneuropathy (DPN), the latter assessed using a multi-level diagnostic approach.

**Methods** In 51 diabetic patients, CAN, symptoms and signs of DPN, vibration perception threshold (VPT), cold (CTT) and warm thermal perception thresholds (WTT) were measured. Neuropad response was determined as normal (complete colour change) or abnormal (absent or incomplete colour change). The time until the complete colour change (CCC time) was recorded.

**Results** CCC time showed significant correlations with all the neurological parameters, the strongest of which were with Valsalva ratio ( $\rho = -0.64$ ,  $P < 0.0001$ ), symptoms of DPN ( $\rho = 0.66$ ,  $P < 0.0001$ ), postural hypotension ( $\rho = 0.54$ ,  $P = 0.0001$ ) and CTT ( $\rho = -0.54$ ,  $P = 0.0001$ ). CCC time showed moderate diagnostic accuracy for both CAN and DPN: the areas under the receiver operating characteristic (ROC) curves were 0.71 and 0.76, respectively. The diagnostic characteristics of three cut-off values of CCC time, identified by ROC analysis (i.e. 10, 15 and 18 min), were analysed. Compared with 10 min, the 15-min cut-off value provided better specificity (from 27% to 52% and from 31% to 62% for CAN and DPN, respectively) and a better likelihood ratio for negative result (from 0.67 to 0.34 and from 0.58 to 0.33) without lowering sensitivity (from 82% to 82% and from 85% to 80%).

**Conclusions** Neuropad is a reliable diagnostic tool for both CAN and DPN, albeit of only moderate accuracy. Extending the observation period to 15 min provides greater diagnostic usefulness.

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**Keywords** autonomic neuropathy, diabetes, diagnosis, peripheral neuropathy

**Abbreviations** AUC, area under the curve; CAN, diabetic cardiovascular autonomic neuropathy; CCC, complete colour change; CTT, cold thermal perception threshold; DPN, diabetic sensorimotor polyneuropathy; HbA<sub>1c</sub>, glycated haemoglobin; IENF, intra-epidermal nerve fibre; LR, likelihood ratio; MDNS, Michigan Diabetic Neuropathy Score; MNSI-Q, Michigan Neuropathy Screening Instrument Questionnaire; NPV, negative predictive value; PPV, positive predictive value; QST, quantitative sensory testing; ROC, receiver operating characteristic; VPT, vibration perception threshold; WTT, warm thermal perception threshold

### Introduction

There are no clinical tests for the assessment of cardiovascular 'sympathetic' function other than the postural hypotension test which, despite its essential role in standard assessment of cardiovascular autonomic function in diabetes [1,2], has

limited diagnostic accuracy and very low sensitivity for cardiac autonomic neuropathy [3].

Sympathetic sudomotor function is commonly impaired in diabetic patients and abnormalities occur early in diabetes [4]. Loss of sweating in the feet is a recognized risk factor for foot ulceration in diabetic patients [5,6], regardless of other indices of peripheral nerve function [6]. Several limitations have inhibited the widespread adoption of different methods of assessing sudomotor function, such as low sensitivity and reproducibility for the sympathetic skin response or low availability for the

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Quantitative sudomotor axon reflex test [4,7–9]. The need for a simple and reliable test of sudomotor function in the lower limbs remains unmet.

Most diagnostic modalities available for diabetic sensorimotor polyneuropathy (DPN) measure mainly large-fibre function. The new techniques assessing small-fibre function, such as quantitative sensory testing (QST) for thermal sensation [10], nerve axon reflex [11] and punch-skin biopsy for intra-epidermal nerve fibre (IENF) density [12] are not easily accessible. Moreover, it is possible that, if damage to small somatic nerve fibres occurs concomitantly with that to sympathetic fibres in the lower limbs, sudomotor dysfunction in the feet might be more strongly related to the impairment of small somatic nerve fibres than to cardiovascular autonomic tests [13]. In this case, assessment of sudomotor function would become a tool to determine the overall function of small nerve fibres.

