

Diagnosis of Diabetic Neuropathy Using Simple Somatic and a New Autonomic (Neuropad®) Tests in the Clinical Practice

The modified Neuropathy Disability Score and the new test for sudomotor dysfunction Neuropad stratify patients according to their general risk profile and can be used for complex evaluation of diabetic somatic and autonomic neuropathy

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Key words

- diabetic neuropathy
- sudomotor dysfunction
- NDS
- neuropad

Abstract



Aim: The global spread of diabetes (DM) and the importance of early therapeutic intervention determine the need of simple, inexpensive and sensitive methods for diagnosis of diabetic complications in the general practice. The aim of this study was to assess a new instrument – the plaster Neuropad in diagnosing the sudomotor diabetic dysfunction and to investigate the correlates of Neuropad data with diabetic complications.

Patients and methods: In this cross-sectional study participated 264 inpatients (M/F=126/138) with DM type 1/2 (61/203), mean age 55.4 ± 12.0 and DM duration of 9.3 ± 7.1 years. According to hospital records were registered: anthropometric data; fasting plasma glucose and HbA1c; presence of micro-(retino-, nephro-, neuropathy), and macrovascular (arterial hypertension, coronary artery disease and/or brain vascular disease) complications, and neuropathic symptoms were evaluated. For investigation of somatic DN a modified Neuropathy Disability Score (NDS) and for sudomotor autonomic DN – Neuropad were used.

Results: Neuropad showed the highest between-feet correlation of 0.91 compared to all other individual tests and the NDS. Neuropad was able to separate patients in groups with different general risk profile, including age, duration of DM, presence of coronary and/or brain vascular disease, nephropathy, and retinopathy. Moreover, Neuropad differentiated patient groups by their stage of DN, evaluated by symptoms, diagnosis, the individual somatic tests and with the highest significance – by NDS. Most sensitive for detecting DN was $NDS \geq 3$, followed by Achilles reflexes, vibration perception (128 Hz tuning fork) and Neuropad. A borderline or abnormal result of Neuropad showed sensitivity=76.3/79.3, specificity=56.1/42.9, positive=86.3/62.8 and negative=39.5/63.0 predictive values, and diagnostic accuracy 72.2/62.9%, compared to the indices for presence of somatic DN ($NDS \geq 3$)/foot at risk ($NDS \geq 6$) respectively.

Conclusions: Screening for DN must cover somatic and autonomic disturbances. Neuropad is a new sensitive and appropriate for everyday clinical use test for detecting sudomotor DN and identification of patients at higher risk for chronic diabetes complications.

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Introduction



The global spread of diabetes and the importance of early intervention determine the need of simple, inexpensive methods for early diagnosis of diabetes complications in the general practice. The most common chronic diabetic complication is diabetic neuropathy (DN) with its most prevalent form – distal symmetric sensory motor diabetic polyneuropathy (DSN). It is often accompanied by distal (sympathetic) autonomic neuropathy, and signs of autonomic dysfunction are often apparent on examinations: these mainly include dry skin [Boulton et al., 2004]. DSN and

distal (sympathetic) autonomic neuropathy have important role in diabetic foot syndrome pathology and associated with high risk of foot ulceration [Boulton, 2004; Tentolouris et al., 2009]. Various simple instruments for diagnosing of different sensory modalities or motor functions have been introduced from the neurological into the general practice – Semmes-Weinstein monofilament, Rydel-Seiffer tuning fork, thermal discrimination devices, tactile circumferential discriminator, muscle power handgrip dynamometer, neurological hammer [for review Boulton et al., 2004; Grant et al., 1999].

Diabetic autonomic neuropathy (DAN) develops in most cases simultaneously with DSN and is seldom an object of investigations. One aspect of DAN is the sudomotor dysfunction – a predisposing factor to fissures, infection and ulceration. There are several tests for evaluation of sudomotor function [Low, 2003]. Significant work has been done using tests like Thermoregulatory Sweat Test (TST) [Guttmann, 1947], modified by Fealey RO [Fealey, 1993], Quantitative Sudomotor Axon Reflex Test (QSART) [Lång et al., 1995], Skin Potential Recording [Shahani et al., 1984] of the Sympathetic skin response (SSR). Recently V.A.Low et al. using TST, autonomic reflex screen (ARS), and nerve conduction studies and electromyography in patients with distal small-fiber neuropathy (DSFN) including diabetic etiology, concluded that sudomotor examination is a highly sensitive detection tool in DSFN. Autonomic involvement is mainly distal, and additionally may involve adrenergic and the long cardiovagal fibers [Low et al., 2006]. Although very sensitive, these tests are not applicable for everyday outpatient practice. Therefore simple and reliable methods for assessing Sudomotor dysfunction are needed. Neuropad has been developed recently [Papanas et al., 2005] as an accessible tool for determining the sudomotor function (sweating) on the soles [for review Schnell et al., 2008]. The references about this device in the literature are still scarce. Recently, it has been shown, that the responses with Neuropad correlate with different somatic and autonomic tests and nerve fiber density [Liatis et al., 2007; Quattrini et al., 2008]. The method is reliable and simple for use [Tentolouris et al., 2007] with excellent reproducibility [Papanas et al., 2005]. Recently we tested Neuropad in diabetic men with erectile dysfunction – a typical example of complex neuronal and macrovascular involvement, showing that it is linked more to microangiopathic complications and neuropathy than to macroangiopathic disturbances [Kamenov et al., 2007].

