

## Original Article

# Usefulness of a new indicator test for the diagnosis of peripheral and autonomic neuropathy in patients with diabetes mellitus

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Accepted 19 May 2007

### Abstract

**Aims** The aim of the present study was to assess the performance of a new indicator test (NIT), based on the measurement of sweat production after exposure to dermal foot perspiration, in the diagnosis of both peripheral sensorimotor polyneuropathy (PSN) and autonomic neuropathy in patients with diabetes.

**Methods** One hundred and seventeen diabetic patients were examined. PSN was assessed using the neuropathy symptoms score, the neuropathy disability score and the vibration perception threshold. Cardiac autonomic neuropathy (CAN) was assessed using the battery of the four classical standardized tests proposed by Ewing *et al.*, *Diabetes Care* 1985; 8: 491–498. Sudomotor dysfunction was assessed using the NIT.

**Results** Fifty patients (42.7%) had PSN and 44 patients (37.6%) had CAN. Of the 50 patients with PSN, 43 had a positive NIT (sensitivity 86%) and, out of the 67 patients without PSN, a negative NIT was obtained in 45 patients (specificity 67%). The positive and the negative predictive value of the NIT in detecting PSN were 66.2 and 86.5%, respectively. The sensitivity and specificity of NIT in detecting CAN was 59.1 and 46.5%, respectively. In the case of severe CAN, the sensitivity was increased to 80.9% and the specificity to 50%.

**Conclusions** The NIT has good sensitivity and negative predictive value for diagnosis of PSN and can be used as a screening method for detection of this complication in patients with diabetes. In addition, the test has a low sensitivity for detection of autonomic neuropathy in patients with milder forms of CAN.

Diabet. Med. (2007)

**Keywords** autonomic neuropathy, diabetic neuropathy, neuropad, peripheral sensorimotor polyneuropathy, sudomotor dysfunction

**Abbreviations** CAN, cardiac autonomic neuropathy; NIT, new indicator test; NDS, neuropathy disability score; NSS, neuropathy symptoms score; PSN, peripheral sensorimotor polyneuropathy; QSART, quantitative sudomotor axon reflex test; VPT, vibration perception threshold

### Introduction

Diabetic peripheral neuropathy (DPN) is a common and troublesome complication of both Type 1 and Type 2 diabetes [1,2]. Peripheral sensorimotor polyneuropathy (PSN) is one of the strongest risk factors for diabetic foot complications such as ulceration, neuro-osteoarthropathy and amputation [3,4]. Peripheral neuropathy implies a heavy burden of morbidity of people with diabetes and increases the economic cost of diabetes management [4].

PSN is the commonest risk factor for diabetic foot ulceration [4]. Early diagnosis is important in order to identify patients at high risk for foot complications. Consequently, screening for PSN is recommended in clinical practice guidelines [5]. However, the methods of testing to identify loss of protective sensation have been quite variable. Clinical assessment often relies on the skill, experience and interpretation of the individual clinician. Nerve conduction studies, although most sensitive, are not suitable for widespread clinical use because of the need for special equipment and expert personnel. In routine community clinical practice there is a need for a simple assessment to rapidly screen large numbers of patients. The optimal screening tool should be simple, practical, and inexpensive, least subjective

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and reliable. Several reports have discussed the potential clinical use of the Semmes–Weinstein 10-g monofilament in identification of patients at risk for foot ulceration [6,7]. Measurement of the vibration perception threshold (VPT) has also been used for screening [7,8]. In addition, in order to assess patients for PSN and identify those at risk, neuropathic symptoms and signs have been used to create scores such as the neuropathy symptom score (NSS) and neuropathy disability score (NDS) [9,10].