Recently, Neuropad® (miro Verbandstoffe, Wiehl, Germany), an adhesive indicator test able to detect sweating through colour change, has been proposed as an easy, practical and cheap test for the assessment of sudomotor function in the feet [14]. A few studies demonstrated high sensitivity and limited specificity of Neuropad in detecting DPN [15–18]. In these studies, diagnosis was based on screening tools [15–17,19,20] or on both clinical examination and nerve conduction studies [18,21]. Moreover, accuracy in diagnosing DPN was greater than that for diabetic cardiovascular autonomic neuropathy (CAN) [16]. An association between IENF density and graded Neuropad response was also reported [17].

The present study aimed to determine in diabetic patients (i) the diagnostic accuracy of Neuropad for the presence of CAN, (ii) the presence of DPN, the latter assessed using a multi-level diagnostic approach, i.e. symptoms, deficits, and quantitative sensory testing and (iii) the differential relationship of Neuropad response to large- and small-fibre function.

## Patients and methods

### Patients

We consecutively recruited 51 diabetic patients (29 male) among outpatients attending the diabetic clinic of the Tor Vergata University, Rome. Inclusion criteria were the diagnosis of Type 1 or Type 2 diabetes and age between 18 and 70 years. Exclusion criteria were: conditions or drugs affecting the autonomic nervous system or sudomotor function (impaired kidney function, respiratory failure, beta blockers, diuretics, tricyclic antidepressants, anticonvulsants etc.); any cardiovascular disease with the exception of hypertension; any other clinically significant disease; non-diabetic peripheral neuropathies; peripheral arterial disease (detected by the presence of claudication, absence of palpable dorsalis pedis or posterior tibial pulses, or ischaemic foot ulcers); active foot ulcers. The study was approved by the Ethics Committee of Tor Vergata University and informed consent was obtained from all participants.

Age was  $44.9 \pm 13.7$  years (mean  $\pm$  SD), diabetes duration  $14.7 \pm 10.7$  years, body mass index  $27.2 \pm 5.2$  kg/m<sup>2</sup>, glycated haemoglobin (HbA<sub>1c</sub>)  $7.9 \pm 1.7\%$ , serum creatinine  $83.1 \pm 21.2$   $\mu$ mol/l, casual blood pressure was  $124/77 \pm 16/10$  mmHg. Twenty-four patients (47%) had Type 1 diabetes, 22 (44%) had retinopathy, 13 (25%) had microalbuminuria, 21 (41%) were smokers and 17 (33%) had hypertension and were treated with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or calcium-channel blockers.

### Methods

Neurological assessment was performed in a quiet room, with ambient temperature in the range of 22–25°C. Blood glucose was measured at the beginning of the neurological examination and, if  $< 3.3$  or  $> 22.2$  mmol/l, the testing was rescheduled.

#### Cardiovascular autonomic neuropathy assessment

Autonomic function was assessed by deep breathing, lying to standing, Valsalva manoeuvre and postural hypotension tests, performed according to standard procedure [22] using age-related reference values [23] and by using a computerized system for data acquisition and analysis (DAN test; Microlab Elettronica Sas, Padua, Italy). An autonomic score was obtained from the sum of scores given to each test (0 for a normal, 1 for a borderline and 2 for an abnormal result, overall range 0–8) [24]. CAN was defined as the presence of at least two abnormal tests.

#### Peripheral neuropathy assessment

Neurological assessment included ascertainment of neuropathic symptoms and deficits using validated scored systems, i.e. the Michigan Neuropathy Screening Instrument Questionnaire (MNSI-Q) and the Michigan Diabetic Neuropathy Score (MDNS) [25]. No electro-diagnostic studies were performed. Vibration perception threshold (VPT) was measured using the Biothesiometer (Biomedical Instruments, Newbury, OH, USA) at the dorsum of the hallux and at the lateral malleolus; age-related normal values derived from literature were used [26]. Cold (CTT) and warm thermal perception (WTT) thresholds were assessed using the Neuro Sensory Analyzer TSA-II (Medoc, Ramat Yishai, Israel) at the dorsum of both feet according to the levels test procedure [10,27]. Definition of DPN required the presence of at least two abnormalities among symptoms, deficits, VPT and CTT and/or WTT.

