Scientific Publications

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N°	Study Title	Presented as	Presented in	Date	Country	
1	Zick R., Schäper T., Deeters U. *St. Bonifatius Hospital Lingen, Academic Hospital of the Medical University Hanover, Germany* "Measurement of perspiration in the diabetic foot"	□ Oral Presentation X Poster □ Complete Study X Published	Annual Meeting German Diabetes Association, Bremen. P-160 "Der Klinikarzt"	05/2003 05/2003	Germany	
2	Ch. Manes, G. Piggas, K. Mikoudi, G. Skaragas D. Karagianni, D. Skoutas, E. Tsotsia, *2nd Department of Internal Medicine, Diabetes Unit, General Teaching Hospital "PAPAGEORGIOU", Thessaloniki, Greece* "Evaluation of a indicator plaster, neuropad® , for the diagnosis of peripheral neuropathy in diabetics patients (Preliminary report)"	X Oral Presentation □ Poster □ Complete Study □ Published	17th Diabetological Congress of North Greece	11/2003	Greece	
3	Ioan Andrej Vereşiu, Monica Negrean, Eva Fülöp Diabetes Center & Clinic Cluj-Napoca, Romania "neuropad® in the screening of the diabetic syndrome"	□ Oral Presentation X Poster □ Complete Study □ Published	30th National Congress of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases, Romania	05/2004	Romania	
4	Grzegorz Rosinski, Arkadiusz Krakowiecki *Department of Gastroenterology and Metabolic Diseases, Medical University of Warsaw, Poland* "Evaluation of usefulness of the early detection plaster (neuropad®) for diabetic foot syndrome in view of early differentiating between subtypes of neuropathic diabetic foot"	□ Oral Presentation □ Poster □ Complete Study □ Published		09/2004	Poland	
5	Kren K, Slak M. Urbančič V. *University Medical Centre, Dept. Od Endocrinology, Ljubljana, Slovenia* "neuropad® as a screening tool for sudomotoric dysfunction"	X Oral Presentation □ Poster □ Complete Study □ Published	4th Scientific Meeting of the Diabetic Foot Study Group, OP-59	09/2004	Slovenia	

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N°	Study Title	Presented as	Presented in	Date	Country	
6	C. Manes, K. Mikoudi, E. Sossidou, G. Pigas, D. Karagianni, D. Skoutas, S. Fotiadis, *Diabetes Unit, General Hospital "PAPAGEORGIOU", Thessaloniki, Greece* "Evaluation of a new indicator plaster in identifying diabetic patients at risk of foot ulceration"	X Oral Presentation X Poster □ Complete Study □ Published	4th Scientific Meeting of the Diabetic Foot Study Group (1105) 40th Annual Meeting EASD (PS-103 Diabetic Foot and Charcot foot: Poster 1049 + Symposium)	09/2004 09/2004	Greece	
7	Tae Seo Sohn, M.D.[1], Hyun Shik Son, M.D.[1], Jae Myung Yu, M.D., Bong Soo Cha, M.D.[3], Kyung Wan Min, M.D.[4], Sei Hyun Baik, M.D.[5] *1 Division of Endocrinology and Metabolism, Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea* *2 Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Hallym University, Seoul, Korea* *3 Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Korea* *4 Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Eulji University, Seoul, Korea* *5 Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Korea University, Seoul, Korea* "Evaluation of the indicator test (neurocheck™) in diagnosis of peripheral neuropathy among Type 2 diabetic patients in Korea"	□ Oral Presentation X Poster □ Complete Study □ Published	Official symposium on the occasion of the 40th Annual Meeting EASD: "Autonomic neuropathy of the diabetic foot: Recent clinical findings and outlook"	09/2004	South Korea	
8	S. Pruna, C. Ionescu-Tirgoviste *Electrophysiology Lavoratory, Institute of Diabetes "N. Paulescu", Bucharest, Romania* "A comparative study between color change plaster for diabetic foot syndrome and electrical impedance change of plantar skin sweat secretion	□ Oral Presentation X Poster □ Complete Study □ Published	40th Annual Meeting EASD (Poster session: PS 103 – Diabetic foot: management and Charcot foot: Poster 1050)	09/2004	Romania	

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N°	Study Title	Presented as	Presented in	Date	Country	
9	N. Papanas *Second Department of Internal Medicine, Democritus University of Thrace, Greece* "Sudomotor dysfunction is associated with duration of Type 2 diabetes mellitus"	X Oral Presentation □ Poster □ Complete Study □ Published	Official symposium on the occasion of the 40th Annual Meeting EASD: "Autonomic neuropathy of the diabetic foot: Recent clinical findings and outlook"	09/2004	Greece	
10	N. Papanas[1], K. Papatheodorou[1], D. Christakidis[2] D. Papazoglou[1], G. Giassakis[3], H. Piperidou[3], , Ch. Monastiriotis[1], E. Maltezos[1] *[1]Second Department of Internal Medicine, Democritus University of Thrace, Greece* *[2]Diabetic Department, General Hospital of Alexandroupolis, Greece* *[3]Department of Neurology, Democritus University of Thrace, Alexandroupolis, Greece* "Evaluation of the new indicator plaster (neuropad®) in the diagnosis of peripheral neuropathy among Type 2 diabetic patients"	□ Oral Presentation X Poster □ Complete Study X Published	40th Annual Meeting EASD (PS 098 – Somatic neuropathy No. 10156) Experimental & Clinical Endocrinology Journal 04/2005: 113: 1-4	09/2004 04/2005	Greece	
11	Thomas Schäper *Department of Internal Medicine (Professor R. Zick) St. Bonifatius Hospital Lingen, Academic Teaching Hospital of the Medical University Hanover, Germany* "Test for autonomic neuropathy in the diabetic foot"	□ Oral Presentation □ Poster X Complete Study □ Published	Doctoral Thesis for the Degree of Doctor of Medicine at Hanover Medical University Accepted by: Professor Bitter-Suermann	02/2005	Germany	
12	N. Papanas[1], K. Papatheodorou[1], D. Papazoglou[1], D. Christakidis[2], Ch. Monastiriotis[1], E. Maltezos[1] *[1]Second Department of Internal Medicine, Democritus University of Thrace, Greece* *[2]Diabetic Department, University Hospital of Alexandroupolis, Greece* "Reproducibility of the new indicator test for sudomotor function (neuropad®) in patients with type 2 diabetes mellitus"	□ Oral Presentation X Poster □ Complete Study X Published	9th Greek Diabetological Congress, Rhodes, Greece Experimental & Clinical Endocrinology Journal 10/2005: 113 577-581	03/2005 10/2005	Greece	

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13	S.Liatis, S. Maggana, K. Marinou, A. Nikolopoulos, H. Housos, G. Marathonitis, N. Tentolouris, N. Katsilambros *1th Department of Internal Medicine, Diabetological Clinic of General Hospital Laiko, Athens Greece* "Evaluation of new indicator test neuropad® for the diagnosis of peripheral neuropathy (PN) and for the diagnosis of autonomic neuropathy (AN) in diabetic patients"	□ Oral Presentation X Poster □ Complete Study □ Published	9th Greek Diabetological Congress, Rhodes, Greece	03/2005	Greece	
14	C. Manes, K. Mikoudi, S. Papantoniou, D. Karagianni, D. Konstantinidou, D. Skoutas *Diabetes Unit, General Hospital "PAPAGEORGIOU", Thessaloniki, Greece* "Evaluation of clinical examination and neuropad for the assessment of peripheral nervous dysfunction in patients with diabetes mellitus type II"	□ Oral Presentation X Poster □ Complete Study □ Published	9th Greek Diabetological Congress, Rhodes, Greece	03/2005	Greece	
15	K. Marinou, S. Maggana, S.Liatis, A. Nikolopoulos, Diakoumopoulou, N. Tentolouris, N. Katsilambros *1th Department of Internal Medicine, Diabetological Clinic of General Hospital Laiko, Athens Greece* "Usefullness of the indicator plaster neuropad® for the diagnosis of peripheral and autonomic neuropathy in patients with diabetes mellitus"	□ Oral Presentation X Poster □ Complete Study □ Published	41st Annual Meeting EASD, (Poster session: PS 92 – Poster 995)	09/2005	Greece	
16	S. Chon[1], Y.-S. Kim[1], S. Oh[1], J.-T. Woo[1], S.-W. Kim[1], J.-W. Kim[1], B.-K. Choe[2] *[1]Dept. of Endocrinology, Kyung Hee Univ. Hospital, Seoul, Republic of Korea,* *[2]Dept. of Preventive Medicine, Kyung Hee University, Seoul, Republic of Korea.* "Assessment of diabetic autonomic neuropathy in type 2 diabetic patients using neuropad®: A new indicator plaster for detection of disturbed sweat secretion"	□ Oral Presentation X Poster □ Complete Study □ Published	41st Annual Meeting EASD, (Poster session: PS 92 – Poster 996)	09/2005	South Korea	

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N°	Study Title	Presented as	Presented in	Date	Country	
17	M. Pietrogrande, M. Meroni *U.O. Medicina, Policlinico San Marco, Zingonia, Dipartimento di Medicina, Chirurgia & Odontoiatria, Ambrosiani degli Studi di Milano, Italy* Disautonomia nella neuropatia crioglobulinemica: Studio preliminare	X Oral Presentation □ Poster □ Complete Study □ Published	Congresso Nazionale della Società' Italiana di Neurofisiologia Clinica, Turin, Italy	05/2006	Italy	
18	Piaggesi A., Ambrosiani Nobili l., Civitelli A., Leporati E., Macchiarini S., Scire V., Teobaldi I. *Azienda Ospedaliera Universitaria Pisana (University Hospital of Pisa)* *U.O. Malattie Metaboliche e Diabetologiche, Sezione Piede Diabetico (Metabolic and Diabetic Disease Department, Diabetic Foot Unit)* "Usefulness of the new neuropad® medical device for the screening of ulcer risk in diabetic patients"	X Oral Presentation □ Poster □ Complete Study □ Published	Congresso Nazionale della Società' Italiana di Diabetologia (SID) Milan, Italy	05/2006	Italy	
19	N. Papanas[1], K. Papatheodorou[1], D. Papazoglou[1], D. Christakidis[3], Ch. Monastiriotis[1], E. Maltezos[1] *[1]Second Department of Internal Medicine, Democritus University of Thrace, Greece* *[3]Diabetic Department, University Hospital of Alexandroupolis, Greece* The new indicator test (neuropad®): A valuable diagnostic tool for small fibre impairment in Type 2 diabetic patients	□ Oral Presentation □ Poster □ Complete Study X Published	In print in the "The Diabetes Educator"	03/2007	Greece	
20	N. Papanas[1], G. Giassakis[3], K. Papatheodorou[1], D. Papazoglou[1], C. Monastiriotis[1], D. Christakidis[3], H. Piperidou[2], E. Maltezos[1] *[1]Second Department of Internal Medicine, Democritus University of Thrace, Greece* *[2]Department of Neurology, Democritus University of Thrace, Greece* *[3]Diabetic Department, University Hospital of Alexandroupolis, Greece* Sensitivity and specificity of the new indicator test (neuropad®) for the diagnosis of peripheral neuropathy in type 2 diabetic patients: A comparison with clinical examination and nerve conduction study	□ Oral Presentation □ Poster □ Complete Study X Published	Accepted for publication in "Journal of Diabetes and its complications"	03/2007	Greece	

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21	Manes C., Kirlaki E., Papantoniou S., Sossidou E., Skoutas D., Tsotoulidis S., Kefalogiannis N., Sion M. *Diabetes Unit, 3rd Dptm of Medicine, "PAPAGEORGIOU" Hospital – Thessaloniki, Greece* *Diabetes Unit, Venizelion Hospital, Heraklion Kreta, Greec* *Health Center of Thasos – Kavala, Greece* "neuropad®: Validation of a new indicator plaster as a screening tool in identifying patients at risk of foot ulceration – a multicenter study"	X Oral Presentation □ Poster □ Complete Study □ Published	Oral presentation at DFSG 2006	09/2006	Greece	
22	N. Papanas[1], G. Giassakis[2], K. Papatheodorou[1], D. Papazoglou[1], C. Monastiriotis, D. Christadkidis[3] H. Piperidou[2], E. Maltezos[1] *[1]Second Department of Internal Medicine, Democritus University of Thrace, Greece* *[2]Department of Neurology, Democritus University of Thrace, Greece* *[3]Diabetic Department, University Hospital of Alexandroupolis, Greece* "Use of the new indicator test (neuropad®) for the assessment of the staged severity of neuropathy in type 2 diabetic patients"	X Oral Presentation X Poster □ Complete Study X Published	Oral presentation at DFSG 2006 Poster presentation at EASD 2006 "Experimental & Clinical Endocrinology and Diabetes", 01/2007; 115(1), 58-61	09/2006 01/2007	Greece	
23	T. Didangelos, I. Zografou, A. Papageorgiou, M. Koukourikou, V. Athyros, K. Kontotasios, D. Karamitsos *Diabetes Center, 2nd Propeudetic Department of Internal Medicine, Aristotle University, Hippocration Hospital, Thessaloniki, Greece* "Validation of a new diabetic autonomic neuropathy bedside test (plaster) versus the cardiovascular reflex tests"	□ Oral Presentation X Poster □ Complete Study □ Published	Poster presentation at Neurodiab 2006 Poster presentation at EASD 2006	09/2006	Greece	
24	Rayaz Malik *Senior Lecturer and Consultant Physician, Department of Medicine, Manchester Royal Infirmary, Manchester, UK* "The Neuropad: a highly sensitive test to evaluate small and large fibre neuropathy in diabetic patients"	X Oral Presentation □ Poster □ Complete Study □ Published	7th International Symposium on Diabetic Neuropathy, Somerset West, South Africa	12/2006	United Kingdom	

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25	T. Didangelos, I. Zografou, A. Papageorgiou, T. Kechidou, V. Athyros, S. Stergiopoulos, D. Karamitsos *Diabetes Center, 2nd Propeudetic Department of Internal Medicine, Aristotle University, Hippocration Hospital, Thessaloniki, Greece* "Validation of a new diabetic autonomic neuropathy bedside test (plaster) vs. the MNSI, monofilament of 10gr and biothesiometer"	□ Oral Presentation X Poster □ Complete Study □ Published	19th World Diabetes Congress, IDF 2006, Cape Town, South Africa P 186	12/2006	Greece	
26	TAN Ping, LI Jing, LUO Qiao-yun, HU Qing-Xiang, LIU Zhi-hong *Second Dept. of Medicine, The No. 458 Hospital of PLA Guangzhou 510602, China* "Evaluation of neuropad in early detection of diabetic peripheral neuropathy"	□ Oral Presentation □ Poster □ Complete Study X Published	Chinese Journal of Diabetes 2006 Vol.14 No.6 P.468-469	12/2006	P.R. of China	
27	Z. Kamenov, V. Christov, T. Yankova *Clinic of Endocrinology, Alexandrovska University Hospital, Medical University, Sofia, Bulgaria* "Prevalence of neuropathic disturbances and their predictive value for erectile dysfunction in diabetic men"	X Oral Presentation □ Poster □ Complete Study X Published	9th Congress of the European Society for Sexual Medicine, Vienna, Austria Podium Session Abstract P-02-016 page 6 The Journal of Sexual Medicine 2006	12/2006 12/2006	Bulgaria	
28	SHEN Jie ,CAO Ying, XUE Yao-Ming,et al. *Department of Endocrinology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China* "Preliminary evaluation of test for the function of foot autonomic nerve in the early diagnosis of peripheral neuropathy in diabetic patients"	□ Oral Presentation □ Poster □ Complete Study X Published	Submitted for publication	01/2007	P.R. of China	
29	Zdravko A. Kamenov, Vladimir G. Christov, Tsanka M. Yankova *Clinic of Endocrinology, Alexandrovska University Hospital, Medical University, Sofia, Bulgaria* "Erectile dysfunction in diabetic men is linked more to microangiopathic complications and neuropathy than to macroangiopathic disturbances"	□ Oral Presentation □ Poster □ Complete Study X Published	Accepted for publication in: "International Journal of Mens' Health and Gender"	01/2007	Bulgaria	

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N°	Study Title	Presented as	Presented in	Date	Country	
30	Renming Hu, Bin Lu *Institute of Endocinology and Diabetology* *Huashan Hospital, Fudan University, Shanghai, China* "Assessment of chronic diabetic complications in type 2 diabetic patients using neuropad®: A new indicator plaster for detection of disturbed sweat secretion"	□ Oral Presentation □ Poster □ Complete Study □ Published		Ongoing	P.R. of China	
31	Dr. Kramer *Centre for Diabetes and Endocrinology Johannesburg, South Africa* A multi-centre, cross-sectional, correlation healthy subject controlled study to investigate the use of neuropad® test as a possible detection method for early autonomic and sensory neuropathy in Diabetes Mellitus.	□ Oral Presentation □ Poster □ Complete Study □ Published		Ongoing	South Africa	
32	V. Spallone *University of Tor Vergata, Rome, Italy* "Assessment of the usefulness of neuropad® as a diagnostic tool for overall peripheral diabetic neuropathy, sympathetic nerve function and small fibres' function"	□ Oral Presentation □ Poster □ Complete Study □ Published		Ongoing	Italy	
33		□ Oral Presentation □ Poster □ Complete Study □ Published				
34		□ Oral Presentation □ Poster □ Complete Study □ Published				