The aim of this study was to assess a new instrument – the plaster Neuropad in diagnosing the sudomotor diabetic dysfunction

and to investigate the correlates of Neuropad data with diabetic complications.

Patients and Methods



In this cross-sectional study participated 264 consecutive inpatients with diabetes type 1 and 2 from the contingency of a University endocrine clinic, where all following procedures are routinely performed. This is the largest study with Neuropad published in the literature. The inclusion criteria were: Diagnosis of DM type 1 or 2 according to the ADA (2004) criteria and independently of the duration of the disease. Fasting plasma glucose (FPG) should have been stable the last week and in the range 3.5–12 mmol/L.

Exclusion criteria:

- ▶ Another type of neuropathy (exclusion made by neurologist).
- ▶ Unstable diabetes. More important was the lack of high excursions (>5 mmol/L for corresponding points in the blood sugar profile), than the particular value.
- ▶ Recent acute diabetic complications – ketoacidosis, severe hypoglycemia.
- ▶ Drugs, which can interfere with the neuropathic examination – analgesics, tricyclic antidepressants, anticonvulsants, etc.

Patient database

According to the hospital records and diagnoses, a simple and applicable also for outpatient offices database was developed for every patient. It consisted of several parts:

1. Demographic and anthropometric data, HbA1c and FPG (Table 1).
2. Diabetes data – type, duration since diagnosis and treatment – diet, tablets, insulin, insulin+tablets.
3. Arterial hypertension (yes/no) was defined, if blood pressure was > 140/90 mmHg and/or the patient was already on anti-

Table 1 Demographic, anthropometric and diabetes data of the patients.

Parameter	All patients	Men			Women		
		total	DM1	DM2	total	DM1	DM2
N	264	126	36	90	138	25	113
age (years)	55.4±12.0	53.5±13.1 C	35.6±9.2 a	60.6±8.3	57.1±11.1	37.6±9.3 a	61.4±8.1
height (cm)	166.1±7.5	172.5±5.7 A	173.5±5.6	172.1±5.8 A	160.3±6.0	163.5±6.5 b	159.5±5.6
weight (kg)	82.2±14.5	85.2±14.4 B	78.9±14.1 b	87.7±14.2 C	79.4±14.6	65.0±10.1 a	82.6±14.2
BMI (kg/m ²)	29.9±5.1	28.6±4.4 B	26.2±4.1 b	29.6±4.1 A	31.0±5.6	24.3±3.5 a	32.5±5.4
waist (cm)	99.5±13.0	100.7±12.4	92.1±11.0 Aa	104.1±10.7	98.4±13.6	79.2±10.3 a	102.7±11.5
hip (cm)	106.9±10.1	102.9±8.0 A	97.1±7.6 a	105.2±7.4 A	110.6±11.0	100.1±7.5 a	112.9±10.7
W/H	0.93±0.08	0.98±0.06 A	0.94±0.06 b	0.99±0.05 A	0.89±0.07	0.79±0.07 a	0.91±0.06
duration of DM (y)	9.3±7.1	8.7±6.8	8.5±6.3 A	8.8±6.2	9.9±7.2	13.2±8.5 c	9.2±6.7
FPG (mmol/L)	7.3±1.8	7.1±1.6	7.5±1.5 B	6.9±1.5	7.5±1.9	8.0±2.4	7.4±1.7
HbA1c	9.3±1.7	9.0±1.7 C	9.7±1.5 Bc	8.7±1.5 B	9.6±1.7	9.1±1.4	9.7±1.7

C=p<0.05; B=p<0.01; A=p<0.001 between men and women in corresponding groups

c=p<0.05; b=p<0.01; a=p<0.001 between DM1 and DM2 inside the men and women groups

hypertensive treatment. The duration since diagnosis and number of drugs used were recorded.