#### Assessment of sudomotor function

An independent operator unaware of the previous test results assessed sudomotor function with the Neuropad system. Patients were requested to lie down for 10 min with their feet bare. Thereafter, two adhesive indicator tests were applied on the plantar surface of the first or second metatarsal head of both feet. Any change of colour from blue to pink was assessed at 10 min. Results were classified as normal (complete colour change in both

feet) or abnormal (absent or incomplete change of colour in at least one foot). The time in seconds elapsed until the complete colour change from blue to pink (CCC time) was also recorded (with a maximum duration of observation of 30 min).

### Statistical analysis

Data are expressed as means  $\pm$  SD. Unpaired Student's *t*-test and the chi-square test for categorical variables were used. Spearman coefficients and multivariate regression analysis were used to determine the independent relationship between CCC time (average of the CCC time values at right and left foot) and other neurological parameters. All statistical analyses were carried out using the program STATVIEW V (SAS Institute, Cary, NC, USA) on a Macintosh iBook G4 computer. A value of  $P < 0.05$  was considered significant.

Receiver operating characteristic (ROC) analysis was used to assess the diagnostic accuracy of Neuropad in distinguishing between patients with and without CAN and with and without DPN, through the measurement of the area under the curve (AUC), which incorporates both components of accuracy, i.e. sensitivity and specificity, into a single measure [28].

Moreover, using the chi-square test, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the likelihood ratio for a positive result ( $LR^+$ ), which is the ratio of sensitivity to 1-specificity and for a negative result ( $LR^-$ ), which is the ratio of specificity to 1-sensitivity [29]. Fagan's nomogram was used to obtain a simple estimation of post-test probability from pretest probability through the  $LR^+$  [29].

## Results

Eleven patients (22%) had CAN. In 10 of these patients, one or more autonomic symptoms were present, i.e. sweating abnormalities—gustatory sweating and/or upper body compensatory hyperhidrosis—in six patients, urinary symptoms in four patients, erectile dysfunction in six patients, gastrointestinal symptoms—gastroparesis and/or diabetic diarrhoea—in two patients, orthostatic symptoms in two patients. Thirty-six (71%), 12 (23%), 21 (41%) and 14 patients (27%) showed abnormal results of MNSI-Q, MDNS, VPT and CTT and/or WTT, respectively. Twenty patients (39%) had DPN and 13 patients (25%) had small-fibre neuropathy, defined as the presence of loss of pain sensation on pinprick and/or abnormal thermal thresholds.

Thirteen patients (26%) showed a normal response of Neuropad at 10 min and 35 and three patients an incomplete or absent response, respectively (74%). There were no differences in any clinical variable in patients with abnormal or normal Neuropad responses (data not shown). The Valsalva ratio was significantly lower ( $1.57 \pm 0.38$  vs.  $1.97 \pm 0.42$ ,  $P = 0.003$ ) and the fall in blood pressure on standing significantly higher ( $13.0 \pm 10.9$  vs.  $5.77 \pm 11.1$  mmHg,  $P = 0.045$ ) in the group of patients with abnormal 10-min response. There was no significant association between

abnormal 10-min response and any other autonomic or sensorimotor variables, including the presence of CAN ( $\chi^2 = 0.39$ ,  $P = 0.53$ ), DPN ( $\chi^2 = 1.91$ ,  $P = 0.167$ ) and of small-fibre neuropathy ( $\chi^2 = 1.65$ ,  $P = 0.198$ ).

CCC time was significantly related to HbA<sub>1c</sub>, and to all the neurological parameters (Table 1, Fig. 1), with all the latter correlations remaining significant in a multivariate regression analysis including HbA<sub>1c</sub> as independent variable, with the exception of autonomic score and lying to standing ratio. The strongest correlations were observed with Valsalva ratio, symptoms, postural hypotension and CTT.