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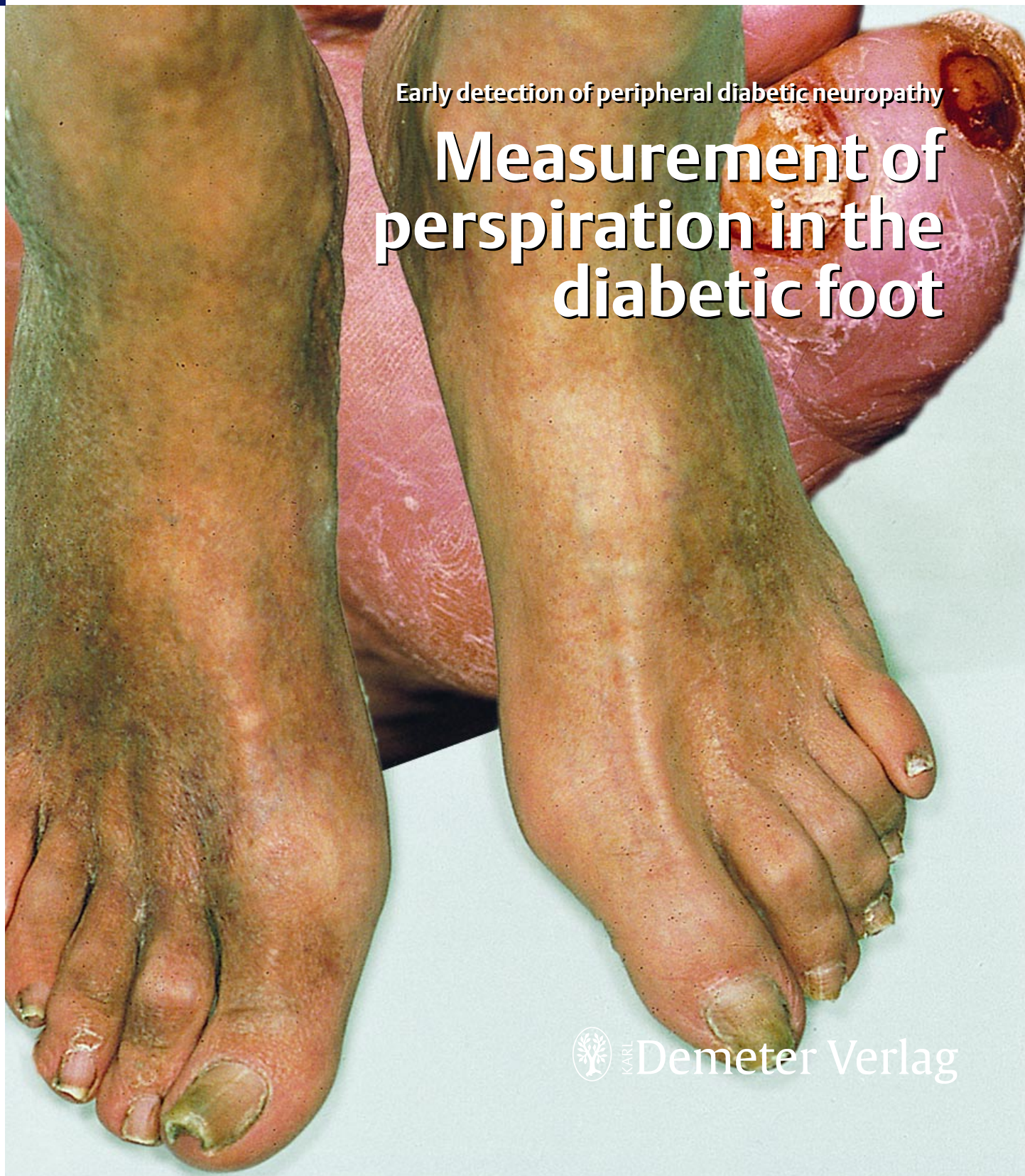

Early detection of peripheral diabetic neuropathy

Measurement of perspiration in the diabetic foot

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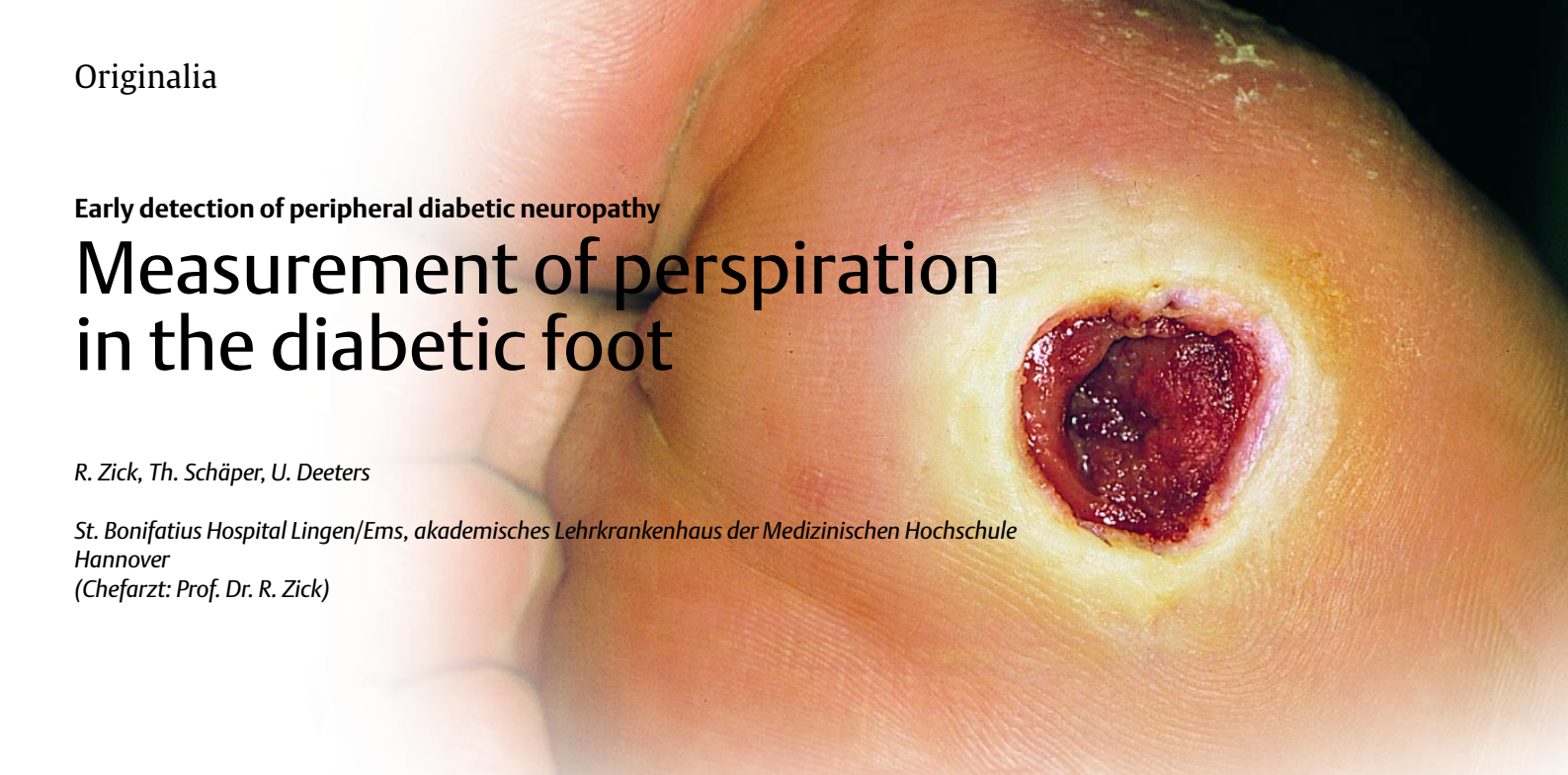


Early detection of peripheral diabetic neuropathy

Measurement of perspiration in the diabetic foot

R. Zick, Th. Schäper, U. Deeters

St. Bonifatius Hospital Lingen/Ems, akademisches Lehrkrankenhaus der Medizinischen Hochschule Hannover
(Chefarzt: Prof. Dr. R. Zick)

The indicator plaster, neuropad® – which utilizes the water-induced colour change of a cobalt II compound from blue to pink – now enables us for the first time to investigate the diabetic foot, simply and reliably, for alterations in perspiration caused by autonomic neuropathy. In an initial clinical study, we used the indicator plaster to examine the plantar perspiration of the feet of 20 diabetics with and without peripheral sensory neuropathy, at metatarsal heads (MTH) I and II - the usual site of neuropathic ulcers. In healthy controls, the standardised colour change was complete after 10 minutes. It was found that changes in perspiration occurred even in diabetics with no manifest sensory neuropathy. The neuropad® thus enables early detection of peripheral diabetic neuropathy – the underlying cause of diabetic foot – and initiation of preventive measures.

Diabetic neuropathy is the major underlying cause of the diabetic foot syndrome (1, 2). To date, the autonomic nerve component of this neuropathy, which is associated with changes in plantar sweating has, for methodological reasons, received little diagnostic or therapeutic attention. This situation might be about to undergo a change – thanks to the indicator plaster neuropad®, which signals the presence of water or sweat on the basis of a colour change of a cobalt II salt from blue to pink.

The aim of this first clinical study of the indicator plaster was to determine the degree of moisture on the skin at the level of the metatarsal heads I/II (MTH I/II) – where neuropathic ulcers typically develop - in control persons and diabetics with and without confirmed peripheral sensory neuropathy. Also investigated was the question whether disordered secretion of sweat parallels sensory loss or, as preliminary studies suggest, precedes it (5).

Patients and Methods

Recruited to the study were 40 diabetics (21 women and 19 men) aged 30–70 years (average age 55.5 years), 9 of whom had type 1, and 32 type 2 diabetes. The average duration of the diabetes was 14.5 years. The control group comprised 27 healthy subjects (11 women and 16 men) aged between 22 and 63 years (average age 41.5 years). Exclusion criteria were: chronic alcohol abuse, hyperthyroidism or hypothyroidism (TSH < 0.23; > 4.0 µIU/ml), known allergic or eczematous skin disease, peripheral arterial perfusion disorders (Doppler pressure index less than 1), and age below 18 or above 70 years.

Sensory neuropathy affecting the lower extremities was established with the aid of the standardised tuning fork, the 10 g monofilament and the Vibrameter (Somedic AB, Sweden). A pathological finding was diagnosed as present when two of the three diagnostic tests were positive for neuropathy. In borderline cases, the final decision was taken on the basis of an additional neuropathy score. The degree of wetness of the plantar skin was determined for both feet at the level of metatarsal heads I and II using the neuropad® (Miro Verbandstoffe GmbH, Wiehl).

During the examination, the seated patient was required to place his/her feet on a second chair arranged in front of him/her. In order to exclude artefacts, we allowed an interval of 5 minutes between the removal of shoes and socks and measurement with the indicator plaster. Using a standardised colour scale, the time taken for a complete change in colour from blue (HKS 46 K 55%) to pink (HKS 17 K 30%) (colour change duration) (Fig. 1) was determined.

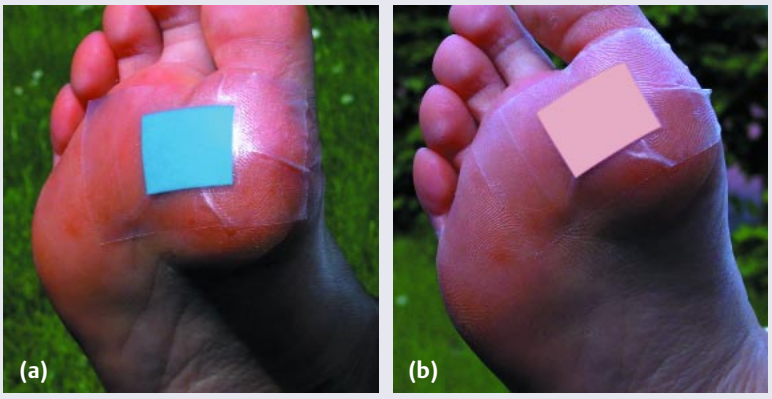
The statistical evaluation of the data was kindly performed by the Diplommathematiker (graduate mathematician) H. Geerlings from the Institute for Biometrics at the Medical College Hanover using the SPSS (Statistical Packet for the Social Sciences) programme.

Results

Of the 40 diabetics investigated, one-half proved to have peripheral sensory neuropathy. No significant differences were found between the right and left foot in terms of the moisture film/sweat secretion of the plantar skin at the level of metatarsal heads I and II in any of the three groups – controls, diabetics with

neuropathy, and diabetics without neuropathy. The parameter measured was the mean time in seconds taken for the indicator plaster to change colour. There was, however, a significant difference between the three study arms (Fig. 2). In 95% of the healthy controls, colour change was complete after only ten minutes, as shown by the percentile calculation. A comparison of the percentiles of the mean colour change durations for the three groups revealed that a number of the patients with no manifest sensory neuropathy also had a perspiration disturbance, which may be interpreted as an expression of an autonomic nerve disorder. As shown in figure 3, this was the case in seven of the 20 diabetics investigated. Virtually all of the diabetics with sensory neuropathy (18 out of 20) were found to have dry feet, reflecting a disordering of sweat production.

Fig. 1 Standardised colour change of the indicator plaster



In healthy subjects, colour change is complete in about ten minutes. A longer duration is indicative of neuropathy

Discussion

Every fifth diabetic develops associated problems with the feet. This drives up the costs of hospital care, accounting for almost one-quarter of the overall costs incurred by the underlying disease. In Germany alone, more than 25 000 diabetics continue to have a lower-limb amputation every year – and the tendency is rising rather than falling. Accordingly, care of the diabetic foot should be a priority aim of modern diabetes management.

The major cause of the diabetic foot syndrome is peripheral neuropathy. In the past, little diagnostic or therapeutic attention was directed towards the autonomic element of this neurological disorder, which is associated with a change in the secretion of sweat by the extremities. For methodological reasons in particular, interest was focussed mainly on the sensory and motoric aspects of the disease. With the indicator plaster, we now have available a means of rapidly and simply identifying diabetics in whom the cutaneous film of moisture is reduced or absent as a result of a disturbance of perspiration.

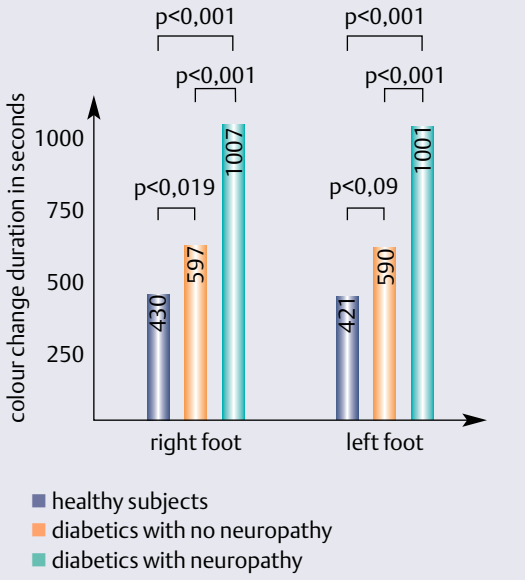
Unlike the tuning fork, the Tip-Therm and the 10 g monofilament that are usually employed to diagnose peripheral diabetic neuropathy, the indicator plaster is not a sensory test, and thus does not require the cooperation of the patient. Nor has the investigator any influence on the results obtained with this new test system. These are advantages that favour the indicator plaster also for use within the framework of the new disease management programmes that require objective, independently checkable quality standards for the diagnosis and treatment of the diabetic foot syndrome.

Among the healthy subjects, the 95% percentile of complete colour change from blue to pink in the plantar region of the foot at the level of the metatarsal heads I/II was ten minutes. If, therefore, longer colour change durations are measured in diabetics, an autonomic neuropathy must be assumed to be present – provided other causes of dry skin have been excluded. The severity of the neuropathy is oriented to the colour change duration in comparison with the healthy control group. If the time required for complete colour change is appreciably longer, the diabetic should be treated rigorously with moisturizing foams or creams as a prophylactic measure against the development of the diabetic foot syndrome.

The fact that seven out of ten diabetics revealed a disturbance of sweat secretion in the absence of a peripheral sensory neuropathy indicates that the latter develops in the foot before sensory loss becomes manifest. This means that the indicator plaster can enable diabetics at risk to be identified – and thus to be started on prophylactic measures – earlier than is possible with currently available methods.

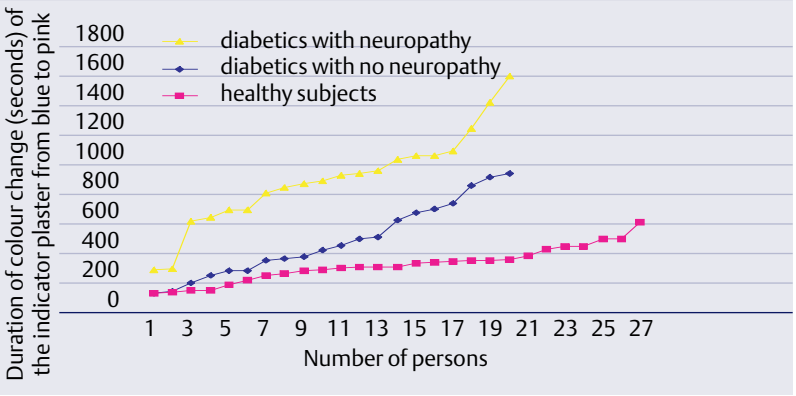
Risse (3) drew attention to the fact that in many diabetics with peripheral sensory neuropathy own-body perception is altered. In particular, they experience a sensation as though their feet „no longer belong to them“. This may explain why dia-

Fig. 2 Mean duration of indicator plaster colour change



Colour change duration differs significantly among all three study arms (diabetics with neuropathy; diabetics without neuropathy, healthy subjects)

Fig. 3 Indicator plaster colour change durations



The colour change durations also exceed the critical threshold of 10 minutes in some of the diabetics with no sensory signs of neuropathy. This indicates that autonomic neuropathy develops before sensory disorders become manifest

betics with neuropathy frequently delay consulting their doctor, even when they already have severe lesions of the foot. Many diabetics fail to relate the results of test methods based on sensory perception to themselves. This means that such tests cannot serve such patients as a motivational basis for efforts to bring about a desirable change in habitual behaviour. This may be the reason why so many amputations of the foot are still having to be carried out in Germany, despite intensive efforts to instruct and motivate patients.