4. Dyslipidemia (yes/no) was accepted if total cholesterol > 5.2 mmol/L, and/or HDL-C < 1.0 mmol/L for men and < 1.3 for women, and/or triglycerides > 1.7 mmol/L and/or the patient was already on antilipemic treatment. The duration and treatment of dyslipidemia were recorded.
5. Coronary artery disease and/or brain vascular disease (CAD/BVD) (yes/no) were defined as presence of corresponding symptoms, and/or already documented diagnosis from a cardiologist or neurologist, and/or specific treatment for CAD/BVD.
6. Nephropathy (yes/no) – microalbuminuria > 30 mg/24 h or proteinuria > 0.5 g/24 h, attributed to diabetes, and taking into consideration other kidney diseases; duration.
7. Retinopathy (yes/no) – all stages of diabetic retinopathy, diagnosed by an ophthalmologist; duration.
8. Neuropathy. A multilevel approach with different diagnostic aspects was used:
 - ▶ Are actual symptoms of diabetic neuropathy presented (yes/no): positive – including different types of painful complaints, and negative – numbness or “feet feel dead” etc.?
 - ▶ Has the diagnosis “diabetic neuropathy” been set before by a neurologist and documented in patients records (yes/no)?
 - ▶ Further DN was investigated with objective methods – NDS and Neuropad.

Modified NDS

Detailed description of modified NDS is given elsewhere [Boulton et al., 2004]. In a large prospective study patients with NDS ≥ 6 points had a sixfold increased risk for developing a foot ulcer [Abbott et al., 2002]. We used a modified NDS, including 4 items tested on both feet.

1. Vibration perception (VP) threshold estimated with 128 Hz Rydel-Seiffer tuning fork (Thio-Vib), graduated in 8 stages on both vibrating branches. Application – on the apex of the big toe. Normal (can distinguish $\geq 6/8$) = 0 points. Abnormal (cannot distinguish $\leq 5/8$) = 1 p.
2. Thermal perception (TP) with an instrument (Thio-Term), based on the difference of thermal conductivity (metal and plastic) causing different subjective feeling. Application – on the skin of the sole 1–2 cm distally of the place between metatarsal heads I-II, but not on callus. Normal (can distinguish) = 0 p. Abnormal (cannot distinguish) = 1 p.
3. Semmes-Weinstein monofilament 5.07 (MF) (Thio-Feel) [Kumar et al., 1991]. Applied at the same place like TP. Normal (can feel) = 0 p. Abnormal (cannot feel) = 1 p.
4. Achilles reflex (AR) as routinely examined. Present = 0 p. Present with reinforcement = 1 p. Absent = 2 p.

All procedures have been done in a quiet room with temperature 18–22 °C after the patient has been at rest for 10 min. Every procedure had been performed in at least 3 “active” trials and some “placebo” trials with the patient not seeing the instruments. The maximum NDS for each foot is 5 and for both feet – 10 respectively. We used the accepted two cut-offs for interpretation of NDS results: presence of DN if NDS ≥ 3 and foot at risk if NDS ≥ 6 points. DN was categorized as light, moderate and severe if NDS = 3–5; 6–8, and 9–10 points respectively [Abbott et al., 2002; Young et al., 1993].

Neuropad®

The diagnostic test Neuropad® (Miro Verbandstoffe GmbH, Wiehl, Germany) has been developed for early detection of sudomotor disturbances as a maker of DAN of the feet and for early recognition of the diabetic foot syndrome. It is an adhesive indicator test that changes color when applied to the skin of the foot. The indicator material is a cobalt-containing compound. The results of the test depend on the duration of color change from blue to pink. In trials with permanent registration of the color changes it has been determined [Zick et al., 2003], that 10 min is a sufficient period for differentiating normal from abnormal sweating and this time interval has been set as cut-off for evaluation of the test result. After all NDS-procedures the plaster was stacked on both soles upon the same place, where MF and TP tests were performed. After 10 min the examining person read the color of the indicator and scored it as normal (complete change from blue to pink) = 0 p., borderline (mottled blue/pink color) = 1 p., abnormal (no color change – the test remain blue) = 2 p.

To fulfill the aim of the study we had to answer several questions:

- ▶ Are NDS and Neuropad able to distinguish groups with different diabetes duration and complications – nephropathy, retinopathy, arterial hypertension, dislipidemia and CAD/BVD. What is the particular influence of different demographic, anthropometric, diabetes and its complications factors on NDS and Neuropad?
- ▶ Is Neuropad able to differentiate groups with different stage of DN – comparison with other indices of neuropathy (symptoms, diagnosis and signs including NDS – tests)?
- ▶ Is there difference between both feet for each used test and what are the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of Neuropad, compared to NDS?