A relatively neglected component of peripheral neuropathy is sudomotor dysfunction, which results in reduced sweating and dry sensitive skin, which in turn predisposes to callus and fissure formation [11]. Recent data suggest that, in diabetic subjects with neuropathy, somatic and autonomic small-fibre involvement tend to occur together [12]. Recently, a new indicator test (NIT; NeuroPad® Miro Verbandstoffe GmbH, Wiehl, Germany) has been introduced for the assessment of PSN. This new test measures sweat production based on the colour change of a cobalt II compound (placed on a commercially available sticker) from blue to pink, after exposure to dermal foot perspiration [13]. This is a simple and easy-to-perform test that has been proposed to be useful for the detection of sudomotor dysfunction in subjects with diabetes. Recent data has shown that the new indicator test has a high sensitivity in the detection of PSN when the latter is diagnosed by means of the Diabetes Neuropathy Index (DNI) [14]. However, no data exist on the performance of the new indicator test for the diagnosis of autonomic dysfunction, which is common in subjects with diabetes. The aim of the present cross-sectional study was to assess the performance of the NIT in the diagnosis of both PSN and autonomic neuropathy in patients with diabetes.

## Patients and methods

We have performed a cross-sectional study in 117 consecutive diabetic individuals recruited from the diabetes outpatient clinic of our hospital (Laiko General Hospital, Athens, Greece). Patients with conditions known to affect autonomic function (clinical and/or electrocardiographic signs of coronary artery disease, end-stage renal disease, anaemia, acute illness, or hypoglycaemia in the previous 24 h, chronic alcohol abuse, thyroid disease, lumbar spine disorders or neuropathy from causes other than diabetes) were excluded from the study. In addition, patients taking medications possibly affecting the autonomic nervous system (such as anti-arrhythmic medications, antidepressants, over-the-counter antihistamines and sympathomimetic cough/cold preparations) were also excluded. The ethics committee of our hospital approved the study and informed consent was obtained from all participants.

### Assessment of PSN

Assessment for PSN included examination of clinical symptoms, signs and quantitative sensory testing. Symptoms of somatic neuropathy were assessed using the NSS [15,16]. NSS values  $\geq 3$  were considered abnormal.

The NDS [9,15] was used to quantify the severity of the PSN. The sensations of pain (pinprick), light touch, cold and warm

(using two tubes of cold and warm water) on the dorsum of the feet, and vibration (using a tuning fork at 128 Hz) at the base of the big toes were examined. In addition, Achilles tendon reflexes were examined. NDS values  $\geq 5$  were considered abnormal [9,15,16].

The VPT was measured at the tip of the big toes of both feet using a biothesiometer (Biomedical Instruments, Newbury, OH, USA). The age-related upper normal values were derived from previously published data [17].

PSN was diagnosed when at least two out of the three tests performed (NSS, NDS and VPT) were abnormal [15].

### Assessment of autonomic nervous system function

Autonomic nervous system function was based on the determination of autonomic nervous system activity at the heart level. Cardiac autonomic function was assessed using the battery of the four standardized tests proposed by Ewing *et al.* [18]. In brief, heart rate variation during the Valsalva manoeuvre, the deep breathing test and immediate response to standing (30 : 15 ratio) were assessed by electrocardiogram (ECG) recordings of RR intervals (intervals between two consecutive R waves of the ECG) automatically using the computer-aided examination and evaluation system VariaCardio TF4 (Medical Research Limited, Leeds, UK) [19]. The tests were carried out between 07.00 and 09.00 h, in an environment with a stable temperature of 22–24°C, and the participants were advised not to eat, smoke or drink coffee before the examination. The heart rate-based tests were analysed according to published age-related tables [20,21]. Orthostatic hypotension was diagnosed when a fall in systolic blood pressure  $> 20$  mmHg was observed; a fall of 11–20 mmHg was considered as borderline and a fall of  $< 10$  mmHg as normal response [20]. Each normal autonomic function test was graded as 0.0, each borderline test as 1.0 and each abnormal test as 2.0. On the basis of the sum of this score, we calculated the total cardiac autonomic neuropathy (CAN) score, which is the sum of the partial scores (minimum 0, maximum 10). CAN was diagnosed when at least two out of the four tests performed were abnormal [18,19].