In contrast – as in the case of test strips regularly used by many diabetics to monitor their blood sugar levels – the indicator plaster is a visual system. The clinical experience we have gained to date shows that the confidence placed by patients in the coloured blood sugar test strips is also transferred to the neuropad®, so that patients automatically pay greater attention to their foot problems.

Keywords
peripheral diabetic neuropathy – diabetic foot – prevention – neuropad® indicator plaster

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For more literature please contact the author.

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Evaluation of indicator plaster, neuropad®, for the diagnosis of peripheral neuropathy in diabetics patients. (Preliminary report)

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Introduction

The term " diabetic foot " is used to refer to a variety of abnormal conditions, ulcerations and gangrene, that affect the feet of many diabetic people and could result in foot amputation. It has been suggested that peripheral vascular disease and peripheral nerve damage with the presence of minor trauma are major contributory factors in the aetiopathogenesis of foot ulceration. (AJM Boulton)

Peripheral autonomic neuropathy (absence of sweating) combined with the sensorimotor neuropathy leads to the foot ulceration, as the insensitive dry skin often cracks resulting in minor trauma.

Objective

The evaluation of an indicator plaster, (neuropad®), for diagnosis of peripheral nerve dysfunction. This plaster changes its color if the moisture (sweating) of the feet is intact. Since the dry skin of the feet is due to peripheral autonomic neuropathy (absence of sweating) the stability of the color or the partial change could indicate peripheral autonomic dysfunction.

Patients

Patients who had already been diagnosed with type 2 diabetes mellitus
Forty-three patients (19 men, 24 women) were studied.
Mean age was 64 years (64,4 ± 9,8)
Mean known duration of diabetes was 15,7±9,03 years

Methods

Clinical examination for peripheral neuropathy. The sensations of pain, touch, cold, vibration and the tendons reflexes of knee and ankle were tested in both legs of all the patients.
The sensations of pain, touch, cold, vibration and the tendon reflexes were scored using a modified Neuropathy Disability Score based on the original system proposed by PJ Dyck (table 1 and 2).
The Neuropathy Disability Score (NDS) was used to quantify the severity of diabetic neuropathy on clinical examination.

Sensory Signs	Normal	Abnormal
Pain	0	1
Touch	0	1
Cold	0	1
Vibration	0	1

Table 1

Reflexes of tendons	Normal	Reduced	Absent
Knee	0	1	2
Ankle	0	1	2

Table 2

Stability or partial change of color of the indicator plaster (neuropad®) was considered as a sign of peripheral autonomic dysfunction. During the examination, we allowed an interval of 10´ minutes between patients' removal of shoes and socks and then the pad was stuck to the plantar surface of the feet in the region of metatarsal heads I/II. Initial color was blue. We checked the color again after 10´ minutes. If the color at that time was pink, then the moisture of plantar skin was considered as normal. If not, it was considered as a sign of dryness of plantar skin (disturbance of sweating).
For the statistical analysis was used the chi-square test.

	Group A	Group B
Patients	18	25
NDS	≥2	<2

Table 3

Results

From clinical examination and NDS score we obtained the following patients groups. Group A with peripheral nerve dysfunction NDS≥2 and group B without signs of peripheral dysfunction (table 3)
Stability or partial change of the pad's color from blue to pink was observed in the 84% of group A and 15% of group B $p<0,05$.
The neuropad®´s positive prognostic value for peripheral neuropathy was 83%.
(Truly positive / truly positive + falsely positive)
The neuropad®´s negative prognostic value for peripheral neuropathy was 78%.
(Falsely negative/ falsely negative + truly negative)
The neuropad®´s specificity was 88%.
(Truly negative/ truly negative + falsely positive)
The neuropad®´s sensitivity was 83%.
(Truly positive/ falsely negative + truly positive)

Conclusion

The use of neuropad® can be considered as an important step in diagnosing peripheral nerve damage in diabetic people and identifying those at high risk of foot ulceration.

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neuropad® in the Screening of the Diabetic Foot Syndrome

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Background

Among the chronic complications of diabetes mellitus (DM), foot problems are the most frequent and the most costly. About 40-75% of non-traumatic lower limb amputations are performed in patients with diabetes, and foot ulcers precede 85% of these amputations. The main cause of diabetic foot syndrome is the peripheral diabetic neuropathy.

Aim

The aim of the present study was to assess the sensitivity and specificity of the indicator plaster Neuropad® in the screening of diabetic foot syndrome, by assessing the function of the perspiratory glands of the foot, and thus the autonomous function, based on a shift in color.

Patients and methods

We performed a case-control study in 20 patients with diabetes mellitus, considering as ,,cases" the patients with a personal history of foot ulcers. The indicator plaster Neuropad® was applied on the foot sole, in the area corresponding to the head of the first metatarsal bone (the main site of diabetic foot ulcers) and we assessed the shift in color after 10 minutes (pink-normal test, intermediate or blue-pathological test).

Results

The sensitivity of Neuropad® in identifying the subjects with a history of foot ulcers was very good (80%), comparable with that of other screening methods (10-g monofilament-80%, biothesiometer-70%, calibrated tuning fork-90%). The specificity of Neuropad® was 50% (compared with the 10-g monofilament-100%, the biothesiometer-77.8% and the calibrated tuning fork-40%). The positive and negative predictive values were also comparable with those of the above-mentioned tests.

Conclusions and discussions

Neuropad® is a sensitive, safe, objective, easy-to-use and reproducible screening method for the diabetic foot syndrome. A remarkable advantage is the fact that it allows patient self-screening, abnormal results being reported to the physician. The relatively low specificity for such a screening test cannot be regarded as a disadvantage, the only ,,risk" involved being that of examining and educating more often a patient without neuropathy.

Evaluation of usefulness of the early detection plaster (neuropad®) for diabetic foot syndrome in view of early differentiating between subtypes of neuropathic diabetic foot

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Background

autonomic neuropathy is one of the conditions leading to diabetic foot ulcers. Early signs of development of autonomic neuropathy include decreased skin moisture content. In this study we evaluated the prospect of early differentiation of the Charcot arthropathy and neuropathic perforating foot ulcer by ascertaining the hydrosis status.

Participants

Twenty patients with prior diagnosis of perforating neuropathic foot ulcer and 10 patients with Charcot arthropathy were recruited into the study. The exclusion criterion was having a coexisting disorder that could affect the hydrosis test results. All the subjects were patients of our Outpatient Diabetic Foot Clinic. Their mean age was 56±6 years and the mean period of insulin treatment - 14±3 years. All of them received intensive insulin therapy and antimicrobial therapy if appropriate. Foot x-ray showed abnormalities suggestive either of ostitis or of Charcot arthropathy.

Methods

The plaster was applied according to the manufacturer's directions, 5 minutes after removing shoes/socks. Patients were in supine position and before application of the plaster the feet were inspected to exclude possibility of accidental moistening. Subsequently the plaster was placed on the ball of the great toe or small toe or, in case these areas were damaged, on the skin of the heel. The coloration of the plaster was assessed 10 minutes after its application on the skin.

Results

The test showed abnormalities in almost all patients with Charcot arthropathy (90%) and only in 40% of patients with perforating ulcer.

Conclusions

1. Early detection plaster for diabetic foot syndrome is easy to use and may be therefore applied at home. 2. The plaster's sensitivity in case of Charcot arthropathy is high. 3. A further study on usefulness of the test in predicting imminent Charcot arthropathy a warranted. 4. An abnormal test result should prompt the physician to implement the preventive measures.

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neuropad® as screening tool for sudomotoric dysfunction

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Background and aims

Diabetic autonomic neuropathy, affecting sudomotoric nerve fibers, can cause dry skin which is prone to cracks and fissure. These skin lesions represent a portal of entry for pathogenic microorganisms. In this way, sudomotoric neuropathy is an important risk factor for foot complications. neuropad® is a simple and cheap diagnostic tool for the evaluation of sweat gland function. The aim of our study was to compare the results of neuropad® testing with the other results of standard foot screening procedure (palpation of foot pulses, sensitivity to standardized 10g Semmes – Weinstein monofilament).

Patients and methods

Neuropad was applied to 21 diabetic patients after standard foot screening procedure. The results were evaluated as stated in the instructions in the product package.

Results

Normal sweating was found in 16 patients (76.2%) and absent sweating in 5 (23.8%). Normal sensitivity to 10g monofilament was found in 13/16 patients (81.3%) with normal neuropad® result, and in 4/5 (80.0%) patient with absent sweating as found by neuropad® . 1 patient in each group had absent pedal pulses.

Conclusion

The results of our testing indicate that sudomotoric neuropathy develops independently of sensory nerve damage. Testing with neuropad®, although valuable for detecting sudomotoric nerve dysfunction, does not allow for conclusions regarding sensory nerve function. The latter has to be evaluated by other methods, such as sensitivity to standardized 10g monofilament or vibration with tuning fork.

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Evaluation of a new indicator plaster in identifying diabetic patients at risk of foot ulceration

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Peripheral autonomic neuropathy combined with sensorimotor neuropathy leads to the foot ulceration as the insensitive dry skin often cracks resulting in minor trauma. A new indicator plaster (neuropad) changes its color if moisture is present and the hypothesis is that stability of the color could indicate absence of sweating as a result of peripheral autonomic nerve dysfunction.

Patients – Methods:

To test this hypothesis 74 type 2 diabetic patients were examined (males = 34), mean age and mean duration of diabetes were 65,74±9,25 and 16,92±9,51 (yrs) respectively. Motor and sensory deficits were assessed in both legs and scored using a modified scoring system of this proposed by P. J. Dyck – Neuropathy Disability Score -NDS – (tendon reflexes -maximum 8- and reduced sensation of pain, cold, touch and vibration -maximum 20-). For the diagnosis of small fiber dysfunction (e.g. reduced pain, touch and cold sensation) the NDS1 was used as the sum of these scored sensory deficits and for this of large fiber dysfunction the score of reduced vibration sensation. For the statistical analysis the chi-square test and the multiple regression stepwise model were used. The overall predictive values (positive and negative -PV) for nerve function using this plaster were assessed in all the cases.

Results:

a) In bivariate analysis 79,07% of patients with full change in the plaster's color (Neuropad negative-NN) didn't reveal any significant nerve dysfunction and 58% of patients with partial change or stability of its color (neuropad positive-NP) showed such a dysfunction (NDS>3)-$p<0,05$ and the PV was 68.92%. Small fiber dysfunction (NDS1>2) was established in 61, 29% of NP patients and no significant deficits (NDS1≤2) or their normal function was established in 76, 74% of NN patients, $p<0, 05$, since the PV was in this case 70.27%.Large fiber dysfunction was present in 58% of the NP patients and normal function of these fibers was in 79% of the NN patients ($p<0,05$) and the PV was 70%. b) In the multiple regression analysis the following parameters a) NDS, b) NDS1, c) large fiber dysfunction and d) duration of diabetes were significant factors for neuropad positive or negative results , whereas those reflecting nerve dysfunction were the most powerful.

Conclusion:

Stability or partial change of the color of the new plaster (neuropad) is significantly dependent of peripheral autonomic and somatic nerve dysfunction and could identify in daily practice a significant part of patients at risk of foot ulceration.

EVALUATION OF THE INDICATOR TEST (NEUROCHECK™) IN THE DIAGNOSIS OF PERIPHERAL NEUROPATHY AMONG TYPE 2 DIABETIC PATIENTS IN KOREA

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Introduction

Foot ulcers develop in approximately 15 percent of patients with diabetes, and foot ulcers and amputations are a major cause of morbidity, disability, as well as emotional and physical costs for people with diabetes[1)]. Eighty-five percent of lower-limb amputations in patients with diabetes are preceded by foot ulceration, suggesting that prevention and appropriate management of foot lesions are of paramount importance[2)]. Ulceration is caused by several factors acting together, but particularly by neuropathy[3)]. Diabetic neuropathy is a heterogeneous disorder that encompasses a wide range of abnormalities affecting proximal and distal peripheral sensory and motor nerves as well as the autonomic nervous systems. Diabetic autonomic neuropathy frequently coexists with other peripheral neuropathies and other diabetic complications, but may be isolated, frequently preceding the detection of other complications. Disruption of microvascular skin blood flow and sudomotoric function may be among the earliest manifestations of diabetic autonomic neuropathy and lead to dry skin, loss of sweating, and the development of fissures and cracks that allow microorganisms to enter. These changes ultimately contribute to the development of ulcers, gangrene, and limb loss[4)]. Various aspects of neurovascular function can be evaluated with specialized tests, but generally these tests have not been well standardized and have limited clinical utility. Simple screening tests such as 10-g Semmes-Weistein monofilament examination, superficial pain test, and vibration testing by the on-off method were suggested for the diagnosis of peripheral neuropathy in the diabetes clinic[5)]. But these tests are sensory tests and require the cooperation of the patient and may be under the investigator's influence.

The detection test for DFS and autonomic neuropathy is called neuropad® in most countries of the world with the exception of Korea, where the product is called Neurocheck™.

Nerve conduction studies (NCSs) are strongly correlated with underlying structural changes and are the least subjective and more reliable single criterion standard[6)].
So nerve conduction studies have been used as the gold standard in diagnosing the peripheral neuropathy, but can be time-consuming, expensive, and impractical to operate in a primary care clinic.

More recently, a new indicator test (Neurocheck™ (CJ)), which utilizes the water-induced color change of a cobalt compound from blue to pink, has been introduced. This new test is an easy-to-perform measure of the sudomotoric component of peripheral neuropathy. The aim of the present study was to evaluate this new indicator test in the diagnosis of peripheral neuropathy among type 2 diabetic patients.

Patients and Methods

This study included 124 patients (45 men, 79 women) with diabetes mellitus. These patients were recruited from 5 diabetic centers in South Korea. The patients aged 26 - 70 years (average age 54.94 ± 9.24 years), 3 of whom had type 1, and 121 type 2 diabetes mellitus. The average duration of diabetes was 11.62 ± 6.16 years. The average level of HbA1c was 8.3 ± 1.4% (Table 1). The degree of patient's symptom was checked as total symptom score (TSS) from 0 to 14.4 point. The total symptom score was about the frequency and severity of pain, burning sense, paresthesia, and numbness on patients' feet. Exclusion criteria were: hyperthyroidism, hypothyroidism, chronic alcohol abuse, vitamin B12 depletion, lumbar spine disorders or known allergic skin disease.
In electrodiagnostic test, motor conduction velocities, distal motor latencies and distal compound muscle action potential amplitudes of the median, ulnar, peroneal and tibial nerves were studied. Additionally, sensory parameters, such as sensory conduction velocities and amplitudes of sensory nerve action potentials of the median, ulnar, peroneal and sural nerves were measured according to standard procedures. Room temperature was maintained at 20-25 to avoid any environmental variations.
Autonomic sudomotoric neuropathy was assessed by means of the new indicator test (Neurocheck™ (CJ)). Patients were allowed to rest in constant room temperature for 5 minutes after they had taken off their shoes and socks. During the examination, the seated patients were required to place his/her feet on a second chair arranged in front of him/her. The degree of wetness of the plantar skin was determined for both feet at the level of metatarsal head I and using the Neurocheck™. The degree of color change in 10 minutes was assessed as complete color change, incomplete color change or no color change. Patients in whom the degree of color change of the indicator test was incomplete or none in 10 minutes were considered to have sudomotoric neuropathy.
And, we scored the color change of each foot as 0: complete color change, 1: incomplete color change, 2: no color change, so the total score ranged from 0 to 4 point.
Data were analyzed by SPSS program. The result of new indicator test and nerve conduction study were compared using Fisher's exact test and the measure of agreement was described as κ statistic ($-1 \leq \kappa \leq 1$) using kappa analysis. The duration of diabetes and total symptom score were compared using a two sample t-test. The HbA_{1C} of patients with and without neuropathy were compared Wilcoxon rank sum t-test. P value of <0.05 was required for statistical significance.

The detection test for DFS and autonomic neuropathy is called neuropad® in most countries of the world with the exception of Korea, where the product is called Neurocheck™.

Results

Of the 124 diabetics patients investigated, 109 patients proved to have peripheral neuropathy by nerve conduction study. Autonomic sudomotoric neuropathy by the NeurocheckTM (CJ) was diagnosed in 94 patients with peripheral neuropathy (86.2%) and in 6 patients (40%) without peripheral neuropathy. Overall prevalence of neuropathy was lower using the indicator test (100 patients, 80.65%) than using nerve conduction study (109 patients, 87.9%). The overall measure of agreement between NeurocheckTM and electrodiagnostic test was 0.3673(0.1547, 0.58) (Table 2).

The sensitivity and specificity of Neurocheck™ was higher in women (91.2% and 63.6%) than in men (78.0% and 50.0%). The measure of agreement in men was 0.1567 (-0.1423, 0.4588) and that in women was 0.5093 (0.2396, 0.9601) (Table 3). The level of HbA_{1C} between the patients with neuropathy and without neuropathy showed no difference. But longer duration of diabetes mellitus and higher total symptom score were observed in the patients with sudomotoric/peripheral neuropathy (Table 4). The score determined by the degree of color change of Neurocheck™ was correlated with the possibility of peripheral neuropathy in diabetic patients (Table 5).