Statistical analysis

The data base was processed with the statistical package SPSS 13.0.1. The significance level for rejecting of the null hypothesis was $p < 0.05$. The following statistical methods were applied: descriptive and variation analyses; One sample nonparametric test of Kolmogorov-Smirnov; One-way ANOVA; T-test of Student for two independent samples; Nonparametric tests of Kruskal-Wallis for several independent samples and of Mann-Whitney for two independent samples; Binary logistic regression; Multinomial logistic regression; Chi-square test; Correlation analysis (Kendall's tau-b and Spearman's rho); Criteria of validation of screening test.

Results

Demographic and anthropometric data are presented on **Table 1**. DM2 had 71.4% of men and 81.9% of women. DM1 patients were younger, lighter, slimmer and taller (only for women). DM1-women had the longest duration of the disease. In general, patients were with inadequate diabetes compensation. DM2-men had the “best” and DM1-men – the poorest glucemic control.

We estimated the number of patients who had difference between both feet when performing each test (**Table 2**). In total different VP had 11.7% of the patients, TP – 8.7%, MF – 8.7%, AR – 6.1(2.7+3.4)% and Neuropad – 5.7(4.9+0.8)%. For the AR and

value	VP	TP	MF	AR	Neuropad
0	64 (24.2)	129 (48.9)	171 (64.8)	52 (19.7)	68 (25.8)
0.5	31 (11.7)	23 (8.7)	23 (8.7)	7 (2.7)	13 (4.9)
1	169 (64)	112 (42.4)	70 (26.5)	83 (31.4)	164 (62.1)
1.5				9 (3.4)	2 (0.8)
2				113 (42.9)	17 (6.4)
correlation left/right	0.72	0.82	0.80	0.90	0.91
for total NDS = 0.90					

Data presented as N(%)

Table 2 Patients with different results for the two feet in different tests (groups 0.5 and 1.5) and between-feet correlations.

Parameter	NDS					
	≤ 2	p	≥ 3	≤ 5	p	≥ 6
N	57		207	119		145
age†	45.6 ± 13.8	***	58.0 ± 10.7	50.8 ± 14.5	***	59.1 ± 9.2
men	18 (31.6)	**	108 (52.2)	45 (37.8)	**	81 (55.9)
height (cm)	165.9 ± 7.3	NS	166.1 ± 7.6	165.0 ± 7.5	*	167.0 ± 7.4
weight (kg)	78.1 ± 13.7	NS	83.3 ± 14.6	77.7 ± 12.5	***	85.9 ± 15.7
BMI (kg/m ²)	28.4 ± 4.9	NS	30.2 ± 5.2	28.6 ± 4.8	**	30.9 ± 5.5
waist (cm)	92.9 ± 12.3	***	100.3 ± 12.8	94.7 ± 12.9	***	103.4 ± 12.3
hip (cm)	104.2 ± 9.1	NS	107.7 ± 10.2	104.4 ± 9.0	**	109.0 ± 10.6
W/H ratio	0.89 ± 0.08	***	0.94 ± 0.07	0.91 ± 0.08	***	0.95 ± 0.07
DM 2‡	35 (61.4)	**	168 (81.2)	79 (66.4)	***	124 (85.5)
duration of DM†	5.5 ± 6.0	***	10.4 ± 7.0	6.9 ± 5.9	***	11.3 ± 7.6
FPG (mmol/L)	7.5 ± 1.6	NS	7.2 ± 1.8	7.3 ± 1.7	NS	7.3 ± 1.9
HbA1c (%)	9.1 ± 1.6	NS	9.4 ± 1.7	9.4 ± 1.7	NS	9.2 ± 1.6
AH‡	35 (61.4)	**	168 (81.2)	77 (64.7)	***	126 (86.9)
duration of AH†	7.0 ± 5.2	*	10.5 ± 6.8	8.0 ± 5.8	**	11.0 ± 7.1
N of drugs for AH	1.7 ± 0.7	NS	2.0 ± 0.8	1.8 ± 0.7	NS	2.1 ± 0.8
CHD/BVD‡	7 (12.3)	*	60 (29.0)	22 (18.5)	**	45 (31.0)
dyslipidemia‡	24 (42.1)	NS	91 (44.0)	51 (42.9)	NS	64 (44.1)
nephropathy‡	1 (1.8)	NS	19 (9.2)	3 (2.5)	**	17 (11.7)
retinopathy‡	4 (7.0)	***	72 (34.8)	20 (16.8)	***	56 (38.6)
symptoms of DN‡	19 (33.3)	***	168 (81.2)	64 (53.8)	***	123 (84.8)
diagnosis of DN‡	13 (22.8)	***	120 (58.0)	44 (37.0)	***	89 (61.4)

AH=arterial hypertension; †-data presented in years; ‡- data presented in N (% of patients in the same group); *-p<0.05; **-p<0.01; ***-p<0.001

Table 3 Profiles of patient-groups divided by NDS (cut-off ≤2/≥3 and ≤5/≥6).

