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## Reproducibility of the New Indicator Test for Sudomotor Function (Neuropad<sup>®</sup>) in Patients with Type 2 Diabetes Mellitus: Short Communication

### Abstract

The aim of this study was to examine the reproducibility of the new indicator test for sudomotor function (Neuropad<sup>®</sup>) in type 2 diabetic patients. The study included 142 type 2 diabetic patients (70 men) with a mean age of  $67.3 \pm 7.6$  years and a mean diabetes duration of  $14.2 \pm 6.3$  years. Sudomotor function was assessed by means of colour change in the indicator test. Each patient was examined twice. Moreover, inter-observer variability was assessed in 60 patients (35 patients with sudomotor dysfunction, 25 patients without sudomotor dysfunction). In the right foot, a highly significant ( $r = 0.91$ ,  $p = 0.001$ ) correlation was observed between time until complete colour change of the test on the first ( $910.7 \pm 431.6$  seconds) and second examination ( $935.8 \pm 440.1$  seconds). In the left foot, a highly significant ( $r = 0.89$ ,  $p = 0.001$ ) correlation was observed between time until complete colour change of the test on the first ( $911.6 \pm 430.3$  sec-

onds) and second examination ( $940.5 \pm 441.2$  seconds). Reproducibility was excellent both in patients with sudomotor dysfunction ( $p = 0.001$ ) and in those without sudomotor dysfunction ( $p = 0.001$ ). Agreement in diagnosis of sudomotor dysfunction between the two examinations was 98%. Inter-observer reproducibility was excellent ( $p = 0.001$ ), both in patients with sudomotor dysfunction and in those without sudomotor dysfunction. Intra- and interobserver Coefficient of Variance ranged between 4.1% and 5.1%. **Conclusions:** These results indicate that reproducibility of the new indicator test for sudomotor function is excellent in type 2 diabetic patients with or without sudomotor impairment.

### Key words

Diabetes mellitus · diabetic peripheral neuropathy · diabetic foot · sudomotor dysfunction

### Introduction

Peripheral neuropathy remains one of the most frequent complications of diabetes mellitus (La Cava, 2002; Perkins and Bril, 2003; Petit and Upende, 2003; Duby et al., 2004; Boulton, 2004b). It is linked to the pathogenesis of foot ulcers and contributes to a considerable increase in mortality (Boulton et al., 1998; Reiber et al., 1999; Boulton, 2004a; Boulton, 2004b; Edmonds, 2004). Sudomotor dysfunction, that is diminished sweat

production in the diabetic foot as a manifestation of neuropathy, renders the skin very sensitive to trauma and thus significantly contributes to the pathogenesis of foot ulceration (Reiber et al., 1999; Low, 2003; Boulton, 2004a). However, sudomotor dysfunction has so far been the Cinderella of diabetic complications. This is attributable to the fact that tests required for evaluation of sweat production have been too complicated to be used in everyday clinical practice (Low, 2003; Vinik et al., 2003).

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### Bibliography

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More recently, a new indicator test (Neuropad®) has been introduced, which very easily measures sweat production on the basis of a colour change from blue to pink (Zick et al., 2003). The new test has been reported to yield results that show good correlation with severity of peripheral neuropathy (Papanas et al., 2005). Nonetheless, there is no data on the reproducibility of the new test. Therefore, the aim of the present study was to examine the reproducibility of this new indicator test in the evaluation of sudomotor dysfunction in patients with type 2 diabetes mellitus.

## Materials and Methods

This study included 142 type 2 diabetic patients (70 men, 72 women) with a mean age of  $67.3 \pm 7.6$  years and a mean diabetes duration of  $14.2 \pm 6.3$  years. Patients were recruited from the Second Department of Internal Medicine of Democritus University of Thrace, Greece and from the Diabetic Department of the General Hospital of Alexandroupolis, Greece. The study was approved by the institutional ethics committee and all patients gave their informed consent.

Sudomotor dysfunction was assessed by means of the new indicator test (Neuropad®) (Zick et al., 2003; Papanas et al., 2005). Each patient was examined by the same physician (NP) on two separate visits. On each visit, patients were allowed to rest in constant room temperature ( $25^\circ\text{C}$ ) for 10 minutes after they had taken off their socks and shoes. Indicator tests were applied to both soles at the level of the 1st–2nd metatarsal heads. Time until complete colour change of the test from blue to pink was recorded (Papanas et al., 2005). Time until colour change was measured in seconds with an exactitude of 10 seconds. Complete colour change of the test in both feet within 600 seconds was considered normal response. Sudomotor dysfunction was defined as time until complete colour change of the test exceeding 600 seconds in at least one foot (Zick et al., 2003; Papanas et al., 2005).