Discussion

Diabetic neuropathy is a most common and troublesome complication of diabetes mellitus, leading to the greatest morbidity and mortality and resulting in a huge economic burden for diabetes care. From patients attending a diabetes clinic 25% reported symptoms; 50% were found to have neuropathy after a simple clinical test such as the ankle jerk or the vibration perception test; almost 90% tested positive to sophisticated tests of autonomic function or peripheral sensation[7)].
Neuropathy increases the risk of amputation 1.7-fold; 12-fold, if there is deformity (itself a consequence of neuropathy), and 36-fold, if there is a history of previous ulceration[7)].
It seems that the most rapid deterioration of nerve function occurs soon after the onset of type 1 diabetes and within 2-3 years there is a slowing of the progress with a shallower slope to the curve of dysfunction[8)]. In contrast, in type 2 diabetes, slowing of nerve conduction velocity can be one of the earliest neuropathic abnormalities and often is present even at diagnosis[9)]. Therefore, it is vitally important to make the diagnosis of diabetic neuropathy early so that appropriate intervention can be instituted.
Disruption of microvascular skin blood flow and sudomotoric function may be among the earliest manifestations of diabetic autonomic neuropathy, so assessing sudomotoric function is very important. The tests of sudomotoric function include the quantitative sudomotoric axon reflex test (QSART), the sweat imprint, the thermoregulatory sweat test (TST), and the sympathetic skin response[4)]. But, these tests require expensive equipment and trained personnel. More recently, a new indicator test (Neurocheck™), which utilizes the water-induced color change of a cobalt compound from blue to pink, has been introduced. This test is simple and reliable and does not require the cooperation of the patient. Because it was known that color change of the indicator test was completed within 10 minutes in 95% healthy personnel, we were supposed to assess the degree of color change in 10 minutes in the diabetic patients.
In the present study, sudomotoric neuropathy was diagnosed in 80.6% of total patients and peripheral neuropathy confirmed by nerve conduction study was in 87.9% of total patients. And sensitivity and specificity of the Neurocheck™ test was 86.2% and 60.0%. The sensitivity and specificity was higher in women (91.2% and 63.6%) than in men (78.0% and 50.0%). The overall measure of agreement between indicator test and nerve conduction study was 0.3673 (0.1547, 0.58), which was somewhat low. But, when

compared with gender, the measure of agreement for women was higher (0.5093 (0.2396, 0.9601)) than men (0.1567 (-0.1423, 0.4588)). The reason why Neurocheck™ test was more sensitive in women is unclear, but many factors such as more active physical activity in men and hormone levels may be related, but further investigations are needed in this area.
The level of HbA_{1C} between the patients with neuropathy and without neuropathy showed no difference. But longer duration of diabetes mellitus and higher total symptom score were observed in the patients with sudomotoric/peripheral neuropathy. Because the total symptom score in patients with sudomotoric neuropathy was higher than without sudomotoric neuropathy, it may advisable to evaluate neuropathy in diabetic patients with higher total symptom score.

Even if there was a incomplete color change only on one foot (score 1), the possibility of peripheral neuropathy was 90%, if incomplete color change on both feet (score 2), the possibility was 94.4%, if complete color change on one foot and incomplete color change on the other foot, the possibility was 100%. But in cases of complete color change on both feet, 62.5% of patients were diagnosed with peripheral neuropathy confirmed by nerve conduction study. So, this may explain the low measure of agreement between Neurocheck™ and nerve conduction study. And In cases that Neurocheck™ shows complete color change on both feet, the patients who have high total symptom score need further evaluation on neuropathy.
In conclusion, use of the new indicator test has a high sensitivity in diagnosis of peripheral neuropathy among type 2 diabetic patients, especially in women. It is likely that the new indicator test is useful clinically as a screening and diagnostic device for diabetic neuropathy. Since the specificity of the test is somewhat low, the patients with high total symptom score and without sudomotoric neuropathy may need further diagnostic evaluation on neuropathy.

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Table 1. Characteristics of patients

N	124
Age (years)	54.94 ± 9.24
Male (%)	36.3
Type 2 DM (%)	97.6
Body weight (㎏)	64.82 ± 10.28
Height (㎝)	160.63 ± 8.54
BMI (㎏/㎡)	25.07 ± 3.14
DM duration (years)	11.62 ± 6.16
HbA_{1C} (%)	8.74 ± 1.44

Table 2. The overall sensitivity and specificity of Neurocheck™ in diabetic patients with or without peripheral neuropathy.

By Neurocheck™	By electro diagnostic test		Total
	With neuropathy	Without neuropathy	
With sudomotoric neuropathy	94 (86.2%)	6 (40.0%)	100 (80.6%)
Without sudomotoric neuropathy	15 (13.8%)	9 (60.0%)	24 (19.4%)
Total	109 (100%)	15 (100%)	124 (100%)

Fisher's Exact test : p-value 0.0001

Kappa statistic	**0.3673**
95% lower limit	0.1547
95% upper limit	0.58

Table 3. The sensitivity and specificity of NeurocheckTM in diabetic patients with or without peripheral neuropathy in men and women.

1) Men

By Neurocheck™	By electro diagnostic test		Total
	With neuropathy	Without neuropathy	
With sudomotoric neuropathy	32 (78.0%)	2 (50.0%)	34 (75.6%)
Without sudomotoric neuropathy	9 (22.0%)	2 (50.0%)	11 (24.4%)
Total	41 (100%)	4 (100%)	45 (100%)

Fisher's Exact test: p-value 0.247

Kappa statistic	**0.1567**
95% lower limit	-0.1423
95% upper limit	0.4558

2) Women

By Neurocheck™	By electro diagnostic test		Total
	With neuropathy	Without neuropathy	
With sudomotoric neuropathy	62 (91.2%)	4 (36.4%)	66 (83.5%)
Without sudomotoric neuropathy	6 (8.8%)	7 (63.6%)	13 (16.5%)
Total	68 (100%)	11 (100%)	79 (100%)

Fisher's Exact test: p-value 0.0001

Kappa statistic	**0.5093**
95% lower limit	0.2454
95% upper limit	0.7732

Table 4. The HbA_{1C}, DM duration, and Total Symptom Score in patients with / without sudomotoric neuropathy and with / without peripheral neuropathy.

	Neurocheck™ test			Nerve conduction study		
	With sudomotoric neuropathy (n=100)	Without sudomotoric neuropathy (n=24)		With peripheral neuropathy (n=109)	Without peripheral neuropathy (n=15)	
HbA_{1C} (%)	8.45 (6.9~13.6)	8.4 (6.8~14.1)	p=0.6091	8.4 (6.9-13.6)	7.9 (6.8-14.1)	p=0.9511
DM duration (years)	12.34 ± 5.90	8.63 ± 6.44	p= 0.0076	7.31 ± 2.60	3.14 ± 2.93	p< 0.0001
Total Symptom Score	7.34 ± 2.60	4.62 ± 3.42	p=< 0.0001	7.31 ± 2.60	3.10 ± 2.93	p< 0.0001

Table 5. The Neurocheck™ score and peripheral neuropathy in diabetic patients. (n % of Total % of Row)

Score	Nerve conduction study		Total
	With peripheral neuropathy	Without peripheral neuropathy	
0	15 12.1 **62.5**	9 7.26 37.5	24 19.35
1	18 14.52 **90**	2 1.61 10	20 16.13
2	68 54.84 **94.4**	4 3.23 5.56%	72 58.06
3	4 3.23 **100**	0 0 0	4 3.23
4	4 3.23 **100**	0 0 0	4 3.23
Total	109 87.9	15 12.1	124 100

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A comparative study between color change plaster for diabetic foot syndrome and electrical impedance change of plantar skin sweat secretion

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Background and aims

Lower extremity amputations are the most prevalent and costly of all the diabetes-related complications. We aimed to asses the value of a color change plaster for detecting diabetics who show a high risk of developing neuropathic foot ulcers and to develop a method to store clinical data and digital images to build a secure standardised database.

Material and methods

For diagnostic of diabetic foot syndrome and peripheral neuropathy, based on detection of the plantar site sweat secretion, we have developed a comparative study between color change plaster-NeuropadR for diabetes foot syndrome (blue=abnormal, blue/pink=borderline or pink=normal) and the electrical impedance changes induced by the skin sympathetic sudomotor activity (SSA) detected with an Impedance ReactometerR, a PC-based system developed in our laboratory. This system is based on a self-balancing technique and a lock-in detection, responding to small changes of electro dermal parameters induced by activation of ecrine sweat glands to muscarinic cholinergic agents. Skin impedance fluctuations around equilibrium reflect the dynamics of the functional state of SSA under resting condition or during stimulus induced responses. The AC current, inversely proportional to local skin impedance, is converted to an ac voltage. New software and automated analysis programs were developed for the clinical research. A PowerShot A70 battery-operated with minimal adjustment of the default settings digital camera captured directly JPEG images of the feet at a resolution of 2048 x 1536 pixels with 24-bit colour. Images were stored with patients' data, standardised on the WHO/Europe recommended Basic Information Sheet diabetes dataset and were collected in the Black Sea Tele Diab system an EPR system.

Results

We assesed sweat secretion of plantar site both feet on 34 diabetics (88% type 2 and 12% type 1) aged between 31and 65 years with an average duration of diabetes of 17 years. Using our technique we have shown that the skin sympathetic activity, expressed in mV, has much lower amplitude according to the functional state of SSA due to diabetic neuropathy. A significant correlation could be observed between Neuropad and measurements of impedance changes induced by the SSA which confirmed the relationship between Neuropad and Impedance Reactometer: on 12 diabetics with neuropathy (without foot ulceration) ($r=0.53$, $p<0.001$), on 14 patients at borderline ($r=0.67$, $p<0.001$) and on 8 patients without neuropathy ($r=86.3\%$ $p<0.0001$). These results have indicated that the Neuropad and Impedance Reactometer are equally suitable of use in the diagnosis of the skin sweat secretion impairment at plantar site in diabetes. However, the Neuropad is less expensive than the Reactometer and it is suitable for the patient self-examination use as a control routine examination every 6 months or diagnostic test for medical care. The findings were pathological if both of the examination methods demonstrated the result of disturbed or absence of the sweat secretion and plantar insensitivity to pressure from the 10g-monofilament. Prospective store of clinical data and digital images will be used to monitor the health care outcomes of patients with diabetes.

Conclusion

These preliminary results suggest that colour change plaster for diabetes foot syndrome and the impedance changes induced by the SSA significantly correlate and may be negative prognostic impact of neuropathic foot ulcers.

Sudomotor dysfunction is associated with duration of Type 2 diabetes mellitus

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Summary

Aim of the study

The aim of the present study was to investigate the impact of diabetes duration on sudomotor dysfunction, as diagnosed with the new indicator test (Neuropad®) in type 2 diabetic patients.

Patients and methods

This study included 181 type 2 diabetic patients (87 men) with a mean age of 65.3±6.8 years and a mean diabetes duration of 17.8±7.3 years. Patients were divided into Group I (diabetes duration lower than 10 years), Group II (diabetes duration 10-19 years) and Group III (diabetes duration higher than 19 years). Sudomotor dysfunction was assessed by means of the indicator test applied to both soles.

Results

Frequency of sudomotor neuropathy was 39.70% in Group I, 62% in Group II and 90.19% in Group III (p=0.001). Time until complete colour change of the test was 9.1±2.7 minutes in Group I, 16.8±3.3 minutes in Group II and 26.8±4.3 minutes in Group III (p=0.0001). Among patients with sudomotor dysfunction this time was 13.9±1, .3, 19.7±2.6 and 30.1±4.2 minutes respectively (p=0.0001).

Conclusions

Both frequency and severity of sudomotor dysfunction, as diagnosed with the new indicator test, are associated with duration of type 2 diabetes.

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Evaluation of the new indicator plaster (neuropad®) in the diagnosis of peripheral neuropathy among Type 2 diabetic patients

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Background and aims

Autonomic sudomotor neuropathy is associated with reduction of plantar sweating and contributes to the pathogenesis of diabetic foot ulcers. Early diagnosis of the sudomotor component of peripheral neuropathy may contribute to detection of patients at high risk for diabetic foot complications. Therefore, the aim of the present study was to evaluate the new indicator plaster (Neuropad®) in the diagnosis of peripheral neuropathy among type 2 diabetic patients.

Materials and methods

This study included 104 type 2 diabetic patients (51 men) with a mean age of 64,2±5,6 years and a mean diabetes duration of 12,8±3,7 years. The control group comprised 20 healthy young volunteers (<40 years old). Peripheral neuropathy was diagnosed by means of the Diabetic Neuropathy Index (DNI, normal values: 0-2). Indicator plasters were applied to both soles of patients. Autonomic neuropathy was assessed by means of colour change in the indicator plasters (normal response: colour change within 10 minutes).

Results

Peripheral neuropathy was diagnosed in 71 patients (68,27%). Colour change of the plaster in the right sole was associated with colour change in the left sole (p=0,0001). Autonomic neuropathy was diagnosed in 67 patients (94,36%) with peripheral neuropathy and in 10 patients (30,3%) without peripheral neuropathy (p=0,0001). Compared with DNI, sensitivity of the indicator plaster for diagnosing peripheral neuropathy was 94,36% and specificity was 69,69%. Overall prevalence of neuropathy was higher using the indicator plaster (77 patients, 74,04%) than using the DNI (71 patients, 68,27%). Colour change of the indicator plaster was completed within 10 minutes in 19 volunteers (95%). Time until complete colour change of the indicator plaster was 23,80±6,7 minutes in patients with peripheral neuropathy and 7,67±1,22 minutes in patients without peripheral neuropathy (p=0,001). Among patients with peripheral neuropathy, time until complete colour change of the indicator plaster was 14,20±1,9 minutes in those with a DNI value between 2,5 and 4,5, while it was 32,8±2,6 minutes in those with a DNI value between 5 and 8 (p=0,003).

Conclusions

Use of the new indicator plaster has a very high sensitivity in detection of diabetic peripheral neuropathy. Autonomic sudomotor dysfunction can even be demonstrated in a considerable part of patients with normal DNI. Therefore, the new indicator plaster may prove useful in detection of patients at high risk for diabetic foot complications. Finally, time until complete colour change of the indicator plaster is associated with severity of peripheral neuropathy.

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Evaluation of a New Indicator Test for Sudomotor Function (Neuropad®) in the Diagnosis of Peripheral Neuropathy in Type 2 Diabetic Patients

Abstract

Sudomotor neuropathy is associated with reduction of plantar sweating and contributes to the pathogenesis of diabetic foot ulcers. The aim of the present study was to evaluate the new indicator test for sudomotor function (Neuropad®) in the diagnosis of peripheral neuropathy among type 2 diabetic patients. This study included 104 type 2 diabetic patients (51 men) with a mean age of 64.2 ± 5.6 years and a mean diabetes duration of 12.8 ± 3.7 years. Peripheral neuropathy was diagnosed by means of the Diabetic Neuropathy Index (DNI). Sudomotor neuropathy was assessed by means of colour change in the indicator test. Peripheral neuropathy was diagnosed in 71 patients (68.3%). Sudomotor neuropathy was diagnosed in 67 patients (94.4%) with peripheral neuropathy and in 10 patients (30.3%) without peripheral neuropathy ($p = 0.0001$). Compared with DNI, sensitivity of the indicator test for diagnosing peripheral neuropathy was 94.4% and specificity was 69.7%. Overall prevalence of neuropathy was higher using the indicator test (77 patients, 74.0%) than using the DNI (71 patients, 68.3%). Time until complete colour change of the indicator test was 23.8 ± 6.7 min in patients with peripheral neuropathy and 7.7 ± 1.2 min in patients without peripheral neuropathy ($p = 0.001$). Among patients with peripheral neuropathy, time until complete colour change of the indicator test was 14.2 ± 1.9 min in those with a DNI value between 2.5 and 4.5, while it was 32.8 ± 2.6 min in those with a DNI value between 5 and 8 ($p = 0.003$). **Conclusions:** Use of the new indicator test has a very high sensitivity in detection of diabetic peripheral neuropathy. Sudomotor dysfunction can be demonstrated in a considerable part of patients with normal clinical examination. Time until complete colour change of the indicator test is associated with severity of peripheral neuropathy.

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

Introduction

Peripheral neuropathy is one of the most common chronic complications of diabetes mellitus and leads to considerable increase in morbidity (La Cava, 2002; Perkins and Bril, 2003; Petit and Upender, 2003; Pittenger and Vinik, 2003; Podwall and Gooch, 2004; Duby et al., 2004). It is of crucial importance in the pathogenesis of foot ulcers (Litzelman et al., 1997; Boulton et al., 1998; Jude and Boulton, 1999; Mason et al., 1999; Reiber et al., 1999). A neglected component of peripheral neuropathy is sudomotor neuropathy, which results in reduced sweating and dry, sensitive skin with a propensity towards callus and fissure formation (Reiber et al., 1999; Boulton, 2003).

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Numerous tests of sudomotor function have been devised (Reiber et al., 1999; Boulton, 2003). The most important tests include the quantitative sudomotor axon reflex (QSART), the sweat imprint, the thermoregulatory test and the sympathetic skin response (Low, 2003; Vinik et al., 2003). Studies using these tests have shown that sudomotor neuropathy develops early in diabetic patients and can therefore be demonstrated even in asymptomatic patients with normal clinical examination and nerve conduction study (Kennedy and Navarro, 1989; Caccia et al., 1991; Braune and Horter, 1996■ Not in references ■; Shimada et al., 2001; Hoeldtke et al., 2001). Nevertheless, these tests are not generally available, because they require expensive equipment and trained personnel (Low, 2003; Vinik et al., 2003).

More recently, a new indicator test (Neuropad®) has been introduced, which measures sweat production on the basis of a colour change from blue to pink (Zick et al., 2003). This new test is an easy-to-perform measure of the sudomotor component of peripheral neuropathy. However, its contribution to the diagnosis of peripheral neuropathy has not been investigated in any other study. Therefore, the aim of the present study was to evaluate this new indicator test in the diagnosis of peripheral neuropathy among type 2 diabetic patients.