Neuropad test there were no patients with a very large difference (0 and 2 p on both feet).

NDS

The groups, separated by NDS are presented on **Table 3**. There was significant difference in most parameters between patients with foot at risk (NDS≥6) and the other. The differences between neuropathic (NDS≥3) and non-neuropathic groups were not so pronounced. Further the importance of different risk factors – demographic, anthropometric, diabetes and its complications for having foot “at risk” was evaluated (**Table 4**). The most important complication of DN is the leg amputation, usually preceded by ulceration. In our study each year of age increased the risk for having NDS≥6 with 4.4%, but each diabetes year – with 7%; being male – 2.6 times (included other unfavorable vascular risk factors); each centimeter of the waist (2.8%) and hip (3.8%) or kilogram more (2.4%) etc. Interestingly, the presence of nephropathy (5.1 times) was more important risk factor than having symptoms (4.6 times) or diagnosis DN (2.8 times). Retinopathy was intermediate (3.6 times) and history of CHD/BVD did not increase significantly the risk for NDS≥6.

Neuropad

Next phase of the study was to evaluate if Neuropad is able to stratify patient at different general risk. The test-results of Neu-

ropad (mean from both feet) were divided in 5 categories: 0p. (both feet=0) had 68 patients; 0.5 p. (one leg=1, other leg=0) – 13; 1 p (1/1) – 164; 1.5 p. (2/1) – 2; 2 p. (2/2) – 17 respectively. For statistical reasons the persons with 0 and 0.5 p. were combined in one group (81 pts.) and these with 1.5 and 2 p. in another group (19 pts.), presented on **Table 5**.

Further the importance of each studied factor for having positive result with Neuropad was determined. The significant ORs for borderline (1 p.) and abnormal (2 p.) results are presented in **Table 6**. Although the length of the neurons is an important risk factor for nerve damage in DN, the height was not found to be a significant determinant for NDS and Neuropad. As expected, age, diabetes duration and all microvascular complications were predictors for abnormal responses to Neuropad, but unlikely NDS, having macrovascular disease appeared to be risk factor for Neuropad as well.

NDS & Neuropad

An important point in diabetic neuropathy studies is the possibility to identify the foot at risk for future ulceration and amputation. The predictive value of different diagnostic methods must be determined in prospective studies analyzing the actual incidence of the main endpoint – number of ulcerations and/or amputations. This study was a cross-sectional one and was not aimed to estimate these primary endpoints. We compared Neu-

Factor		OR (CI) for having NDS ≥ 6
age (for each year more)	NA	1.039*** (1.021–1.057)
	ADJ	1.044** (1.026–1.064)
male gender	NA	2.081** (1.269–3.413)
	ADJ	2.633*** (1.543–4.492)
weight (for each kilogram more)	NA	1.026** (1.011–1.041)
	ADJ	1.024** (1.009–1.039)
BMI (for each 1 kg/m ² more)	NA	1.055** (1.015–1.097)
	ADJ	1.060** (1.017–1.105)
waist circumference (for each centimeter more)	NA	1.036*** (1.019–1.053)
	ADJ	1.028** (1.010–1.046)
hip circumference (for each centimeter more)	NA	1.029** (1.009–1.050)
	ADJ	1.038** (1.015–1.061)
waist/hip ratio	NA	1.645*** (1.250–2.164)
	ADJ	1.250 (0.907–1.723)
diabetes duration (for each year more)	NA	1.066*** (1.032–1.100)
	ADJ	1.070*** (1.034–1.108)
insulin treatment	NA	1.089 (0.666–1.779)
	ADJ	1.691 (0.970–2.948)
arterial hypertension (AH)	NA	3.486*** (1.889–6.434)
	ADJ	2.601** (1.289–5.247)
duration of AH (for each year more)	NA	1.049* (1.011–1.088)
	ADJ	1.051* (1.012–1.093)
number of drugs for treatment of AH	NA	1.335* (0.991–1.798)
	ADJ	1.320 (0.969–1.798)
nephropathy	NA	4.423* (1.249–15.667)
	ADJ	5.136* (1.306–20.195)
retinopathy	NA	3.038*** (1.716–5.538)
	ADJ	3.581*** (1.902–6.743)
symptoms of DN	NA	4.805*** (2.692–8.576)
	ADJ	4.565*** (2.419–7.965)
set diagnosis of DN	NA	2.709*** (1.643–4.468)
	ADJ	2.773*** (1.625–4.733)
CHD/BVD	NA	1.984* (1.109–3.549)
	ADJ	1.491 (0.802–2.772)