### Sudomotor dysfunction

Sudomotor dysfunction was assessed using the new indicator test [13]. Patients took their shoes and socks off 10 min before the application of the patches, and then one indicator patch was applied to the sole of each foot at the level of the 1st or the 2nd metatarsal heads and left for 10 min whilst the patients rested at constant room temperature (22–24°C). A complete colour change of the patches from blue to pink in both feet was considered as a normal response. Patients in whom the colour change was partial or incomplete were considered to have an abnormal response. Colour change analysis was performed by an independent observer who was not aware of the neuropathy status of the participants.

### Statistical analysis

Analysis of the data was performed using the SPSS statistical package (version 10; SPSS, Chicago, IL, USA). All data were

**Table 1** Demographic, clinical and biochemical characteristics of the study participants

Age (years)	61.4 ± 11.6
Type 1/Type 2 diabetes	9/108
Body mass index (kg/m <sup>2</sup> )	28.4 ± 4.7
Systolic blood pressure (mmHg)	134 ± 20
Diastolic blood pressure (mmHg)	82 ± 8
Duration of diabetes (years)	10.9 ± 7.1
Current smokers, <i>n</i> (%)	33/117 (28.2)
Fasting serum glucose (mmol/l)	8.5 ± 3.1
Total cholesterol (mmol/l)	5.3 ± 1.1
HDL cholesterol (mmol/l)	1.2 ± 0.3
LDL cholesterol (mmol/l)	3.5 ± 1.0
Triglycerides (mmol/l)	1.6 ± 0.5
HbA <sub>1c</sub> (%)	7.3 ± 1.6
Treatment for diabetes, <i>n</i> (%)	
Diet only	9 (7.8)
Oral glucose-lowering agents	75 (64.7)
Insulin	33 (27.5)
Any diabetic retinopathy, <i>n</i> (%)	40 (34)
Diabetic nephropathy, <i>n</i> (%)	39 (33.3)

Data are shown as mean ± SD, or as *n* (%).

HbA<sub>1c</sub>, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein.

assessed for normal distribution of the values. Skewed data were either log transformed to improve normality for statistical testing, or were compared using non-parametric methods. Categorical data were compared using a  $\chi^2$ -test. Comparisons of normally distributed data, between groups, were performed by the independent samples Student's *t*-test or by ANOVA. Simple correlations were carried using Pearson's or the Spearman's correlation coefficient, as appropriate. *P*-values (two-tailed) < 0.05 were considered statistically significant.

## Results

The demographic, clinical and biochemical characteristics of the study participants are presented in Table 1. In two patients, cardiac autonomic function tests were impossible to obtain because of persistent artifacts. Thus, with regard to

**Table 3** Coexistence of peripheral sensorimotor polyneuropathy (PSN), cardiac autonomic neuropathy (CAN) and severe CAN

	PSN (yes)	PSN (no)	<i>P</i>	Total
CAN (yes)	26	18	0.002	44
CAN (no)	22	49		71
Total	48	67		115
Severe CAN (yes)	17	4	< 0.0001	21
Severe CAN (no)	31	63		94
Total	48	67		115

autonomic function parameters, data from only 115 patients were included in the analysis.

Fifty patients (42.7%; 31 men and 19 women) had PSN, 44 patients (37.6%; 20 men and 24 women) had CAN and 26 patients (22.2%; 15 men and 11 women) had both PSN and CAN (PSN/CAN). Of the 50 patients with PSN, 43 had a positive NIT (sensitivity 86%). In contrast, of the 67 patients without PSN, a negative NIT was obtained in 45 patients (specificity 67%). The positive predictive value (PPV) and the negative predictive value (NPV) of the NIT were 66.2 and 86.5%, respectively (Table 2). An abnormal NSS ( $\geq 3$ ) was associated with a positive NIT in 67% of cases, whilst an abnormal NDS ( $\geq 5$ ) was accompanied by a positive NIT in 86.4% of cases. Both sensitivity and specificity of the NIT in detecting CAN was low (41 and 46%, respectively), although severe CAN (patients with a Ewing test score  $\geq 6$ ) was associated with a positive NIT in 81% of cases (Table 2). Severe CAN was strongly associated with the presence of PSN [of 21 cases with severe CAN, 17 (80.9%) also had PSN, whilst of the 44 patients diagnosed with CAN only 26 (59.9%) had PSN (Table 3)]. PSN/CAN was associated with a more severe form of both PSN and CAN (Table 4).