Materials and Methods

This study included 104 patients (51 men, 53 women) with type 2 diabetes mellitus. Mean age was 64.2 ± 5.6 years and mean diabetes duration was 12.8 ± 3.7 years. These patients were recruited from the Diabetic Department of the General Hospital of Alexandroupolis, Greece and from the Second Department of Internal Medicine of Democritus University of Thrace, Greece. The control group comprised 20 healthy volunteers (< 40 years old). The study was approved by the institutional ethics committee and all patients gave their informed consent.

Exclusion criteria were peripheral arterial occlusive disease, as well as chronic alcohol abuse, thyroid disease, Vitamin B_{12} depletion, lumbar spine disorders or any other cause of peripheral neuropathy.

Peripheral neuropathy was diagnosed by means of the Diabetic Neuropathy Index (DNI), as proposed by the University of Michigan (Feldman et al., 1994; Bax et al., 1996). The DNI is a standardized examination of feet appearance (deformity, dry skin, callus, infection and fissures), neuropathic ulceration, Achilles tendon reflexes and vibration perception at great toe using a 128 Hz tuning fork. This examination is applied separately to each foot. Abnormal findings are added to form the DNI score (normal score ≤ 2, worst score: 8) (Feldman et al., 1994). In the present study patients with a DNI score higher than 2 were considered to have peripheral neuropathy. Peripheral neuropathy was considered moderate in patients with a DNI score between 2.5 and 4.5 and severe in those with a DNI score between 5 and 8.

Sudomotor neuropathy was assessed by means of the new indicator test (Neuropad®) (Zick et al., 2003). Patients were allowed to rest in constant room temperature (25 °C) for 10 min after they had taken off their shoes and socks. Indicator tests were applied to both soles at the level of the 1st – 2nd metatarsal heads and were left until complete colour change from pink to blue. Complete colour change of the test in both feet within 10 min was considered normal response. Patients in whom colour change of the indicator test was completed after 10 min in at least one foot were considered to have sudomotor neuropathy.

Statistical analysis was performed by χ^2 test (using Yates' correction for 2 × 2 contingency tables) for qualitative variables. Quantitative variables had normal distribution and were compared using *t*-test, ANOVA and least significant difference test. Data were expressed as mean ± 1 Standard Deviation ($\bar{x}$ ± 1 SD). Statistical significance was defined at a level of 5% ($p < 0.05$).

Results

Peripheral neuropathy was diagnosed in 71 patients (68.3%). Sudomotor neuropathy was diagnosed in 67 patients with peripheral neuropathy (94.4%) and in 10 patients (30.3%) without peripheral neuropathy ($p = 0.0001$), as shown in Table **1**. Compared with DNI, sensitivity of the indicator test for diagnosing peripheral neuropathy was 94.4% and specificity was 69.7%. Overall prevalence of neuropathy was slightly ($p = 0.44$, NS) higher using the indicator test (77 patients, 74.0%) than using the DNI (71 patients, 68.3%).

In all persons examined time until complete colour change of the test in the right sole did not differ from time until complete colour change in the left sole ($p = 0.99$, Table **2**). Colour change of the indicator test was completed within 10 min in 19 volunteers (95%). Time until complete colour change of the indicator test in healthy volunteers and in diabetic patients with or without peripheral neuropathy is shown in Fig. **1**. Time until complete colour change of the test in patients with moderate vs. severe peripheral neuropathy is shown in Fig. **2**.

Discussion

This study evaluated the sudomotor component of peripheral neuropathy in type 2 diabetic patients using the new indicator test (Neuropad®). Peripheral neuropathy was clinically diagnosed by means of DNI (Feldman et al., 1994; Bax et al., 1996).

Table **1** Sudomotor neuropathy in diabetic patients with or without peripheral neuropathy

Patients	*With peripheral neuropathy*	*Without peripheral neuropathy*	*Statistical evaluation*
With sudomotor neuropathy	67 (94.4%)	10 (30.3%)	$p = 0.0001$ ($\chi^2 = 44.8$)*
Without sudomotor neuropathy	4 (5.6%)	23 (69.7%)	$p = 0.0001$ ($\chi^2 = 44.8$)*
Total	71	33	104

* p value refers to the difference between patients with peripheral neuropathy and those without peripheral neuropathy

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Table **2** Time until complete colour change of the indicator test in right vs. left foot

Time until complete colour change of the test (minutes)			
Persons examined	***Right foot***	***Left foot***	***Statistical evaluation***
Controls	4.6 ± 0.7	4.6 ± 0.6	p = 0.99
Patients without peripheral neuropathy	7.7 ± 1.2	7.6 ± 1.2	p = 0.99
Patients with peripheral neuropathy	23.8 ± 6.7	23.9 ± 6.4	p = 0.98

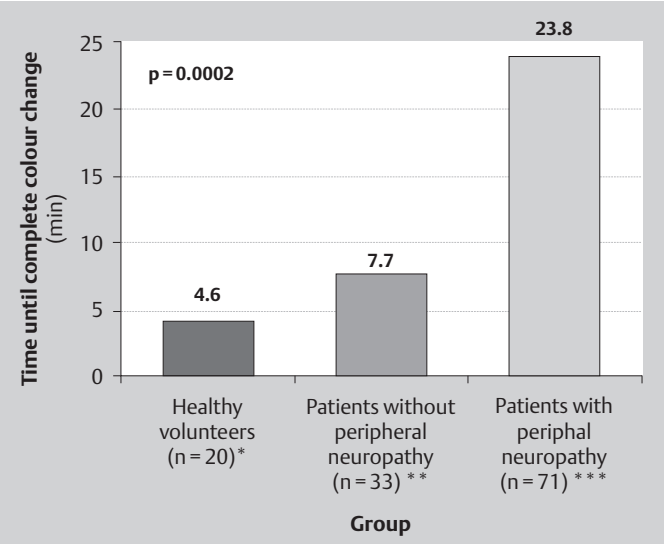

Fig. **1** Time until complete colour change of the test in healthy volunteers (4.6 ± 0.7 min), in diabetic patients without peripheral neuropathy (7.7 ± 1.2 min) and in diabetic patients with peripheral neuropathy (23.8 ± 6.7 min), p = 0.0002. * vs. ** p = 0.033, * vs. *** p = 0.0001, ** vs. *** p = 0.001.

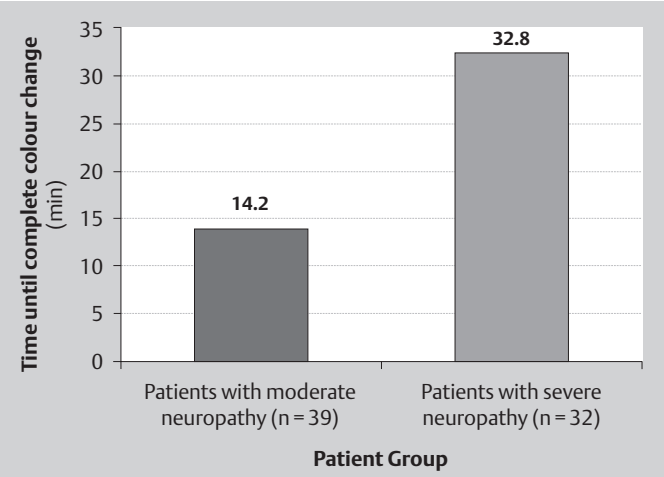

Fig. **2** Time until complete colour change of the test in diabetic patients with moderate (14.2 ± 1.9 min) vs. severe peripheral neuropathy (32.8 ± 2.6 min), p = 0.003.

Frequency of peripheral neuropathy was 68.3%. Prevalence of diabetic peripheral neuropathy is known to differ considerably, according to type of patients studied and diagnostic criteria (Pirart, 1978 a; Pirart, 1978 b; Ziegler et al., 1987; the DCCT Research Group, 1988; Maser et al., 1989; Young et al., 1993; Ahroni et al., 1994; Dyck et al., 1997; Forrest et al., 1997; Fedele et al., 1997; Boulton, 1998). Fedele et al. used the DNI as a screening test in an epidemiological study and reported that 32.3% of patients had neuropathy (Fedele et al., 1997). The higher prevalence of neuropathy in our study as compared to Fedele et al. may be attributed to the fact that we included a smaller number of patients and recruited patients who were attending the Diabetic Department or were hospitalized. Therefore, our results are not representative of the epidemiology of neuropathy in a diabetic population.

Sudomotor neuropathy was diagnosed in 94.4% of patients with peripheral neuropathy and in 30.3% of those without peripheral neuropathy. This difference was highly significant (p = 0.0001). Compared with DNI, sensitivity of the indicator test for diagnosing peripheral neuropathy was 94.4% and specificity was 69.7%. Specificity was only 69.7%, because sudomotor dysfunction was also diagnosed in 30.3% of patients with normal DNI score. This is probably due to the fact that sudomotor dysfunction develops early in the course of diabetes and can be detected even in patients with normal clinical examination and nerve conduction study (Kennedy and Navarro, 1989; Caccia et al., 1991; Braune and Horter, 1996■ Not in references ■; Shimada et al., 2001; Hoeldtke et al., 2001). Our results are in accordance with those of Zick et al. (2003). However, we used a more homogeneous group of patients, enrolling exclusively type 2 patients. Besides, we employed the standardized and validated DNI score for a more precise diagnosis of peripheral neuropathy.

Prevalence of diabetic neuropathy was insignificantly higher using the indicator test than using the DNI. We therefore believe that the indicator test may prove sensitive in detection of patients at high risk for diabetic foot complications. The fact that the difference in prevalence did not attain statistical significance is perhaps attributable to the small number of patients. Larger prospective studies are needed to investigate the contribution of Neuropad® to detection of high-risk patients and prevention of foot ulcers.

Time until complete colour change of the indicator test differed significantly between diabetic patients with peripheral neuropathy, diabetic patients without peripheral neuropathy and healthy volunteers. Time until complete colour change was significantly higher in diabetic patients with peripheral neuropathy than in those without peripheral neuropathy. It was significantly lower in healthy volunteers than in either group of diabetic patients (with or without peripheral neuropathy). These results are in agreement with the study by Zick et al. (2003).

Furthermore, time until complete colour change in the present study was examined in relation to the DNI score. Analysis showed that time until complete colour change was significantly higher in patients with severe peripheral neuropathy (DNI score between 5 and 8) than in those with moderate peripheral neuropathy (DNI score between 2.5 and 4.5). Consequently, the indica-

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tor test was a reliable diagnostic tool not only of the presence but also of the severity of peripheral neuropathy. This result suggests that the indicator test may find a novel use in clinical practice, helping to quantify the reduction of sweat production due to neuropathy and thus – indirectly – the risk for foot ulceration. This is particularly important, since application of the test is easy and interpretation of results does not require patient cooperation. The easy applicability of the test in our study, even in patients of low educational level, was in contrast to vibration perception (as included in the DNI score).

Moreover, time until complete colour change of the test did not differ between the two soles. This association was demonstrated in all persons examined, regardless of the presence of diabetes and regardless of the diagnosis of peripheral neuropathy. Thus, the test showed very good intra-individual reproducibility. This finding offers further evidence that the test is a reliable index of sudomotor function per se and is independent of minor local skin factors which may be different in one foot. Further studies might examine if the test can consistently be applied to one foot only, which is expected to reduce cost and time needed for the procedure.

In conclusion, use of the new indicator test has a very high sensitivity in diagnosis of peripheral neuropathy among type 2 diabetic patients. Sudomotor dysfunction can even be demonstrated in a considerable part of patients with normal clinical examination. Furthermore, time until complete colour change of the test may be used to assess severity of peripheral neuropathy. Therefore, the new indicator test may prove useful in detection of patients at high risk for diabetic foot complications.

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Tests for autonomic neuropathy in the diabetic foot

Doctoral Thesis
for the degree of Doctor of Medicine
at Hanover Medical University

by
Thomas Schäper
from Ibbenbüren

Accepted by the Senate of Hanover Medical University
on

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Rector: Professor Dr.
Thesis supervisor: Professor Dr. med. R. Zick
Examiner:
Second examiner:

Date of oral examination:
Doctoral examining committee members:

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1. Introduction

A large proportion of the population of western industrialised nations suffers from the widespread disease diabetes mellitus. Today, the incidence of this metabolic disease is also on the increase in so-called emerging countries. According to statistical calculations from WHO, the number of diabetics world-wide is expected to be 350 million in 2025. In 2001 the number of diagnosed diabetics in Germany was put at 5.7 million [1].

The number of people suffering from type 2 diabetes mellitus has risen in recent decades; up by approx. 43% compared to 1988 [2]. While the incidence of diabetes in the population was 0.6% at the beginning of the sixties, the latest estimates for the nineties were 5-8%. These figures are for the total population. Among older people the prevalence of diabetes reaches up to 25% depending on age group [3, 4].

Part and parcel of diabetes mellitus are its complications. These late symptoms have considerable clinical consequences and are generally underestimated. The informative CODE 2 (Costs of Diabetes in Europe) study revealed that diabetes mellitus causes around 6,000 instances of loss of eyesight, 8,000 renal failures requiring dialysis, 28,000 cases of amputation of limbs [5, 6], 27,000 heart attacks [7] and 44,000 strokes [8] annually, making diabetes mellitus the most expensive and major widespread disease in Germany – with the problem on the increase. The study states that the total costs of type 2 diabetes mellitus in the Federal Republic come to 15.8 bn. euros annually. On the other hand, the UKPDS (United Kingdom Prospective Diabetes Study) was able to prove that early, rigorous treatment and prophylaxis could reduce the later complications of diabetes [9].

One frequent complication is diabetic foot syndrome. The significant undesired consequences of foot problems result in foot ulcers and amputations. When discussing the pathogenesis for the development of a diabetic foot it is imperative to differentiate between factors relating to peripheral neuropathy and peripheral arterial occlusive disease as well as those connected with the development of foot ulcers. What we are dealing with therefore is a sequence of events which comprises a combination of factors, as the following Figure 1 illustrates [10].

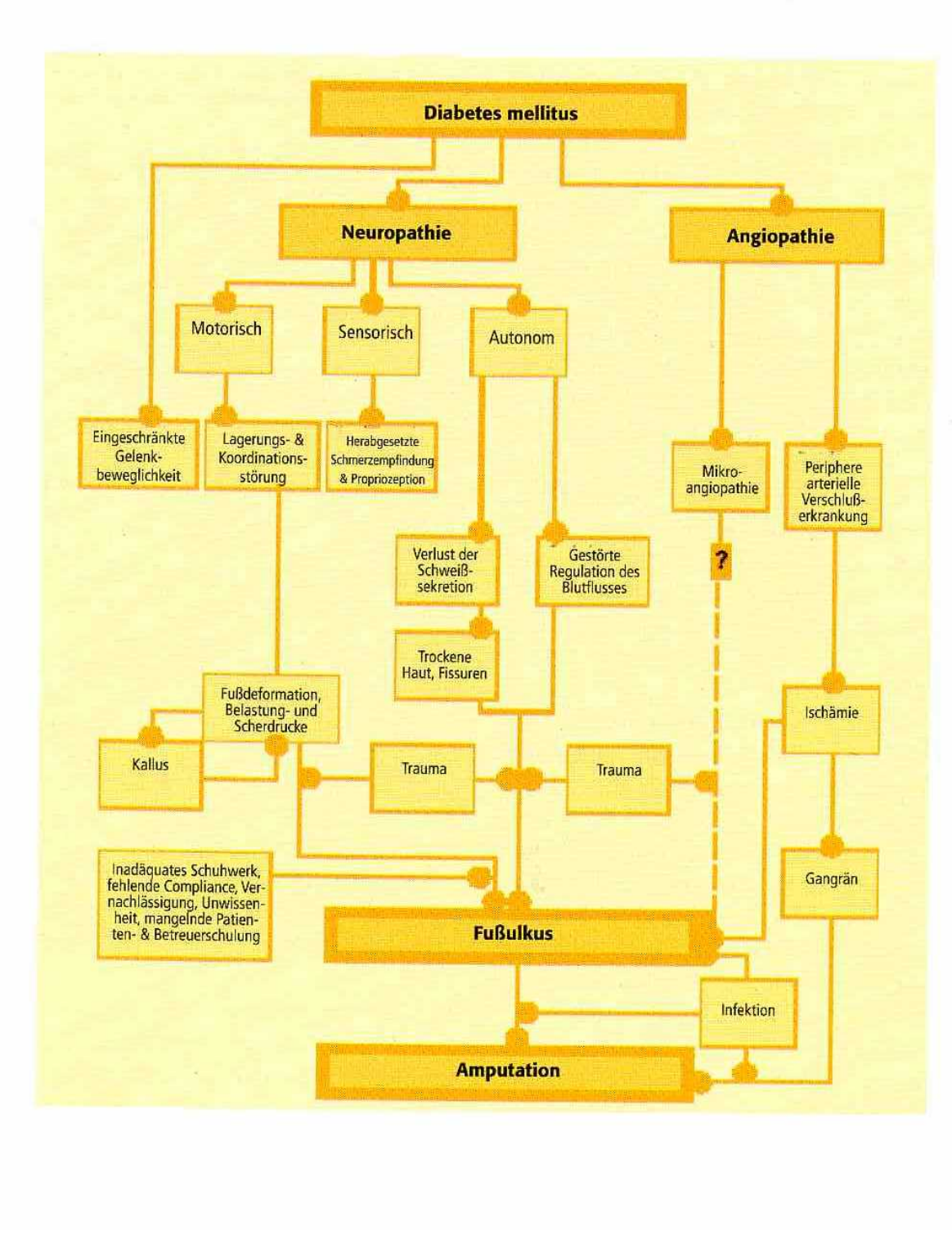


Fig. 1: Pathogenesis of diabetic foot ulcerations; modified as in [10].

Circulatory disturbance of the lower limb is one of the significant causes of foot lesion with diabetes mellitus [11]. The success of treatment and healing depends largely on the circulatory situation [12, 13]. Peripheral arterial occlusive disease develops alone in 20% of cases, but otherwise always in conjunction with neuropathy.