Only the significant factors are presented. ADJ – adjusted for gender and age (for the factor gender – adjusted for age and for age – adjusted for gender); NA – not adjusted. * -p<0.05; ** -p<0.01; *** -p<0.001

Table 4 Importance of different risk factors – demographic, anthropometric, diabetes and its complications for having NDS ≥ 6 .

ropad to proven risk-assessment score like NDS. The next analysis was focused on the differences in neurological parameters – symptoms, diagnosis and all tests of NDS in the three Neuropad groups (**Table 7**). Patients with normal Neuropad differed significantly in all indices for DN from those with borderline and abnormal Neuropad response. The results for sensitivity, specificity, positive and negative predictive value are presented on **Table 8**. To investigate the concordance of (1) presence of actual neuropathic symptoms (2) presence of neuropathy in the patient's records and (3) results of different neurological tests, the patients were divided in two groups – 187 (70.8%) symptomatic and 77 (29.2%) asymptomatic (**Table 9**). For each group the proportion of patients with a diagnosis of DN in the history was estimated and the proportion with pathological tests.

Discussion

In this study, we analyzed different simple neurological tests, which are used routinely. When choosing the test for diagnosing of DN we took into consideration that it should be objective, informative for sensory function, broadly accepted and used, not time-consuming and simple to be performed by a trained non-specialist in the community as well. The modification of NDS, created by MJ Young et al. [Young et al., 1993] and used in sev-

eral large studies [Zick et al., 2003; Cabezas-Cerrato, 1998] was recommended by the expert group (Neurodiab) of the European Association for the Study of Diabetes [Boulton et al., 1998 Jun; Boulton, 1998 Nov]. It has been shown NDS ≥ 6 to be the best predictor for foot ulceration and the best neuropathy end point in a large prospective community study [Kumar et al., 1991]. Neuropad is a relatively new test for autonomic dysfunction based on sudomotor evaluation. One advantage of this test is its independence from the cooperation of the patient. The patient himself can also apply the plaster. The base of the comparison of both types of tests is that in most cases the distal small fiber somatic and autonomic DN develop and progress in parallel [Singer et al., 2004]. The small fiber orientation of this study and the good predictive value of monofilaments were the reasons for using them [Kumar et al., 1991; Pham et al., 2000]. For methodological reasons it was necessary to determine the proportion of patients with between-feet difference in the tests. There were no patients with very high difference in the 3-grades tests (AR and Neuropad). The between-feet correlation of Neuropad was highest. The groups with difference of ≤ 1 point were small and were further included in the respective larger neighbor groups. The results of this between-feet comparison support the cheaper and less time-consuming single-foot investigation. Another point of view is that a larger between-feet difference could stress attention to other types of neuronal damage. Our

Table 5 Comparison of Neuropad to demographic, anthropometric and diabetes indices.

Group	2	p for 2/1 groups	1	p for 1/0 groups	0	p for 0/2 groups
N	19		164		81	
age†	64.1 ± 15.6	*	57.7 ± 14.0	***	48.6 ± 15.4	***
male gender‡	10 (52.6)	NS	81 (49.4)	NS	35 (43.2)	NS
height (cm)	165.3 ± 10.9	NS	165.7 ± 9.3	NS	167.0 ± 9.2	NS
weight (kg)	77.6 ± 14.6	NS	82.9 ± 19.2	NS	81.8 ± 18.6	NS
BMI (kg/m ²)	28.7 ± 6.3	NS	30.2 ± 6.7	NS	29.5 ± 6.9	NS
waist (cm)	98.9 ± 13.5	NS	100.7 ± 16.8	NS	97.1 ± 15.9	NS
hip (cm)	106.0 ± 15.3	NS	107.5 ± 13.1	NS	105.9 ± 12.5	NS
W/H	0.94 ± 0.07	NS	0.94 ± 0.10	NS	0.92 ± 0.09	NS
DM 2‡	14 (73.7)	NS	137 (83.5)	***	52 (64.2)	NS
duration of DM†	15.2 ± 10.2	*	10.0 ± 9.0	**	6.5 ± 7.1	***
FPG (mmol/L)	7.6 ± 2.4	NS	7.1 ± 2.2	NS	7.5 ± 2.3	NS
HbA1c (%)	9.5 ± 2.4	NS	9.3 ± 2.1	NS	9.3 ± 2.1	NS
hypertension‡	16 (84.2)	NS	130 (79.3)	NS	57 (70.4)	NS
CHD/BVD‡	3 (15.8)	*	56 (34.1)	***	8 (9.9)	NS
dyslipidemia‡	6 (31.6)	NS	74 (45.1)	NS	35 (43.2)	NS
nephropathy‡	3 (15.8)	NS	15 (9.1)	*	2 (2.5)	**
retinopathy‡	10 (52.6)	*	47 (28.7)	NS	19 (23.5)	**