Inter-observer variability was assessed in 60 patients (35 patients with sudomotor dysfunction, 25 patients without sudomotor dysfunction). These were also examined by a second physician (KP), who was blinded to the results of the examination by the first physician. The same room was used for examination by both physicians.

Moreover, in each of 20 patients (10 patients with sudomotor dysfunction and 10 patients without sudomotor dysfunction) Neuropad was applied 10 times by the first and 10 times by the second physician. For each patient, we calculated intra- and inter-observer Coefficient of Variance (CV %) of time until complete colour change on examination by the first and by the second physician. CV % was calculated by the formula:  $\text{CV \%} = \text{SD} / \bar{x} \times 100$  (SD = Standard Deviation;  $\bar{x}$  = average of measurements).

Exclusion criteria were as follows: age  $< 17$  years or  $> 75$  years, peripheral arterial occlusive disease, other potential causes of neuropathy (end-stage renal failure, alcohol abuse, Vitamin B<sub>12</sub> depletion, malignancy), thyroid disease, drugs (corticosteroids, antihistaminic and psychoactive drugs, which may affect sweating), peripheral nerve lesions (traumatic lesions, plexus paresis,

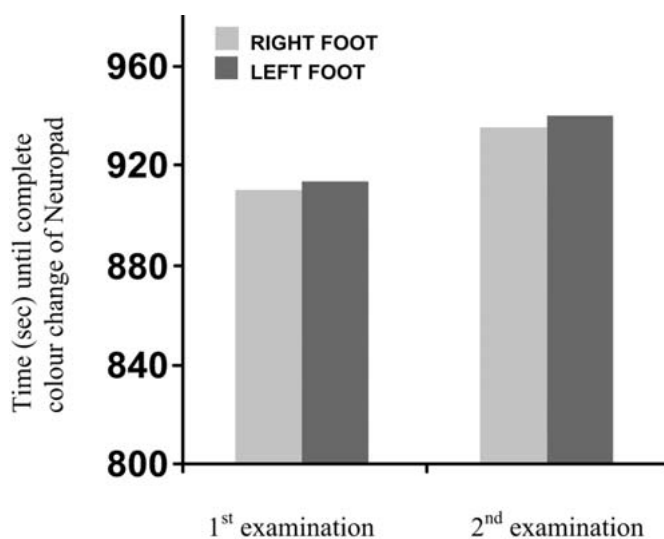


Fig. 1 Time until complete colour change of the test in the right foot (1st examination:  $910.7 \pm 431.6$  sec, 2nd examination:  $935.8 \pm 440.1$  sec,  $r = 0.91$ ,  $p = 0.001$ ) and in the left foot (1st examination:  $911.6 \pm 430.3$  sec, 2nd examination:  $940.5 \pm 441.2$  sec,  $r = 0.89$ ,  $p = 0.001$ ). On each examination, time until colour change showed a highly significant correlation between the two feet ( $r = 0.96$ ,  $p = 0.001$ ).

spinal root compression, herpes zoster, polyradiculopathy), skin diseases (neurodermatitis, psoriasis, scleroderma, allergy to metals, Raynaud syndrome, hyperhidrosia, acrocyanosis).

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) 11.0. Time until complete colour change of the test was a quantitative variable with normal distribution. Paired *t*-test was used to compare time until complete colour change of the test on each examination, as well as time until complete colour change of the test assessed by the two independent physicians. Data were expressed as mean  $\pm$  Standard Deviation ( $\bar{x} \pm 1\text{SD}$ ). Statistical significance was defined at a level of 5% ( $p < 0.05$ ).

## Results

In the right foot, a highly significant ( $r = 0.91$ ,  $p = 0.001$ ) correlation was observed between time until complete colour change of the test on the first ( $910.7 \pm 431.6$  seconds) and second examination ( $935.8 \pm 440.1$  seconds). In the left foot, a highly significant ( $r = 0.89$ ,  $p = 0.001$ ) correlation was observed between time until complete colour change of the test on the first ( $911.6 \pm 430.3$  seconds) and second examination ( $940.5 \pm 441.2$  seconds). On each examination, time until colour change showed a highly significant correlation between the two feet ( $r = 0.96$ ,  $p = 0.001$ ). These results are depicted in Fig. 1.

In each foot, further analysis investigated the correlation between time until complete colour change of the test on the first and second examination both in patients with sudomotor dysfunction and in those without sudomotor dysfunction. Results are summarized in Table 1.