Neuropathies can be divided into three categories, depending on the type of nerve affected. Peripheral neuropathy is defined as a clinically manifest or subclinical disease which develops in people with diabetes mellitus and cannot be traced back to any other cause of peripheral neuropathy [14, 15]. Neuropathic disease is differentiated by manifestations in the somatic and autonomic areas of the peripheral nervous system.

Due to the abundance of clinical pictures, which overlap in parts, no generally accepted classification has yet been established. However, the following Table 1, compiled by Sima et al. [14], which takes aetiopathogenesis into account, gives a good overview.

1.) Rapidly reversible:

Hyperglycaemic neuropathy

2.) Persistent symmetric polyneuropathies:

a) distal somatic sensory/motor polyneuropathies involving predominantly large fibres
b) Autonomic neuropathies
c) Small-fibre neuropathies

3.) Focal/multifocal neuropathies:

a) Cranial neuropathies
b) Thoracoabdominal radiculopathies
c) Focal limb neuropathies
d) Proximal neuropathies
e) Compression and entrapment neuropathies

Tab. 1: Classification of neuropathies by Sima et al [14]

The clinical complaints of the individual neuropathies can be described as follows:

Sensory neuropathy leads to a loss in perception of temperature, touch and vibration. Harmful trauma and stimuli are no longer accurately felt, which can lead to the formation of ulcera [16, 17, 18, 19].

Motor neuropathy causes a flexion deformity of the toes ("claw toe") and, as a result, a changed gait. Areas of increased pressure arise under the metatarsal heads and toes [20]. Along with the changed gait pattern and foot deformities comes ultimately, probably due to protein glycosylation in joints, tendons and soft tissue, reduced flexibility of joints, which increases abnormal biomechanical stress on the foot involving changed distribution of pressure on the plantar area [21].

Autonomic neuropathy finally leads to dry, cracked skin due to loss of sudomotor function. Fissures and rhagades are the result. For micro-organisms like bacteria and fungi, the loss of the skin's protective function represents an ideal point of entry, meaning infections can develop very easily, which often constitute an early symptom of DFS that goes unnoticed. Increased blood flow due to enlarged arteriovenous shunts leads to a warm, and only seldom edematous, foot. Autosympathectomy leads to vasodilatation with dilated instep veins [20]. Other consequences of diabetic autonomic neuropathy are cardiopathy [22], gastroparesis and erectile dysfunction [23, 24].

2. Objectives

To date diabetic neuropathy has always been initially diagnosed in the clinic. An essential element of diagnosis is a detailed case history, where the subjective symptoms of neuropathy are specifically ascertained: smarting or stabbing pain, paraesthesia such as tingling or numbness, temperature paraesthesia or hyperaesthesia. The complaints, which especially tend to exacerbate at night, are recorded in the form of scores (NSS: Neurological Symptom Score) [25].

A clinical assessment should be followed up by a neurological examination [10]. The electrophysiological methods like motor and sensory nerve conduction velocity and action potential have the benefit of objectivity as well as higher sensitivity and reproducibility. Their limitations are, however, the complexity of the method and the fact that they are restricted to recording large myelinated fibres. The function of the autonomic nervous system goes unobserved.

The objective of this thesis, therefore, was to look into autonomic neuropathy of the diabetic foot and in particular to test changes in nerve-stimulated sweat secretion using newly available methods (Neuropad indicator plaster, selective hydrometry) and an established standard procedure (flat-electrode hydrometry). In concrete terms, the following questions were to be answered:

1.) Is the Neuropad indicator plaster as a new diagnostic agent capable of detecting changed sweat secretion as the manifestation of a loss of sudomotor function? Do differences arise in results for diabetics with and without verified sensory neuropathy?

2.) Is selective hydrometry as a new test procedure capable of detecting changed sweat secretion as the manifestation of a loss of sudomotor function? Do differences arise in results for diabetics with and without verified sensory neuropathy? Can a difference in sweat secretion between the forefoot and heel area be detected with selective hydrometry and, if so, does this difference change once sensory neuropathy develops?

3.) Can the measurements taken in the plantar area of the foot with the Neuropad indicator plaster, selective hydrometry and flat-electrode hydrometry in control individuals and

diabetics with and without sensory neuropathy be compared, and what practical conclusions can be drawn from this?

4.) In terms of sensitivity, specificity and positive and negative predictive value, do the Neuropad indicator plaster and selective hydrometry meet clinical test requirements, and what practical conclusions can be drawn from this?

3. Patients and methods

3.1. Patient population

Three populations were formed to facilitate a comparison of practicability and clinical relevance of the measuring methods for determining skin dryness with the existing examination methods. Participants in the study were divided into diabetics with positively verified peripheral sensory neuropathy, diabetics without neuropathy and a healthy, normal population.

Before testing began, all participants in the study were informed in detail, both verbally and in writing, about the point of the study and the forthcoming measurements [Appendix 1]. A written informed consent with the patients' and testing physician's signature was obtained [Appendix 2]. The subjects were able to withdraw from the study at any time without having to give reasons. No remuneration or compensation of any kind was paid. No outside funding was provided.
Before the start of tests, all aspects of the clinical study were presented to the ethics commission of Hanover Medical University, under the chair of Prof. Dr. H.D. Tröger, and approved by this same body.

In accordance with the definition of diabetic neuropathy, which occurs in persons with diabetes mellitus and cannot be traced back to any other cause of peripheral neuropathy [14], the participants in the study were examined before being categorised. To eliminate the possibility of non-diabetogenous peripheral neuropathy a detailed case history was drawn up first of all on the basis of the patient record [Appendix 3]. Entered here were, besides the age and gender of the participant, the type of diabetes, duration of diabetes and form of metabolic control. It was also established whether there was a prior history of secondary diseases like retinopathy, nephropathy, angiopathy and diagnosed neuropathy. Questions were asked about foot care routine, allergies, atopies, skin type and UV sensitivity, the footwear mainly worn and adjuvant medication. Measurement of blood pressure using the Riva-Rocci method and heart rate followed. Subsequent laboratory tests to record the current metabolic condition and potential indirect parameters for exclusion from the study were carried out in the laboratory of St. Bonifatius Hospital in Lingen (Head Physician Prof. Dr. med. R. Zick): determined here were HbA1c, creatinine, urea, TSH, GOT, GPT, albuminuria.

Excluded from the study were minors and those over 75. Subjects with manifest peripheral arterial occlusive disease (PAOD), established by the tibiobrachial index < 0.9 [26], or mediasclerosis [27], were excluded from the study. Since both PAOD and mediasclerosis can lead to trophic foot disturbances, these criteria were included in the exclusion criteria.

Patients who were taking part in another clinical study at the time were not examined. By definition the presence of uraemia, renal insufficiency requiring dialysis, toxic neuropathies due to alcohol, severe hepatopathy and neoplasia, as well as the taking of certain drugs, for instance, corticosteroids, psycho-active drugs and antihistamines, which could bring undesired pharmacological effects to bear on the results of the study, resulted in exclusion. Cases of traumatic peripheral nerve lesions, plexus pareses, spinal root compression syndromes, herpes zoster and polyradiculopathies also resulted in exclusion. Naturally, no subjects suffering from dermatological illnesses which per se are accompanied by a trophic disturbance of the affected area of skin could be included in the study. These include neurodermatitis, psoriasis, Raynaud's syndrome, acrocyanosis, hyperhidrosis, sclerodermatitis and even allergies. Appendix 4 gives a full overview of the exclusion criteria.

If one or more of the above criteria was applicable, the patient was excluded from the study. For the purpose of further objectification the renal parameters were determined in detail by means of serum creatinine and urea, also the transaminases GOT and GPT, the basal TSH value as a marker of thyroid dysfunction and the HbA1c value to express the level of blood sugar metabolism.

3.2. Classification criteria

After taking the inclusion and exclusion criteria into account the patients were examined for the presence of peripheral sensory neuropathy using the “basic examination methods”. The four methods of examination for determining sensory neuropathy, into which this thesis will go in detail in 3.5., were performed on all patients. Vibratory sensibility, sensitivity to pressure or sense of touch, and temperature perception were qualitatively and quantitatively tested. PAOD was determined by means of Doppler measurement. The recommendations of the San Antonio Consensus Conference [15] on the diagnosis of diabetic neuropathy as well as the criteria set out by Ziegler [27] and Dyck [29, 30] were used to decide whether peripheral sensory neuropathy existed. On this basis it was defined that for the purposes of this study a peripheral sensory neuropathy existed if and when two of the four tests carried out (in 3.5.) returned pathological findings. If there was an ulcus on the foot, the presence of sensory neuropathy was also assumed.

If the existence of sensory diabetic neuropathy could not be conclusively decided on and established, the results in relation to the Neuropathy Disability Score (Appendix 5) and the Total Symptom Score found by the case history (Appendix 6) were used to ensure reliable classification [10] .

3.3. Group composition

Having considered the exclusion criteria and taken the neuropathy measurement 96 individuals were included in the study. They break down into 34 healthy control individuals and 62 diabetics, who we recruited both through out-patient visits to the special unit of the Diabetes Centre at St. Bonifatius Hospital in Lingen as well as during in-patient stays in the Department of Gastroenterology and Diabetology.

Of the population of diabetics, 47 patients have type 2 diabetes mellitus; the remaining 15 patients have type 1 diabetes mellitus. The gender breakdown among diabetics was 27 females to 35 males. The average age was 56.8 years, while the average duration of illness was 13.5 years. The HbA1c value was 8.3% on average.

The healthy control group of 34 individuals was made up of 24 females and 10 males with an average age of 47.6 ± 11.7 years altogether. The average HbA1c value of the healthy individuals was 5.2% ± 0.4%.
The three Groups were called Group A, Group B and Group C.

- Group A: Diabetics without peripheral sensory neuropathy
- Group B: Diabetics with peripheral sensory neuropathy
- Group C: Control comprising healthy individuals

The 62 diabetics were divided into Groups A and B, depending on the existence of sensory neuropathy. The decision as to which of the two Groups (A or B) a patient was assigned was governed by the result of the test methods determining the existence of peripheral sensory neuropathy. These are listed in detail in Section 3.5.
The 34 healthy participants made up the control population, Group C.

Table 2 gives an overview of the Group characteristics:

Group	**n**	**Age (years)**	**Standard deviation**	**Male**	**Female**	**Type of diabetes**	**Duration of diabetes**	**HbA1c value**
A	36	54.3	11.5 years	19 (52.8%)	17 (47.2%)	13 type 1 23 type 2	11.61 years ± 8.9 years	8.1% ± 1.4%
B	26	58.9	11.0 years	16 (61.5%)	10 (38.5%)	2 type 1 24 type 2	16.08 years ± 8.8 years	8.7% ± 1.8%
C	34	47.6	11.7 years	10 (29.4%)	24 (70.6%)			5.2% ± 0.4%

Tab. 2: Group characteristics of the three populations, giving age, standard deviation, gender, type and duration of diabetes and HbA1c value.

3.4. Methods

Early diagnosis of diabetic neuropathy is of great importance. Studies show that by the time of diagnosis of type 2 diabetes mellitus, defined under the WHO criteria for hyperglycaemia [31], in around 80% of cases a changed vibratory sensibility and in just under 16% of cases a nerve conduction impairment already exists. There was already a loss of muscle proprioceptive reflex in 13.6% of the newly diagnosed patients [32].

Objective methods of measuring peripheral neuropathy are apparative and very time-consuming. Electrophysiological tests like nerve conduction velocity can only reliably pick up disturbances in the large, rapidly-conducting nerve fibres [33]. In the case of motor nerve lesions the electromyograph delivers valid results.
Just as time-consuming are the procedures for measuring sudomotor function such as Minor's chemical reaction-based iodine-starch test and Moberg's ninhydrin test. The same goes for quantitative test methods such as the axon reflex test. The microneurographic single-fibre recording of sympathetic nerve fibres is not eligible for routine diagnostics. To this day there is still no generally accepted procedure for diagnosing autonomic neuropathy [34].

The extensive apparative diagnostics of these objective examination methods are unnecessary for diagnosis in typical clinical cases of diabetic neuropathy [35]. The examinations explained in the following, which are used as "basic" methods to compare the new measuring methods, suffice for routine clinical purposes.

3.5. Description of the procedures for testing for sensory neuropathy

The sensory fibres of the peripheral nervous system are responsible for the perception of pressure, temperature, pain and vibration. However, the clinical symptoms of patients affected by sensory neuropathy are rather unspecific. They therefore go undetected for a long time, and thus untreated. Solid verification of sensory neuropathy thus relies on valid and comparable, clinically applicable, simple routine test methods, based essentially on perceptual-physiological tests. These include tests with the Rydell-Seiffer c-128 tuning fork, the standardised Semmes-Weinstein 10g monofilament, the Tip-Therm and quantitative thermoreception.

3.5.1. Rydell-Seiffer c-128 tuning fork

Pallaesthesia was checked for using the principle of "bone sensibility", as described by Rydell and Seiffer [36]. The tuning fork consists of a solid metal fork of 128 Hz, whose frequency is reduced to 64 Hz by screw-on weights. The vibrations of the Rydell-Seiffer tuning fork are transmitted to bone structures, in this instance the head of the first metatarsal bone, and the patient asked about his perception of the vibrations [37, 38, 39]. The procedure was as follows for both feet:

The subjects sat down on a chiropodist's chair and removed all clothing from their feet. After briefly informing them about the test about to be carried out, the tuning fork was stimulated by striking it with the hand, and placed first of all on the subject's distal head of the radius for purposes of demonstration, so as to differentiate between vibration and the contact pressure of the tuning fork and to give a reliable perception of the test stimulation. Then the tuning fork, set once more in vibration, is placed on the lateral metatarsophalangeal joint under light pressure. Now the participant is asked whether vibration can be felt and instructed to indicate the moment when he no longer feels the vibration. To rule out any errors due to visual influence, the subjects are asked to close their eyes during the test and to concentrate solely on the vibration.

At the ends of the tuning fork there is a scale from which the amplitude of the vibration can be read off using overlapping triangles. The scaling is in eighths, where 8/8 reflects the maximum vibration, and 1/8 the minimum vibration. As vibratory sensibility decreases with age, values ≥ 6/8 in patients under 40 were regarded as normal. Subjects who are older than 40 should be able to sense a vibration level between 5/8 and 8/8. If values under this benchmark were measured, the result was deemed pathological.

Vibration measurement was carried out separately on both feet twice – above the metatarsophalangeal joint (malleolus medialis) and the instep (dorsum pedis) – and the arithmetic mean documented in the appropriate place in the patient record (Appendix 7). Figure 2 shows a picture of the test.

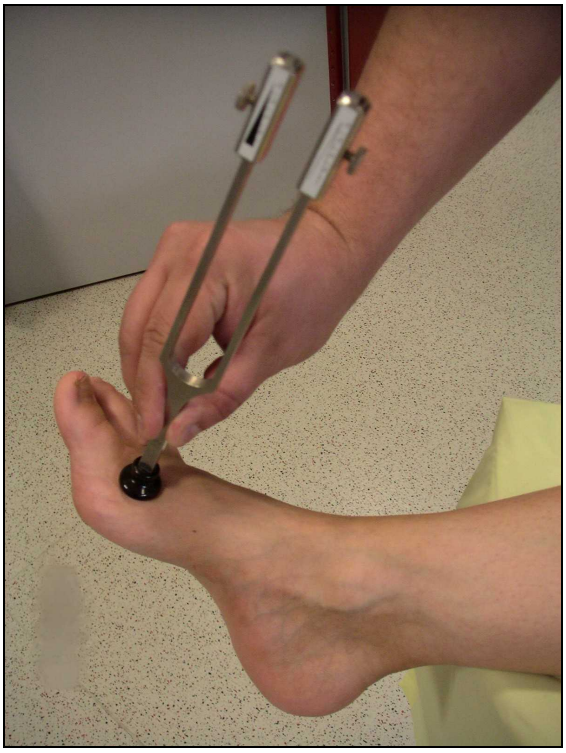


Fig. 2: Testing vibratory sensibility using the Rydell and Seiffer tuning fork [36].

3.5.2. Semmes-Weinstein 10g monofilament

To evaluate the sensory qualities of touch and pressure the subjects were tested with the 10g monofilament. Here a standardised nylon filament (Semmes-Weinstein monofilament) with a contact pressure of exactly 10g was pressed onto the plantar points of the metatarsal heads MTH I, II, V as well as onto the heels and the insteps [40]. The monofilament was placed vertically onto the point on the skin to be tested and was pressed down until it bent, to thereby exert a spot pressure of 10g. Before the foot was actually tested, the procedure was demonstrated on the subject's hand. The subject was instructed to indicate perceived touch by calling out. Once it was certain that the participant understood the test method, the foot was tested. Any hyperkeratoses present were removed beforehand. The subject was made to close his eyes again to rule out the possibility of visual influences. In addition, the examiner varied the sequence of the skin areas to be tested. The examiner also tested whether the patient's statements were true by sometimes not applying the monofilament but still inquiring about the perception of pressure. If the subject then did state that he felt pressure he was reminded of the need to give an honest response.

Sensation of touch was tested on both feet separately. The results were noted as “positive” or “negative” for the individual areas in the patient record (Appendix 7). The test was deemed pathological if the subject failed to notice touch on one point on the plantar skin of the foot.
Figure 3 demonstrates application of the 10g monofilament. Both the Semmes-Weinstein procedure and the Rydell-Seiffer method test disturbances of nerve function, which at most could be regarded as epiphenomena of diabetic foot lesions, namely touch perception and vibratory sensibility. These procedures do not, however, test the reduction in pain sensation as the actual disturbance which leads to foot lesions. Pain sensation was thus tested on both balls of the big toe by pricking them, without causing injury, with the tip of a toothpick. The subject stated whether he perceived the stimulus as painful. The result was documented in the record.