†-data presented in years; ‡- data presented in N (% of patients in the same group); *-p<0.05; **-p<0.01; ***-p<0.001. Neuropathy data are given separately in **Table 6**

Factor		OR (CI) for having Borderline (1 point)	OR (CI) for having Abnormal (2 points)
age (for each year more)	NA	1.041*** (1.021–1.060)	1.081*** (1.037–1.128)
	ADJ	1.043*** (1.024–1.063)	1.085*** (1.040–1.131)
diabetes duration (for each year more)	NA	1.056** (1.018–1.096)	1.115*** (1.054–1.178)
	ADJ	1.056** (1.016–1.096)	1.110*** (1.048–1.117)
insulin treatment	NA	0.871 (0.508–1.491)	6.955* (1.506–32.115)
	ADJ	1.467 (0.792–2.718)	16.075** (3.251–79.489)
nephropathy	NA	3.926 (0.876–17.607)	2.167 (0.186–25.223)
	ADJ	4.933* (1.027–23.689)	3.013 (0.232–39.131)
retinopathy	NA	1.290 (0.696–2.388)	3.567* (1.264–10.066)
	ADJ	1.364 (0.717–2.596)	4.104* (1.378–12.221)
symptoms of DN	NA	2.746** (1.551–4.861)	3.000 (0.916–9.830)
	ADJ	2.092* (1.132–3.867)	2.033 (0.586–7.046)
diagnosis of DN	NA	2.542** (1.463–4.417)	2.603 (0.939–7.213)
	ADJ	2.491** (1.402–4.429)	2.802 (0.966–8.129)
CHD/BVD	NA	4.731*** (2.130–10.510)	1.711 (0.408–7.171)
	ADJ	3.576** (1.574–8.123)	1.038 (0.239–4.515)

Only the significant factors are presented. ADJ – adjusted for gender and age (for the factor age – adjusted for gender);

NA – not adjusted. *-p<0.05; **-p<0.01; ***-p<0.001

Table 6 Importance of different risk factors – demographic, anthropometric, diabetes and its complications for having positive results with Neuropad.**Table 7** Comparison of other indices of DN between the groups, divided by Neuropad.

Group	2	p for 2/1 groups	1	p for 1/0 groups	0	p for 0/2 groups
N	19		164		81	
neuropathy symptoms	15 (78.9)	NS	127 (77.4)	***	45 (55.6)	*
diagnosed neuropathy	11 (57.9)	NS	94 (57.3)	***	28 (34.6)	*
NDS	7.8 ± 2.0	**	5.8 ± 2.8	***	4.0 ± 3.1	***
VP	17 (89.5)	***	108 (65.9)	*	44 (54.3)	**
TP	13 (68.4)	NS	73 (44.5)	*	26 (32.1)	**
MF	9 (47.4)	NS	48 (29.3)	**	13 (16.0)	**
AR	18 (94.7)	*	138 (84.1)	***	49 (60.5)	***

Data presented as N (%) of patients with abnormal test-results from the respective groups, separated by Neuropad. Exception: NDS - mean ± SD. *-p<0.05; **-p<0.01;

***-p<0.001 between group differences

patients were pre-selected not to have other types of neurological diseases.

Neuropad was able to separate patients in groups with different general risk profile, including age, duration of DM, presence of

CHD/BVD, nephropathy, and retinopathy. In particular, age was an important determinant of Neuropad (and all other somatic tests). For this reason, we eliminated this confounding factor when further calculated the ORs. Moreover, Neuropad differen-

tiated patient groups by their stage of DN, evaluated by symptoms, diagnosis, all individual somatic tests and with the highest significance – by NDS (Table 6 and 7). In this analysis the difference between the group with normal Neuropad and borderline/abnormal Neuropad-groups was highly significant. This observation was the background for our decision to use this cut-off (negative/any abnormal result of Neuropad) to compare further Neuropad to NDS (Table 8 and 9). Microvascular complications appeared to be stronger predictor for DN (measured by NDS and Neuropad), than macrovascular.