**Table 1** Time until complete colour change of the indicator test (Neuropad®) on each patient examination, according to the presence or absence of sudomotor dysfunction

Foot examination	Time until complete colour change (seconds, mean ± SD)		Correlation coefficient	p value
	1st examination	2nd examination		
Right foot (with sudomotor dysfunction, n = 101)	1160.8 ± 241.5	1179.6 ± 262.4	r = 0.91	p = 0.001
Right foot (without sudomotor dysfunction, n = 41)	327.8 ± 117.6	343.1 ± 120.1	r = 0.89	p = 0.001
Left foot (with sudomotor dysfunction, n = 101)	1161.4 ± 245.1	1180.8 ± 250.2	r = 0.9	p = 0.001
Left foot (without sudomotor dysfunction, n = 41)	326.7 ± 115.9	344.9 ± 117.8	r = 0.89	p = 0.001

**Table 2** Time until complete colour change of the indicator test (Neuropad®) in the right and left foot, assessed by the two physicians

Foot examination	Time until complete colour change (seconds, mean ± SD)		Correlation coefficient	p value
	1st physician	2nd physician		
Right foot (total, n = 60)	880.7 ± 351.5	900.6 ± 376.5	r = 0.9	p = 0.001
Right foot (with sudomotor dysfunction, n = 35)	1250.7 ± 249.5	1269.8 ± 255.8	r = 0.91	p = 0.001
Right foot (without sudomotor dysfunction, n = 25)	300.9 ± 120.5	316.7 ± 116.7	r = 0.9	p = 0.001
Left foot (total, n = 60)	877.3 ± 358.3	902.3 ± 360.1	r = 0.92	p = 0.001
Left foot (with sudomotor dysfunction, n = 35)	1248.7 ± 247.6	1271.2 ± 230.1	r = 0.89	p = 0.001
Left foot (without sudomotor dysfunction, n = 25)	301.8 ± 111.5	317.4 ± 121.3	r = 0.92	p = 0.001

Sudomotor dysfunction was diagnosed in 101 out of 142 (71.1%) patients on first examination and in 99 out of 142 (69.7%) patients on second examination. Thus, agreement in diagnosis of sudomotor dysfunction between the two examinations was 98%.

A highly significant correlation was found between time until complete colour change of the test, assessed by the two observers (p = 0.001). This correlation was demonstrated both in patients with sudomotor dysfunction and in those without sudomotor dysfunction, as shown in Table 2. Sudomotor dysfunction was diagnosed in 35 out of 60 patients diagnosed by the first physician and in 34 out of 60 patients by the second physician, with a 97.1% agreement between the two physicians.

In patients with sudomotor dysfunction, intra-observer CV % ranged between 4.2% and 5.1% (right foot) and between 4.1% and 5% (left foot), while inter-observer CV% ranged between 4.3% and 4.9% (right foot) and between 4.3% and 4.9% (left foot) (Table 3). In patients without sudomotor dysfunction, intra-observer CV % ranged between 4.1% and 4.8% (right foot) and between 4.1% and 4.7% (left foot), while inter-observer CV% ranged between 4.3% and 4.7% (right foot) and between 4.2% and 4.5% (left foot) (Table 3).

## Discussion

This study investigated the reproducibility of the new indicator test for sudomotor function (Neuropad®) in patients with type 2 diabetes mellitus. Patients were examined on two separate visits. On each visit, time until complete colour change of the test from blue to pink was recorded (Papanas et al., 2005). In the right foot, a highly significant (r = 0.91, p = 0.001) correlation was observed between time until complete colour change of the test on the first and second examination. Similar results were obtained in the left foot. Hence, the test showed very good reproducibility in both feet.

Further analysis examined the influence of sudomotor dysfunction on the reproducibility of the test. In each foot, it was shown that the significant correlation between time until complete colour change of the test on first and second examination was observed both in patients with sudomotor dysfunction (p = 0.001) and in those without sudomotor dysfunction (p = 0.001). Accordingly, the excellent reproducibility of the test was not dependent on the presence of sudomotor dysfunction.