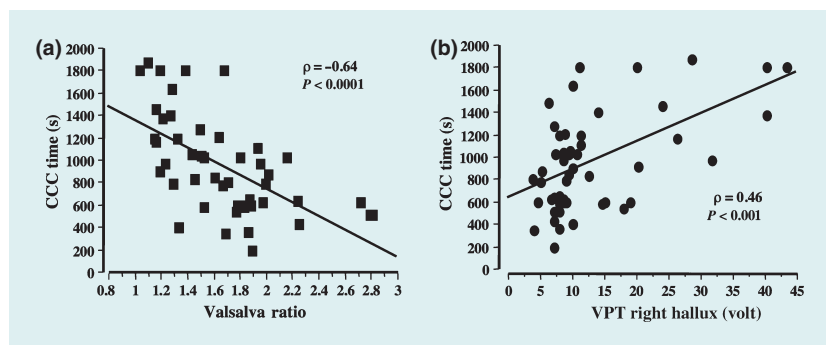
Figure 2 shows the two ROC curves describing the ability of CCC time for diagnosing CAN and DPN, respectively. ROC analysis also identified the CCC time cut-off values of 600 s (10 min), 900 s (15 min) and 1080 s (18 min) as those having the best sensitivity, the best balance between sensitivity and specificity and the best specificity, respectively, in diagnosing CAN or DPN. Table 2 shows the diagnostic characteristics for CAN and DPN of these different cut-off values of CCC time. NPV was higher than PPV, in particular for CAN. The cut-off value of 18 min had the highest  $LR^+$  and the cut-off value of 15 min had the lowest  $LR^-$  for both CAN and DPN. Using Fagan's nomogram and assuming a pretest probability of 20%

**Table 1** Spearman correlation between complete colour change time (CCC time) of Neuropad test and clinical and neurological parameters

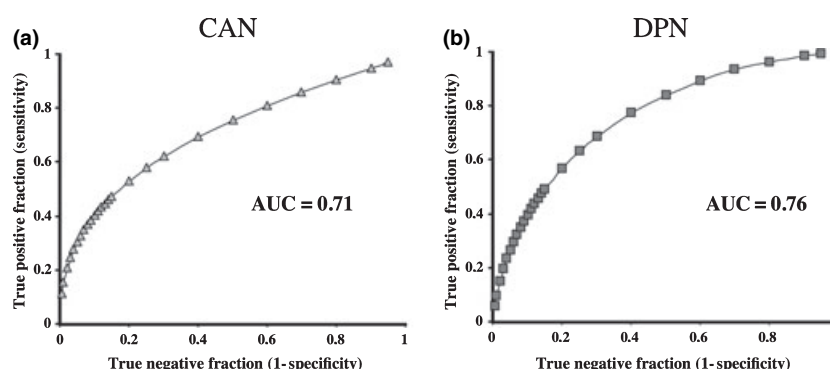
Variable	Spearman's $\rho$	<i>P</i>
Age	0.18	0.20
BMI (Kg/m <sup>2</sup> )	0.04	0.79
Duration of diabetes (years)	0.24	0.09
HbA <sub>1c</sub> (%)	0.32	0.029
Creatinine ( $\mu$ mol/l)	0.15	0.30
LDL cholesterol (mmol/l)	0.15	0.37
Systolic BP (mmHg)	0.10	0.46
Diastolic BP (mmHg)	-0.10	0.94
Autonomic score	0.38	0.0074
Expiration/inspiration ratio	-0.43	0.0022*
Lying to standing ratio	-0.30	0.0335
Valsalva ratio	-0.64	< 0.0001*
Postural hypotension (mmHg)	0.54	0.0001*
MNSI-Q	0.66	< 0.0001*
MDNS	0.38	0.0077*
VPT right hallux (Volt)	0.46	0.001*
VPT left hallux (Volt)	0.45	0.0014*
VPT right malleolus (Volt)	0.46	0.0012*
VPT left malleolus (Volt)	0.48	0.0007*
CTT right foot ( $^{\circ}$ C)	-0.54	0.0001*
CTT left foot ( $^{\circ}$ C)	-0.37	0.0093*
WTT right foot ( $^{\circ}$ C)	0.33	0.0186*
WTT left foot ( $^{\circ}$ C)	0.40	0.0043*

\*Significance still present in multivariate analysis after correction for HbA<sub>1c</sub>.