The sensitivity (79.3%), specificity (42.9%), positive (62.8%) and negative (63.0%) predictive value of an abnormal Neuropad response, compared to NDS ≥ 6 are similar to the results of C. Quattrini et al. [Quattrini et al., 2008] – 85, 45, 69 and 71% respectively, who used a NDS ≥ 5 cut-off for detecting clinical neuropathy. The ratio between our patients with NDS ≤ 5 and >6 (45/55%) in our population is also similar to their population – 40% with NDS <5 and 60% with NDS ≥ 5 . They concluded in favor of Neuropad as a simple indicator for screening patients with DN. S.Liatis et al. [Liatis et al., 2007] investigated in 117 patients the ability of Neuropad to detect DSN, comparing the test to a more complex aggregate of Neuropathy Symptom Score, Vibration perception (biothesiometer) and NDS (pathological value ≥ 5), diagnosing DSN when two of the three tests were positive. The sensitivity (86%), positive (66.2%) and negative (67.6%) predictive value were similar and specificity (67.2) was higher than in our study where we evaluated patients at higher risk. They also assessed the cardiac autonomic functions with the four standardized tests of Ewing et al. [Ewing et al., 1985] and concluded that the test is more sensitive in patients with severe, than in mild cardiac autonomic DN. Another study, using the corrected QT interval supports a lower specificity (43.1%), but high sensitivity (87.5%) in detecting cardiac autonomic DN [Bilen et al., 2007]. In a very recent study the sensitivity and specificity of Neuropad for diagnosing DSN was 85 and 32%, and for cardiac autonomic neuropathy – 82 and 27% respectively at the originally recommended 10 min time for color assessment [Spallone V. et al., 2009].

Table 8 Evaluation of Neuropad ≥ 1 as a tool for detecting DN (comparison to NDS ≥ 3) and for identification of the foot at risk (compared to NDS ≥ 6) by calculation of sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy (data presented as %).

NDS cut-off	NDS $\leq 2/\geq 3$ Presence of neuropathy	NDS $\leq 5/\geq 6$ Foot at risk
sensitivity	76.3	79.3
specificity	56.1	42.9
positive predictive value	86.3	62.8
negative predictive value	39.5	63.0
diagnostic accuracy	72.2	62.9

Table 9 Conformation of neurological abnormalities with corresponding tests (NDS and Neuropad) in 187 patients with clinical neuropathic symptoms and 77 asymptomatic patients.

Presence of neuropathic symptoms	With diagnosis DN	Neuropad	VP	TP	MF	AR	NDS ≥ 6	NDS ≥ 3
187 patients with symptoms	132 (70.6)	142 (75.9)	141 (75.4)	95 (50.8)	59 (31.6)	163 (87.2)	123 (65.8)	168 (89.8)
77 patients without symptoms	1 (1.3)	41 (53.2)	28 (36.4)	17 (22.1)	11 (14.3)	42 (54.5)	22 (28.6)	39 (50.6)

Data presented as N (%) from the respective groups of 187 symptomatic and 77 asymptomatic patients. Mean value for both feet was used. Cut-off for abnormal test: Neuropad ≥ 1 ; VP=1; TP=1; MF=1; AR ≥ 1 ; NDS foot at risk ≥ 6 ; NDS presence of DN ≥ 3 . All differences between test results of symptomatic and asymptomatic patients are significant ($p < 0.001$)

Diabetic neuropathy (DN) has been defined as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” [Singer et al., 2004]. This statement emphasizes two important messages: (1) A diabetic patient with neuropathic symptoms does not obligatorily have DN. In the Rochester Diabetic Neuropathy Study up to 10% of peripheral neuropathy in diabetic patients was of nondiabetic origin [Dyck et al., 1993]. (2) A diabetic patient without neuropathic symptoms could have DN. In this study, the comparison between patients with actual symptoms with the presence of the diagnosis DN in their records and the results from neurological tests showed, that nine out of ten symptomatic patients had DN evaluated by NDS ≥ 3 , although many of them still were not diagnosed to have DN. From the individual tests AR, VP and Neuropad were most sensitive in this aspect. Every second from the asymptomatic patients also had DN according to NDS. Only one patient in this group has been already diagnosed to have DN. The differences in the test results can be interpreted also with the objectively existing differences in the involvement of different sensory modalities in DN.

In total 207 patients had DN (NDS ≥ 3) and 183 had abnormal Neuropad, representing 78.4 and 69.3% of the whole population respectively. These numbers are higher than the usually reported in the literature because of the hospital origin of our population. Comparing these two different tests, evaluating different aspects of DN, with a sensitivity of 76.3, specificity – 56.1, PPV – 86.3, NPV – 39.5, and diagnostic accuracy of 72.2%, the new test proved to be useful in detecting DN. These findings point out the importance of simple devices for diagnosis of both types DN – somatic and autonomic.

Conclusions

Screening for diabetic neuropathy must include somatic and autonomic disturbances. Neuropad is a new appropriate for everyday clinical use test for sudomotor dysfunction evaluation and sympathetic cholinergic dysfunction estimation. Combination of modified Neuropathy Disability Score and Neuropad represent a sensitive tool for detection of diabetic neuropathy and identification of diabetic foot at risk. Neuropad is able to differentiate patients with different general risk, based on the presence of more advanced micro- and macrovascular chronic diabetic complications.

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