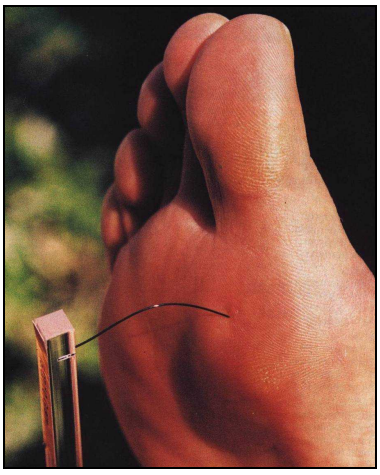
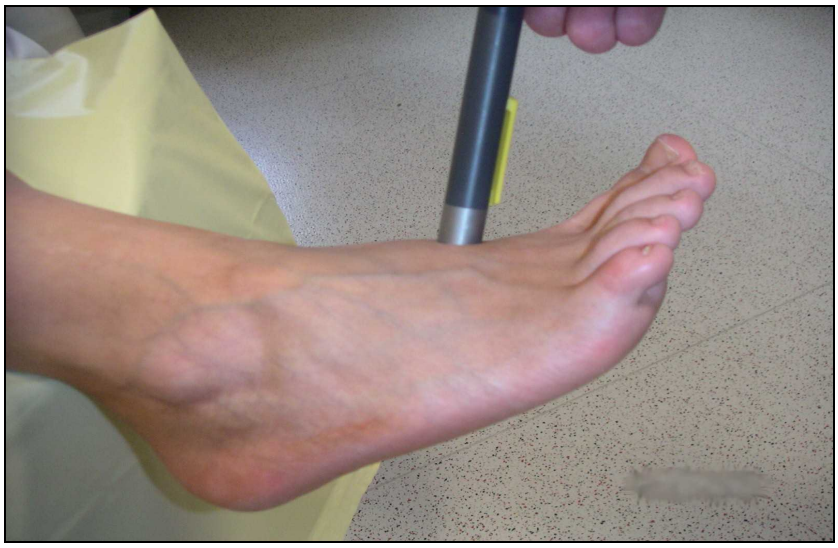


Fig. 3: Semmes-Weinstein monofilament to test sensibility in the forefoot area.

3.5.3. Tip-Therm

By means of Tip-Therm (tip therm GmbH, Düsseldorf) a qualitative conclusion could be drawn as to the patients' temperature perception. The device takes the form of a pen, the material on the ends of which have differing thermal conductivity. One end of the instrument is made of plastic, the other metal. In terms of physics, metal is more thermally conductive than plastic; advantage is taken of this when performing the measurement.
The test consisted in placing the plastic end and the metal end of the Tip-Therm alternately on both insteps and on the lower leg in irregular rotation without the subject seeing. The subject was requested to say whether the surface of the Tip-Therm pressed onto the skin felt cooler or warmer. The end with the metal coating feels cooler than the less conductive (insulating) plastic because of its higher thermal conductivity. To demonstrate and to ensure reliable perception of the test stimulus, this test was also carried out first on the back of the participant's hand and his forearm. If the subject could not tell a difference in temperature or if the reply was incorrect, this was accordingly written down in the record. If no differentiation was made between the different ends of the Tip-Therm at least one point on the instep, then lack of or limited temperature perception was assumed. The following illustration shows the simple and inexpensive Tip-Therm in use.



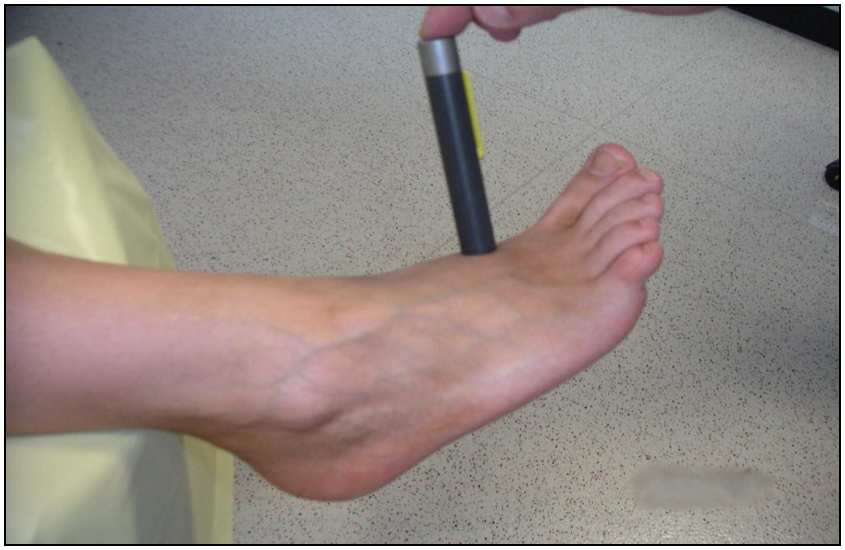


Fig. 4: Qualitative temperature perception using Tip-Therm on the instep, top for the quality "cold" and bottom for the quality "warm".

3.5.4 Quantitative thermoreception

Testing of qualitative temperature perception was followed by a quantitative measurement of thermoreception, in order to be able to draw more precise conclusions about the degree of any existing reduction in temperature perception.

A measuring apparatus consisting of a rod-shaped probe with two identical metal surfaces of the same size was used. Through electrical induction these contacts could be brought to varying differences in temperature. At the beginning of measurement both contacts had exactly the same initial temperature. Using a switch, the temperature of one of the metal surfaces could be graduated up or down in increments of 0.5°C, which the subjects could not see.

The test was performed as follows: Both contact surfaces were placed alternately one after the other on the skin of the patient's instep applying the same amount of pressure each time. The subject had to say afterwards whether the temperature of the two surfaces was different and, if so, which of the two contacts felt warmer. If no difference was noticed, the examiner increased the temperature difference by 0.5°C at a time. The end temperature difference at which the subject perceived a definite difference was noted down. A total of three measurements were taken for each foot separately, and the arithmetic mean written into the

study record (Appendix 7). The maximum temperature was selected such that it would not result in any thermal damage or redness in the tested region of skin. An unperceived temperature difference of at least 3°C was defined as pathological.

3.6. Description of the procedures for testing for skin dryness

Different organ systems can be affected by autonomic diabetic neuropathy: disturbances of the cardiovascular system, of the gastro-intestinal tract and also the urogenital tract are known. Furthermore, pupillary motoricity and sweat secretion can be disturbed, as well as endocrine secretion [34]. This thesis tests the disturbance of sweat release in the skin of the plantar area of the foot as the manifestation of autonomic neuropathy by means of three different test procedures, which will be explained below in detail. The results were then to be compared with those of the sensory neuropathy measurement.
To date, autonomic neuropathy could only be verified using technically complex and very time-consuming procedures, which for that very reason have only be used in an experimental context [41]. The function of the sweat glands was tested using Minor's iodine-starch test or the Ninhydrin test. These function tests are rarely used in neuropathy diagnosis today and have given way to electrophysiological studies. The Quantitative Sudomotor Axon Reflex Test (QSART), considered to be the gold standard of sweat gland function measurement, however, involves substantial investment [42, 43] and is only available is specialised centres.
This thesis therefore looks into the question of whether this situation can be changed by new and easy-to-handle measuring methods to determine skin moisture.

On trial here were the Neuropad indicator plaster, flat-electrode hydrometry and specific moisture measurement at certain areas of skin in the plantar region of the foot.

3.6.1. Neuropad

The Neuropad (Miro Verbandsstoffe GmbH) is a plaster containing the salt complex cobalt II chloride, and is very well tolerated by the skin. It utilises the chemical property of the salt, which causes a water-induced colour change from blue to pink. The indicator plaster enables a direct assessment of sweat secretion to be made on the basis of the colour change. Since sweat is composed almost entirely of water, which the plaster absorbs, this can be determined by the plaster.

The time it took for a complete, standardised colour change in the indicator plaster from blue (HKS 46K 55%) to pink (HKS 17K 30%) to take place was measured in seconds, as illustrated in Figure 5. Very frequently diabetic foot syndromes manifest themselves in the form of an ulcus under the heads of the metatarsal bones (MTH) I and II, and so in this study the skin moisture was tested at this point with the Neuropad.

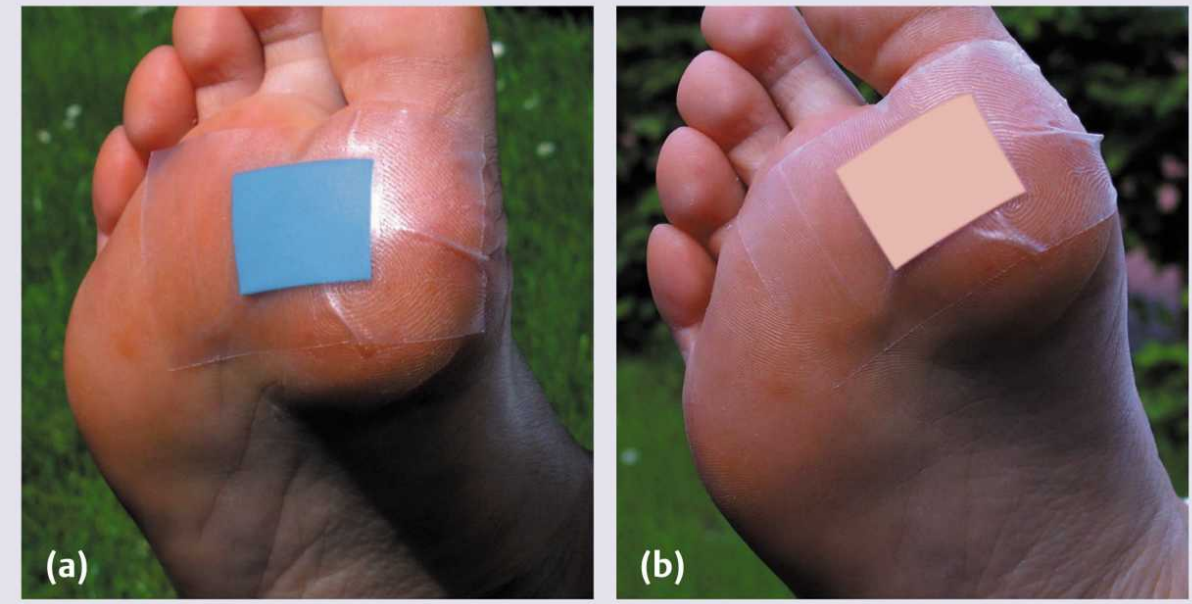


Fig. 5: Neuropad indicator plaster at MTH I/II; blue at the start of measurement (a) and pink at the end of measurement with positive colour change (b).

To guarantee similar test conditions, during this study the subjects were always tested in the same room. The ambient conditions were identical throughout; this was verified with a room thermometer and a hygrometer.

Before starting the test with Neuropad the bare feet had to become acclimatised to the ambient room conditions. Any moisture from footwear and physical and emotional strain were thereby

eliminated. The time span required for the feet to become accustomed to the ambient temperature and to form a natural film of moisture was estimated at an acclimatisation time of 10 minutes.

The indicator plaster was simultaneously stuck to the MTH I/II plantar region of both feet and the time taken for the colour to change completely measured with a stopwatch. The change times for both feet were subsequently documented in the patient record (Appendix 8).

3.6.2 Flat-electrode hydrometry

Flat-electrode hydrometry is another method for determining skin moisture, where the level of moisture is measured by electrical conductance. Being a dielectric medium, the skin presents a rise in moisture due to increased sweat gland activity with a consequent fall in skin resistance, something which the studies of Féré and Tarchanoff showed 100 years ago [44]. Other works on conductance determination certify the cogency of hydrometry regarding skin moisture measurement [45].

Unlike the indicator plaster, which is a chemical method, flat-electrode hydrometry applies the physical principle of conductivity [46]. The more moist the skin, the higher its electrical conductance. Skin moisture was measured both on the hands and feet of the participants. To take the measurement the subject placed both bare feet under slight pressure on the electrode surfaces of the flat-electrode hydrometer, as depicted in Figure 6.

Once the conductance measured had stabilised, it could be read off the device display. Moisture on the palms of the hands was tested in a similar manner, but with two hand electrodes.

The standards of Hilling et al [45] were used. At a conductance of < 50 µS (microSiemens) on the feet and < 40 µS on the hands a pathological result, and thus over-dry skin, was assumed, and the result entered in the record (Appendix 8).

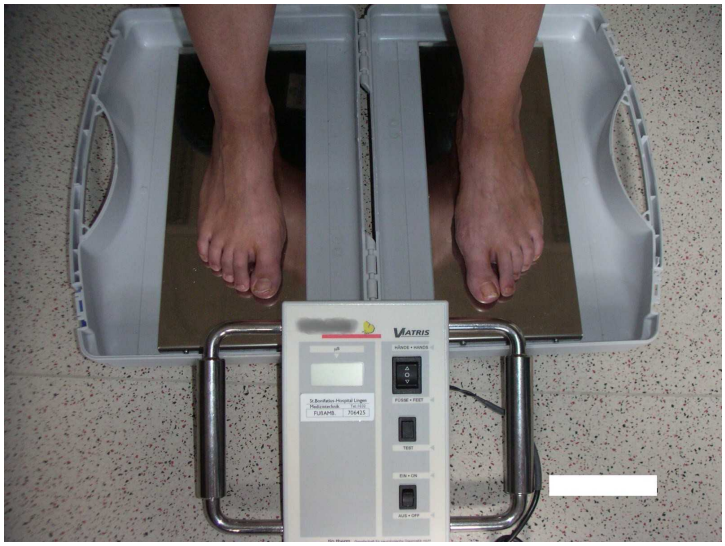


Fig. 6: **Flat-electrode hydrometry of both feet.**

3.6.3. Selective hydrometry

Unlike conventional hydrometry, which determines the overall skin moisture of the areas of skin touching the electrode surfaces, with the new test procedure of so-called selective hydrometry individual, defined points of the skin of the plantar region of the foot can be tested selectively. The underlying principle of measurement is the determination of skin resistance. Here, however, it is not a flat-electrode plate that is used, but a single, pen-shaped probe consisting of two metal contacts set just a few millimetres apart. Using this, 12 points on each sole are tested for their local moisture content. Figure 7 gives an idea of how this method is performed. The benefit of this new method is that a conclusion can now be drawn as to the status of sweat secretion based on specific areas of skin that cannot be tested with flat-electrode hydrometry.

The resistance is calculated using the following formula:

$$Rx = Rg \times Rs / Rs - Rg$$

Here Rx stands for the existing skin resistance, Rg is the value displayed by the measuring instrument (0-19) and Rs is the constant internal device resistance of 20 MOhm.

The 12 test points, shown in the picture, record the skin resistance of each toe, at points MTH I, III and V in the forefoot region, the medial and lateral border of the foot and the skin in the middle above the calcaneum and the distal medial heel.

To guarantee a uniform contact pressure of the measuring apparatus during the test, the probe is equipped with a spring mechanism. This made comparable, reproducible results possible, which were accordingly documented in the patient record (Appendix 8).

One by one the probe was pressed vertically onto the subjects' skin at the 12 test points and the value read off the display (Figure 8).

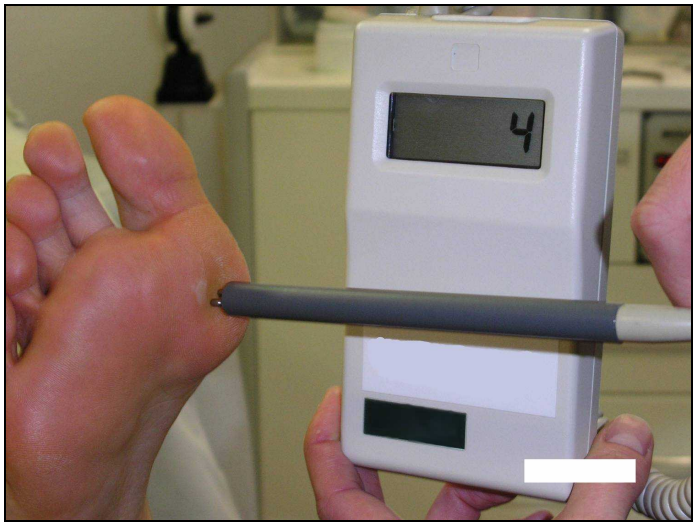


Fig. 7: Specific hydrometry on the skin of the foot in the plantar region MTH I/II.

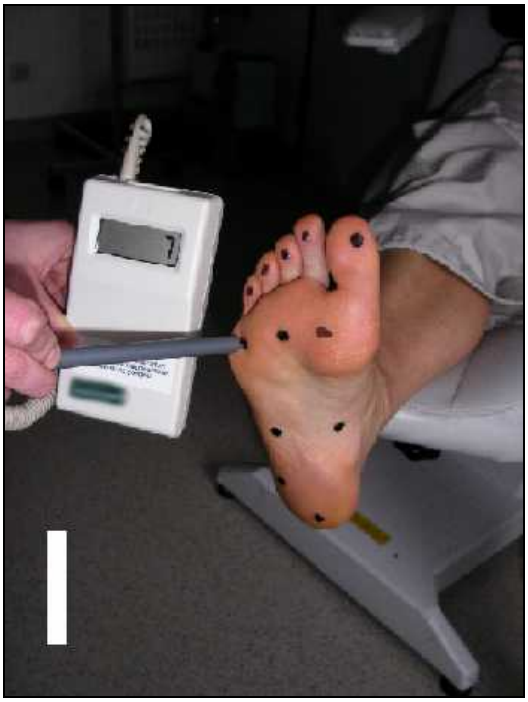


Fig. 8: The 12 test points measured using specific hydrometry (black dots) in the plantar region of the foot.