MNSI-Q, Michigan Neuropathy Screening Instrument Questionnaire; MDNS, Michigan Diabetic Neuropathy Score; VPT, vibratory perception threshold; CTT, cold thermal perception threshold; WTT, warm thermal perception threshold.



**FIGURE 1** Relationship between complete colour change time (CCC time) of Neuropad test and Valsalva ratio (a) or vibration perception threshold (VPT) (b) at the right hallux: both correlations were significant on Spearman's correlation, and remained significant after adjustment for glycated haemoglobin (HbA<sub>1c</sub>) in multivariate regression analysis.



**FIGURE 2** Binormal receiver operating characteristic (ROC) plots for complete colour change time (CCC time) of Neuropad test in distinguishing between patients with and without diabetic cardiovascular autonomic neuropathy (CAN) (a) and between patients with and without diabetic sensorimotor polyneuropathy (DPN) (b). The areas under the curves (AUC) were  $0.71 \pm 0.09$  [95% confidence interval (CI) 0.51–0.87] for CAN and  $0.76 \pm 0.07$  (95% CI 0.62–0.87) for DPN.

**Table 2** Diagnostic characteristics of different cut-off values of complete colour change time (CCC time) of Neuropad test for diabetic cardiovascular autonomic neuropathy (CAN) and diabetic sensorimotor polyneuropathy (DPN)

Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR <sup>+</sup>	LR <sup>-</sup>
For CAN						
10 min	82	27	24	85	1.13	0.67
15 min	82	52.5	32	91	1.73	0.34
18 min	73	75	44	91	2.92	0.36
For DPN						
10 min	85	32	45	77	1.25	0.47
15 min	80	61	57	83	2.05	0.33
18 min	60	74	67	76	2.31	0.54

PPV, positive predictive value; NPV, negative predictive value; LR<sup>+</sup>, likelihood ratio for positive result; LR<sup>-</sup>, likelihood ratio for negative result.

as the most reliable prevalence of CAN [30], very close to the prevalence of CAN in our clinic-based population, the estimate of post-test probability of having CAN was about 30% for a

patient with a positive test and about 8% for a patient with a negative test at 15 min and of about 42% and 8% for a patient with a positive or negative test at 18 min, respectively. In the same way, assuming a pretest probability of 30% as the most reliable prevalence of DPN [30], the estimate of post-test probability of having DPN was about 45% for a patient with a positive test and about 12% for a patient with a negative test at 15 min and of about 48% and 18% for a patient with a positive or negative test at 18 min, respectively.

When dividing the patients according to the response of Neuropad at 15 min, the abnormal response was significantly associated with a greater degree of abnormalities in all neurological parameters and with the presence of CAN and DPN (Table 3). A significant association between the Neuropad response at any cut-off of CCC time and small-fibre neuropathy was not found.

## Discussion

Testing sudomotor function in the lower limbs could be an alternative or supplementary method to the postural hypotension test to explore sympathetic function. It has the potential

**Table 3** Neurological parameters in the diabetic patients divided into two groups according to the response to Neuropad at 15 min