**Table 3** Intra- and inter-observer CV % in patients with sudomotor dysfunction (patients 1–10) and without sudomotor dysfunction (patients 11–20)

Pat. no.	Right foot CV%		Left foot CV%			
	intra-observer		inter-observer		inter-observer	
	1st exam.	2nd exam.	1st exam.	2nd exam.	1st exam.	2nd exam.
1	4.5%	4.6%	4.6%	4.3%	4.5%	4.4%
2	4.2%	4.4%	4.3%	4.5%	4.1%	4.3%
3	4.4%	4.3%	4.3%	4.5%	4.6%	4.6%
4	4.8%	4.6%	4.7%	4.8%	4.4%	4.6%
5	4.2%	4.6%	4.4%	4.4%	4.2%	4.3%
6	4.3%	4.7%	4.5%	4.6%	4.3%	4.4%
7	4.5%	4.7%	4.6%	5%	4.8%	4.9%
8	4.7%	5.1%	4.9%	4.8%	5%	4.9%
9	4.7%	4.3%	4.5%	4.6%	4.4%	4.5%
10	4.7%	4.4%	4.5%	4.3%	4.5%	4.8%
11	4.3%	4.6%	4.4%	4.6%	4.3%	4.4%
12	4.6%	4.8%	4.7%	4.7%	4.3%	4.5%
13	4.7%	4.5%	4.6%	4.3%	4.4%	4.4%
14	4.3%	4.4%	4.4%	4.4%	4.6%	4.5%
15	4.4%	4.1%	4.3%	4.3%	4.7%	4.5%
16	4.5%	4.7%	4.6%	4.6%	4.3%	4.5%
17	4.4%	4.6%	4.5%	4.5%	4.3%	4.4%
18	4.6%	4.5%	4.6%	4.3%	4.5%	4.4%
19	4.5%	4.2%	4.4%	4.1%	4.3%	4.2%
20	4.3%	4.3%	4.3%	4.2%	4.4%	4.3%

Sudomotor dysfunction was diagnosed in 101 out of 142 (71.1%) patients on first examination and in 99 out of 142 (69.7%) patients on second examination. This finding is in accord with two prior studies (Zick et al., 2003; Papanas et al., 2005). There was excellent agreement (98%) in the diagnosis of sudomotor dysfunction between the two examinations. Consequently, the indicator test was very reliable in diagnosing sudomotor dysfunction.

Additionally, a highly significant correlation was found between time until complete colour change of the test, assessed by the two observers ( $p=0.001$ ). This significant correlation was observed both in patients with sudomotor dysfunction ( $p=0.001$ ) and in those without sudomotor dysfunction ( $p=0.001$ ). Agreement in the diagnosis of sudomotor dysfunction between the two physicians was excellent (97.1%). As a result, the test showed very good inter-observer reproducibility, irrespective of sudomotor dysfunction.

Intra- and inter-observer Coefficient of Variance (CV %) ranged between 4.1% and 5.1% in patients with sudomotor dysfunction and between 4.1% and 4.8% in those without sudomotor dysfunction. These good CV % are explicable on the basis of the chemical nature of the test. Indeed, blue Cobalt (II) Chloride be-

comes pink Cobalt (II) Chloride Hexahydrate, the end-product being extremely stable, without being influenced by light and temperature (Budavari et al., 1996; Young, 2003). This chemical reaction does not require patients' or examiners' co-operation, in contrast to clinical examination and quantitative sensory testing (Bax et al., 1996; Boulton, 2004 b).

The clinical implication of our findings is that the new indicator test may reliably be used to evaluate sudomotor function in type 2 diabetic patients. We have previously demonstrated that the test exhibits very good intra-individual reproducibility between right and left foot (Papanas et al., 2005). The highly significant ( $p=0.001$ ) correlation between results in right and left foot was found again in the present study. More importantly, it was shown for the first time that the test yielded results which were highly reproducible on re-examination. Additionally, there was excellent inter-observer reproducibility. Given that reproducibility of diagnostic tests is of paramount importance in the evaluation of diabetic neuropathy, both somatic and autonomic (Valensi et al., 1993; Bax et al., 1996; Kempler, 2003), the findings of the present study suggest an important role for the new test in detecting sudomotor dysfunction. Accurate evaluation of sudomotor function would be consistent with the recommendation of the San Antonio Consensus on diabetic neuropathy, which suggested incorporation of sudomotor examination in the overall assessment of neuropathy (American Diabetes Association and American Academy of Neurology, 1988). This reproducibility even allows us to speculate that the new indicator test might be used to assess whether sudomotor function deteriorates over time and, thus, it might prove of value in the regular evaluation of diabetic complications during patient follow-up. However, prospective studies are needed to address this issue.

In conclusion, the new indicator test for sudomotor function appears to have excellent inter-observer and intra-observer reproducibility in type 2 diabetic patients. Reproducibility is not dependent on the presence of sudomotor dysfunction. These findings suggest that the indicator test yields reliable results. Certainly, further studies are needed to verify the reproducibility of the test, as well as to evaluate its potential role in the prospective evaluation of diabetic complications.

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