## Discussion

The new indicator test is a simple-to-perform test and an abnormal response indicates decreased perspiration of the skin

**Table 2** The performance of the new indicator test (NIT) for the diagnosis of peripheral sensorimotor polyneuropathy using as 'gold standard' the combined measurements of neuropathy symptom score, neuropathy disability score and vibration perception threshold and the diagnosis of cardiac autonomic neuropathy

	Sensitivity	Specificity	PPV	NPV
Peripheral sensorimotor neuropathy	86.0 (80.0–92.0)	67.2 (59.0–75.0)	66.2 (58.0–74.0)	86.5 (80.0–92.0)
Cardiac autonomic neuropathy				
Any CAN	59.1 (50.0–68.0)	46.5 (38.0–56.0)	40.6 (33.0–48.0)	64.7 (55.0–73.0)
Severe CAN	80.9 (73.0–88.0)	50.0 (41.0–59.0)	26.0 (18.0–34.0)	92.1 (89.0–95.0)

Data are expressed as percentage (95% confidence intervals).

CAN, cardiac autonomic neuropathy; NPV, negative predictive value; PPV, positive predictive value.

	Pure PSN	Pure CAN	CAN/PSN	P
<i>n</i>	22	18	26	
Age (year)	61.7 ± 9.4	63.9 ± 8.7	61.4 ± 7.7	0.65
Duration of diabetes (years)	10.8 ± 6.9	12.2 ± 5.7	11.8 ± 6.4	0.64
CAN score (Ewing)*		4.5 (4–6)	6.0 (4–8)	0.002
NDS score*	4 (0–10)		5.5 (0–10)	0.09
NSS score*	6.5 (0–9)		6.0 (0–9)	0.54
VPT	30.2		36.7	0.044
NIT (+/–)	20/2	7/11	22/4	0.0001

Data are expressed as mean values ± SD.

\*Median value (minimum–maximum).

CAN, cardiac autonomic neuropathy; NDS, neuropathy disability score; NIT, new indicator test; NSS, neuropathy symptoms score; PSN, peripheral sensorimotor polyneuropathy; VPT, average VPT value from right and left foot.

**Table 4** Comparison of patients with pure CAN, pure PSN and CAN/PSN

area under investigation (usually the soles of the feet) [13]. Decreased perspiration of the feet is a clinical sign of sudomotor dysfunction, which in turn is a clinical manifestation of diabetic autonomic neuropathy [22]. Sweat glands are innervated by sudomotor, post-ganglionic sympathetic C-fibres (unmyelinated fibres, 0.5–2 µm) which are cholinergic [12]. It has been shown that in PSN there is a perfect concordance between somatic and autonomic C-fibre damage at the local site of the lower extremities [23]. In contrast, autonomic C-fibre function indexes were in modest concordance with the Composite Autonomic Severity Index (CASS), a measure of the severity of generalized autonomic failure [24]. A recent study demonstrated that sudomotor examination is a highly sensitive detection tool of distal small-fibre (C-fibre) neuropathy [25]. Of note in the San Antonio Consensus Statement, tests of sudomotor function have been suggested to be performed (alongside cardiovascular reflex tests) for the diagnosis of autonomic neuropathy [26]. As available methods for sudomotor dysfunction are not widely available and require expensive technical equipment and trained personnel [22], a simple, cheap test, such as the NIT, can be included in the battery of the tests for the diagnosis of diabetic neuropathies in everyday clinical practice. Our study confirms that the NIT has a high sensitivity (86%), but a relatively low specificity (67%) in the detection of PSN. Similar results have been reported in a recent study by Papanas *et al.* [14]. The investigators have used the Diabetic Neuropathy Index [27] as a reference method for the diagnosis of PSN. In that study, sensitivity and specificity of the NIT were 94.4 and 69.7%, respectively.