15 min Neuropad response	Normal	Abnormal	P
N	23	28	
Expiration/inspiration ratio	1.42 ± 0.23	1.21 ± 0.14	0.0002
Lying to standing ratio	1.26 ± 0.17	1.12 ± 0.13	0.0015
Valsalva ratio	1.96 ± 0.38	1.43 ± 0.29	< 0.0001
Postural hypotension (mmHg)	6.30 ± 8.82	15.2 ± 11.7	0.0043
Autonomic score	0.7 ± 1.5	2.6 ± 2.8	0.0055
With CAN (%)	9	32	0.0428*
MNSI-Q	1.04 ± 1.80	3.86 ± 3.18	0.0004
With abnormal MNSI-Q (%)	52	86	0.0089 <sup>†</sup>
MDNS	2.87 ± 2.49	6.25 ± 6.19	0.0175
With abnormal MDNS (%)	9	36	0.0236 <sup>‡</sup>
VPT right hallux (Volt)	8.74 ± 4.13	16.33 ± 11.18	0.0034
VPT left hallux (Volt)	9.54 ± 6.31	16.47 ± 8.58	0.0123
VPT right malleolus (Volt)	11.77 ± 6.75	19.76 ± 11.75	0.0057
VPT left malleolus (Volt)	11.48 ± 6.37	19.40 ± 11.91	0.0061
With abnormal VPT (%)	35	46	0.4004
CTT right foot (°C)	31.38 ± 2.00	25.63 ± 9.89	0.0086
CTT left foot (°C)	30.97 ± 2.63	25.67 ± 10.69	0.0248
WTT right foot (°C)	35.31 ± 2.67	37.96 ± 5.65	0.0436
WTT left foot (°C)	34.33 ± 2.19	37.72 ± 5.51	0.0077
With abnormal CTT and/or WTT (%)	17	36	0.1446
With abnormal small-fibre function (%)	17	39	0.0877
With DPN (%)	17	57	0.0038 <sup>§</sup>

\* $\chi^2 = 4.1$ ;  $^{\dagger}\chi^2 = 6.8$ ;  $^{\ddagger}\chi^2 = 5.1$ ;  $^{\S}\chi^2 = 8.4$ .

Data are mean ± SD.

CAN, diabetic cardiovascular autonomic neuropathy; MNSI-Q, Michigan Neuropathy Screening Instrument Questionnaire; MDNS, Michigan Diabetic Neuropathy Score; VPT, vibratory perception threshold; CTT, cold thermal perception threshold; WTT, warm thermal perception threshold; Small-fibre function, pain sensation (pinprick) and/or thermal thresholds; DPN, diabetic sensorimotor polyneuropathy.

additional advantage of providing information on the overall function of small nerve fibres, thus also becoming a component of DPN assessment. However, because of the lack of a simple and reliable test, the need for testing sympathetic sudomotor function in the lower limbs remained generally unmet. The present study aimed at determining in diabetic patients the accuracy of a simple device for sudomotor function, the Neuropad system, as a diagnostic test for CAN and DPN.

Only two previous studies have explored the relationship between cardiovascular tests and Neuropad [16,17]. They found Neuropad ranking to be correlated to the deep breathing test and associated with the presence of postural hypotension [17] and—rather unexpectedly—a very low sensitivity of Neuropad for CAN, poorer than shown for DPN [16].

In the present study, significant correlations between CCC time and cardiovascular tests and the association between abnormalities in the response of Neuropad at 15 min and those seen in the cardiovascular tests support the use of Neuropad as a diagnostic probe for autonomic function. In addition, ROC analysis documented that CCC time had an acceptable—albeit moderate—diagnostic accuracy for CAN. The limited degree of the association between Neuropad response and CAN, however, supports the view that cardiovascular tests and Neuropad explore different autonomic areas. Nevertheless, given both the limited sensitivity of the postural hypotension test [3] and the

limited specificity of the Neuropad test for CAN, these two tests when used together could adequately compensate for each other and provide wider information on sympathetic function.

ROC analysis indicated the cut-off values of 10, 15 and 18 min as the most interesting for their sensitivity (down from 82% to 73%) and specificity (up from 27% to 75%). It seems that a period of observation longer than the proposed 10 min can improve the diagnostic performance of Neuropad for CAN, with a limited reduction in sensitivity and a remarkable increase in specificity. The estimate of post-test probability of having CAN was about 30% for a patient with a positive test at 15 min and 42% for a patient with a positive result at 18 min, and approximately 7.5% and 8% for a patient with a negative test at 15 and 18 min, respectively.