The usefulness of the new test lies in its high sensitivity, high negative predictive value (86.5%) and its simplicity. It is of note that the low specificity of the NIT for the detection of PSN is because the test may be abnormal in some patients without PSN and not because of its inability to detect PSN. However, it is possible that the NIT may over-diagnose PSN in some patients. In our study, 22 out of the 67 (32.8%) patients without PSN had a positive NIT. Similar results were obtained

in previous studies by Papanas *et al.* (30.3%) [14] and Zick *et al.* (35%) [13]. This might be because sudomotor dysfunction is an early manifestation of diabetic polyneuropathy and can be detected early, even in patients without clinically apparent neuropathy and normal nerve conduction studies [28,29].

Simple and reliable methods are needed to screen patients for PSN in the primary care setting. Screening for PSN is justified for diagnosis, patient education, optimization of glycaemic control and the institution of foot care for the prevention of the foot-related complications in patients with diabetes [5]. Nevertheless, the optimal screening method for this purpose has not been specified. The American Diabetes Association clinical practice guidelines recommend the use of combinations of more than one screening test, such as pinprick sensation, temperature and vibration perception, the Semmes–Weinstein 10-g monofilament pressure sensation and ankle reflexes [5]. It has been reported that combinations of more than one test have > 87% sensitivity in the detection of PSN [5]. Nevertheless, clinical assessment of neuropathy with these instruments relies on the skill and interpretation of the individual clinician [30]. The Semmes–Weinstein 10-g monofilament test is probably a tool that can be most easily used in the diabetic population [31] and has been validated in prospective studies [32]. However, the validity of the NIT in the diagnosis of PSN is not inferior to the monofilament testing as the last has a sensitivity of 30–70% in various studies but its specificity is higher (90%) [30,33]. The NIT was tested against the validated NSS/NDS score and was found to be reliably sensitive in detecting PSN. For this reason, although the NIT is a measure of sudomotor function, which is part of autonomic integrity, because of the high concordance between PSN and sudomotor dysfunction, the NIT may be used as a first screening method for the detection of PSN.

CAN results from damage to the autonomic nerve fibres that innervate the heart and blood vessels and result in abnormalities in heart rate control and vascular dynamics [22]. PSN and CAN do not invariably coexist in diabetes [34,35]. A considerable proportion of patients may have pure

PSN or pure CAN in both types of diabetes [34]. These previous reports are in agreement with our results which have shown that only 50% of patients with PSN also had CAN and 60% of those with CAN concomitantly had PSN (Table 3).

The NIT had a low performance to detect milder forms of CAN (Table 2). This is in accordance with previous observations showing that sudomotor dysfunction of the lower extremities does not necessarily correlate with cardiac autonomic dysfunction. A more precise estimate of NIT's performance in detecting autonomic neuropathy could be obtained by comparing its results with those of the quantitative sudomotor axon reflex test (QSART) [36] or of the sympathetic skin response test [37]. This is a limitation of our study. Nevertheless, both the QSART and the sympathetic skin response test are used as research methods only; they are not widely available and require the purchase of expensive specialized equipment [22]. However, when more severe forms of CAN were present (as indicated by a Ewing score  $\geq 6$ ), the sensitivity of the NIT in detecting CAN increased from 41 to 81%. A subanalysis in the group of patients with severe CAN showed that the latter coexisted with PSN more often (in 81% of the cases) in comparison with the group of patients who had milder forms of CAN (coexistence rates 59%). It might be assumed that, in patients with diabetes, sudomotor dysfunction coexists with CAN more often when the latter is severe or, in other words, severe forms of CAN are more commonly related to a more diffuse form of diabetic neuropathy than the milder forms. Further prospective studies are needed in order to elucidate the possible mechanisms involved in the observed dissociation between the diagnosis of milder forms of CAN and the presence of peripheral sudomotor dysfunction.

In conclusion, the NIT has good sensitivity and negative predictive value for the diagnosis of PSN and can be used as a screening method for the detection of this complication in patients with diabetes. In addition, the test has low sensitivity for the detection of autonomic neuropathy in patients with milder forms of CAN.

## Competing interests

None to declare.

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