The discrepancy between the sensitivity of Neuropad for CAN and for DPN—i.e. 59.1% and 86%, respectively—observed by Liatis *et al.* [16] was not present in our study. Some differences in clinical characteristics of the studied populations, such as a higher mean age (61.4 years), a much greater percentage of Type 2 diabetic patients (92%) or a greater prevalence of CAN (37.6%) in the Liatis study, might partially account for the different results.

A few studies have shown a good level of sensitivity of Neuropad (range 85–100%) and limited specificity (range 45–71%) in detecting DPN [15–18]. In these studies, diagnosis was

mainly based on screening tools [15–17,19,20] and, in a few studies, also on nerve conduction [18] and VPT measures [16]. An association between different thresholds of CCC time and staged severity of DPN was also described [21], as well as a correlation between CCC time and VPT [31] and a correlation between Neuropad response and thermal thresholds [17]. The novelty of the present study was in the use of a multi-level approach to DPN, including assessment of symptoms, deficits and QST for vibration and thermal sensation.

This study showed that the response of Neuropad, determined both as normal/abnormal at 15 min and as CCC time, was associated with or related to all the assessed diagnostic levels of DPN. ROC analysis revealed moderate diagnostic accuracy of CCC time for DPN, with a value of AUC (0.76) very similar to that shown by monofilament when compared against the criterion standard of nerve conduction studies [32]. Also for DPN, a test duration of 15 min provided a better balance between sensitivity and specificity (80% and 61%, respectively), with a higher LR<sup>+</sup> and a lower LR<sup>−</sup> compared with 10 min. The estimate of post-test probability of having DPN was approximately 45% for a patient with a positive Neuropad test at 15 min and approximately 12% for a patient with a negative one.

Papanas *et al.* [20] found a very strong association of Neuropad response with small-fibre impairment in the feet—i.e. abnormal temperature and pain sensation—and high sensitivity and specificity of Neuropad for small-fibre neuropathy (99% and 78%, respectively). In our study, this association was not found. However, temperature sensation was assessed using devices furnished with different level of accuracy [Neuro Sensory Analyzer TSA-II (Medoc) in the present study and Tiptherm in the cited study] and the prevalence of small-fibre impairment was much lower in the present study (34%) than in the cited study (81%) [20]. In any case, when considering the thermal thresholds alone, we found a good association between 15-min Neuropad response and thermal thresholds and a highly significant correlation between CCC time and CTT/WTT at an even greater degree than previously documented—using CASE IV—for heat-as-pain perception threshold and cold detection threshold [17].

Considering all these findings as a whole, it is difficult to derive evidence of a preferential link of Neuropad response to small-fibre measures compared with large-fibre measures. In the cited study [17], although IENF density was associated with 10-min Neuropad response, this relationship was weaker than expected. It has been suggested that an intimate association exists between large- and small-fibre damage in DPN [10] and that separate functional disturbances of small and large fibres cannot be distinguished using QST [10].

In conclusion, Neuropad is a reliable—albeit of only moderate accuracy—diagnostic tool for both CAN and DPN, related to all the standard measures of autonomic and large- and small-fibre function. It can be added to the standard diagnostic package with the advantages of (i) ease of use, (ii) the ability to investigate the two complications together, (iii) the provision of additional

information on sympathetic function in addition to the far less sensitive postural hypotension test, and (iv) the measuring of small-fibre function. Moreover, the ease of use of Neuropad and high inter-rater reliability between the patient and the healthcare provider [19] make it suitable for self-examination, with a possible additional educational value for patients. Because of the balance between sensitivity and specificity, the Neuropad response at 15 min seems to have greater diagnostic usefulness than that seen at 10 min, without being too time-consuming. If a response is normal at 10 min, a longer test is unnecessary.

## Competing interests

VS has received honorarium by the company miro Verbandstoffe, manufacturers of Neuropad, for participating in an Advisory Board Meeting.

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