3.7. Description of the procedure for testing for peripheral arterial occlusive disease

Besides the existence of peripheral neuropathy, circulatory disturbance of the lower limb, referred to as peripheral arterial occlusive disease (PAOD), is one of the most significant aetiologies of a diabetic foot lesion [47]. In diabetics arteriosclerosis and mediasclerosis are the most common arterial diseases. By narrowing and occluding the arteries, arteriosclerosis causes ischemia. Mediasclerosis, also called Mönckeberg sclerosis, is calcification of the tunica media, which causes an incompressible channel without, however, constricting the arterial lumen. So while mediasclerosis does not cause ischemia, it does lead to false high Doppler pressure values [27, 48, 49].

After obligatory palpation of the tibialis posterior artery and the dorsalis pedis artery, a measurement of the ankle artery pressures was taken left and right separately in all subjects using the Doppler ultrasound procedure. Palpable foot pulses do not rule out PAOD [50]; one in three diabetics with palpable foot pulses already has a distinctly reduced pressure value as part of arterial occlusive disease [51].
With the Doppler ultrasound probe the pulsations of the relevant ankle arteries were sought out and represented acoustically. Then, using a blood pressure cuff placed around the distal lower leg, compression was applied. While the air was being let out of the cuff, the arterial pulsations were registered by the Doppler transducer and that value documented (Appendix 7). Figures 9 a) and 9 b) give an illustration of the technique.
Following measurement of the ankle arterial pressure values, blood pressure was determined at the radialis artery and the ankle-arm pressure index calculated as usual. To arrive at this, one divides the systolic ankle pressure by the systolic blood pressure of the radialis artery. A value of <0.9 was regarded as pathological. Subjects with a pathological Doppler index were excluded from the study.
Table 3 gives an overview of Doppler index rating. Paradoxically high values may be found together with mediasclerosis [52].

Condition	**Doppler index**
Healthy individuals	0.9 – 1.1
Patients with pure Mönckeberg sclerosis	>1.2
Patients with critical ischemia (PAOD III°-IV°)	<0.5
Patients with PAOD III°-IV° and Mönckeberg sclerosis	<0.9
Patients with gangrene without diabetes mellitus	0.33 – 0.47
Patients with gangrene and diabetes mellitus	0.54 – 0.56

Tab. 3: Doppler index rating under various conditions [37].

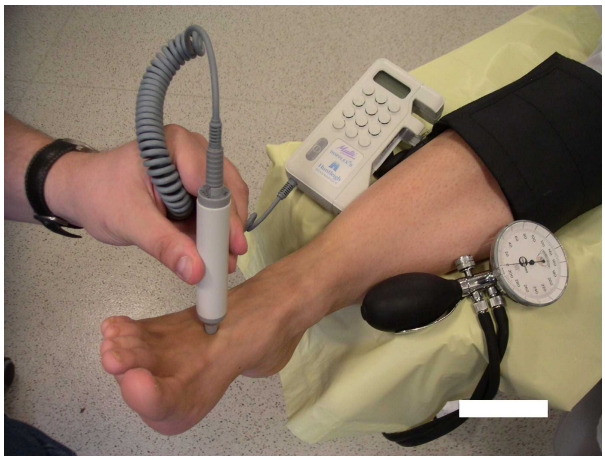


Fig. 9 a): Doppler pressure measurement at the dorsalis pedis artery with the Doppler ultrasound probe.

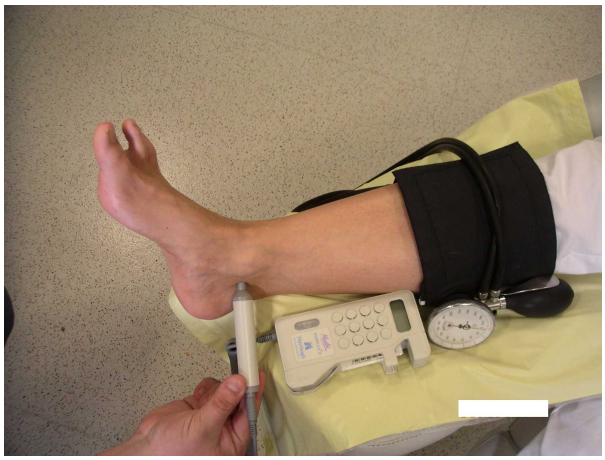


Fig. 9 b): Doppler pressure measurement at the tibialis posterior artery with the Doppler ultrasound probe.

3.8. Statistics

The data gathered were statistically evaluated using computer-assisted statistics programs. Planning of the study and the necessary statistical analyses were discussed with the Institute for Biometrics at Hanover Medical University (MHH), headed by Prof. Dr. Hecker. For specific questions Dr. Hermanns was available in an advisory capacity.

4. Results

Documentation of the case histories and all test results, which were entered by the examiner into a patient record for all participants, made evaluation of the findings, or case histories, clinical findings and neuropathy possible. These are presented in the following in sections 4.1. to 4.7. To guarantee data protection personal information was made anonymous and the subjects allocated a serial number.

4.1. Case history data

On the basis of the case history the duration of diabetes mellitus by the time of testing was calculated for the diabetics. Patients without verified peripheral sensory neuropathy (Group A) had suffered from diabetes mellitus 11.61 ± 8.9 years on average. The Group of diabetics with verified peripheral sensory neuropathy (Group B) had been ill for an arithmetic mean of 16.08 ± 8.8 years. In Group A there are 13 type 1 diabetics and 23 type 2 diabetics. Group B contained 2 type 1 diabetics and 24 type 2 diabetics.

It was further established that the average HbA1c values determined within the population of diabetics did not vary substantially. Group A had an HbA1c value of 8.1 ± 1.4%, and Group B 8.7 ± 1.8%. Understandably the HbA1c values of the healthy individuals (Group C) were well below these values, at 5.2 ± 0.4%.

The gender ratio was similar within the Group of diabetics. In Group A there were 19 males (52.8%) and 17 females (47.2%), in Group B the ratio of men to women was 16 (61.5%) to 10 (38.5%). There was a larger proportion of females to be seen in the healthy control population Group C; 10 males (29.4%) to 24 females (70.6%).

Analysis of the average age distribution of the three Groups gave the following result: in Group A the average age was 54.3 ± 11.5 years, in Group B 58.9 ± 11.0 years, in Group C 47.6 ± 11.7 years. Figure 10 and Table 4 give an overview.

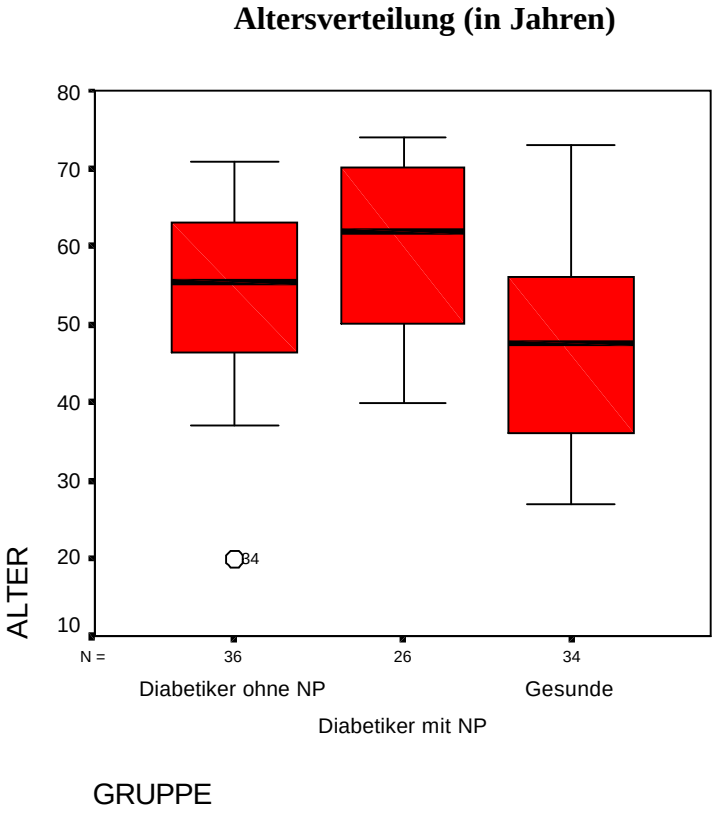


Fig. 10: Age distribution (in years) in the form of a box-whisker plot for diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C).

Age	N	Mean	Standard deviation	Standard error	95% confidence interval for the mean		Mini-mum	Maxi-mum
					Lower	Upper		
Diab.w.o. NP (Group A)	36	54.33	11.472	1.912	50.45	58.21	20	71
Diab. w. NP (Group B)	26	58.96	11.025	2.162	54.51	63.41	40	74
Healthy (Group C)	34	47.56	11.712	2.009	43.47	51.65	27	73
Total	96	53.19	12.210	1.246	50.71	55.66	20	74

Tab. 4: Mean, standard deviation, standard error, 95% confidence interval, minimum and maximum age distribution of the diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and the healthy individuals (Group C).

While filling in the case history the subjects were asked about their foot care. This was broken down into almost daily and weekly care of feet, which was defined as washing and rubbing in care products. In the Group of diabetics without neuropathy one quarter (25%) had an almost daily foot care routine; almost as many patients with sensory neuropathy had such a routine (23%). However, 46.2% of diabetics with neuropathy did not engage in any kind of foot care, compared with 38.9% of diabetics without neuropathy. Appendix 9 presents the case history findings of the three Groups in the form of an overall table.

4.2. Total Symptom Score (TSS)

When taking down the case history the subjects were asked specifically about symptoms of neuropathy. The focus here was on subjective perception and the frequency in each case of smarting, tingling, numbness and pain in the feet. Both the symptom and the frequency of occurrence were given a point rating (0-3), which, when added together, gave the TSS. The maximum possible score was 24 points (Appendix 6).

Defining a TSS up to 3 at most as still physiological and a score of ≥ 4 as pathological, the following observation could be made:

On the basis of the TSS, 91.2% of the healthy Group (C) and over 80% of the diabetics without sensory neuropathy (A) were diagnosed as historically healthy. However, 8.8% (Group C) and 19% (Group A) were classed as pathological on the basis of the TSS, as Figure 11 illustrates.

In the case of diabetics with verified sensory neuropathy (Group B) this ratio was seriously shifted. On the basis of the TSS, 44% of this population were assessed as healthy. Only 56% of diabetics with neuropathy were identified by the TSS as being pathological or as having a sensory neuropathy.

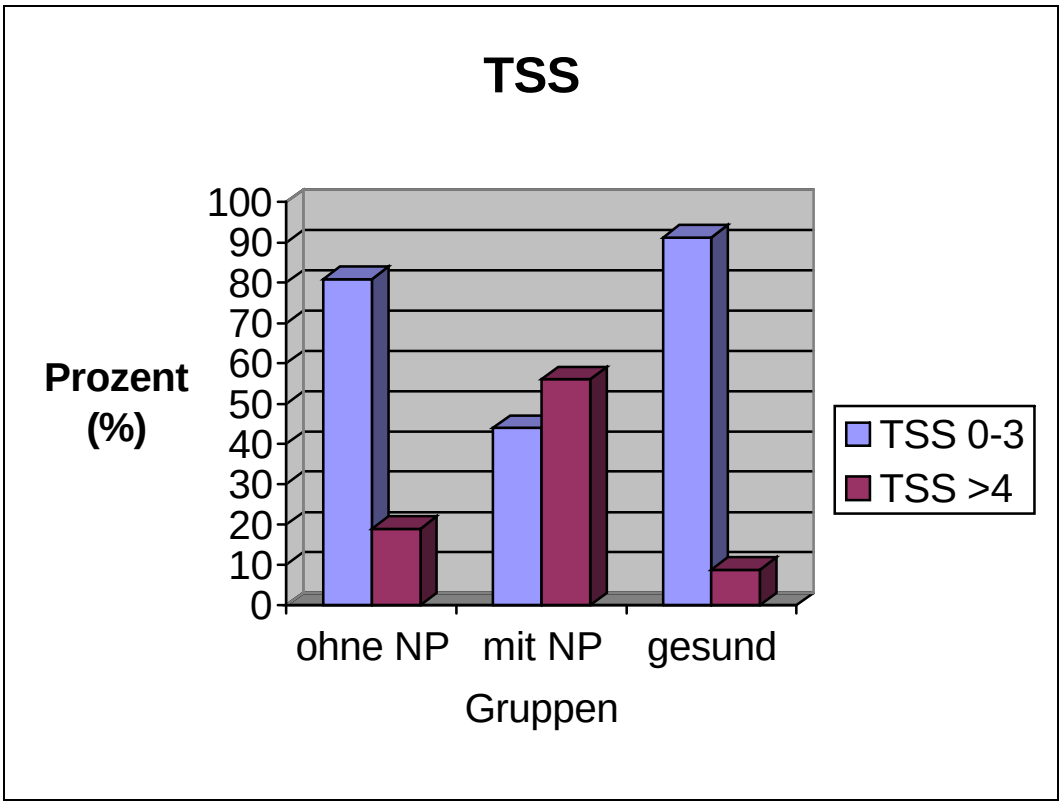


Fig. 11: Results in percent of the Total Symptom Scores (TSS) in diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C).

4.3. Neuropathy tests

Based on the results of the neuropathy tests, described in Section 3.5., the diabetics were divided up into the two Groups, A or B. The criteria for putting them into one of the Groups, with or without sensory neuropathy, comprised having at least two pathological results in the methods described in 3.5.

4.4. Neuropad

The results of moisture determination with the Neuropad indicator plaster were scaled in the "seconds" unit of measurement. The colour change times for both feet in the plantar region of MTH I/II were measured.

4.4.1. Statistical parameters

The mean, the standard deviation, the standard error, the 95% confidence interval and the minimum and maximum value of the colour change times were calculated for the left and right feet separately. Table 5 below presents the results clearly.

The quickest change times were found among the healthy individuals (Group C). Here, the Neuropad showed the requisite change from blue to pink after 500 ± 157 seconds (8.3 ± 2.6 minutes) on average. In diabetics without sensory neuropathy (Group A) colour change was not observed until approximately 683 ± 301 seconds (11.4 ± 5.0 minutes). Fulfilment of the indicator plaster criterion was slowest for Group B, the patients with sensory neuropathy, namely after around 1034 ± 396 seconds (17.2 ± 6.6 minutes).

The means of the Neuropad times for the healthy individuals were 499.8 seconds on the right foot, and 499.5 seconds on the left. The measured change times of Groups A and B with diabetes mellitus differed from this as follows: diabetes without verified sensory neuropathy (Group A) had an arithmetic mean of 679.8 seconds on the right, and 686.9 seconds on the left foot. Much slower colour changes were observed in the Group with sensory neuropathy. Here, complete colour change from blue to pink could not be seen on the right foot until after 1058.1 seconds, and on the left side, after 1009.2 seconds.

No significant variation between the right and left foot within each of the Groups tested was thus observed. Figure 12 shows the colour change times for both the right and left foot in the form of a box-whisker plot.

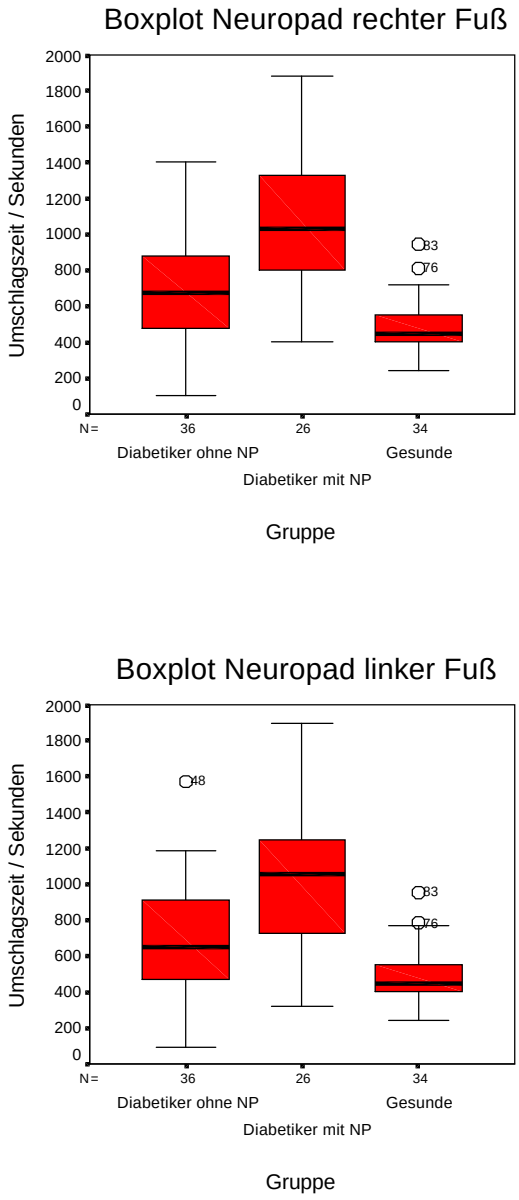


Fig. 12: Box-whisker plot of the colour change times in seconds with the Neuropad plaster for the right (top) and left (bottom) foot at MTH I / II for the diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C).

Neuropad		N	Mean (sec.)	Standard deviation	Standard error	95% confidence interval for the mean		Minimum (sec.)	Maximum (sec.)
						Lower	Upper		
Neuropad Right	Diabetics w.o. NP	36	679.81	290.789	48.465	581.42	778.19	97	1406
	Diabetics with NP	26	1058.12	389.455	76.378	900.81	1215.42	400	1886
	Healthy	34	479.38	146.291	25.089	428.34	530.43	240	943
	Total	96	711.28	362.003	36.947	637.93	784.63	97	1886
Neuropad Left	Diabetics w.o. NP	36	686.94	311.432	51.905	581.57	792.32	88	1576
	Diabetics with NP	26	1009.15	402.792	78.994	846.46	1171.84	315	1900
	Healthy	34	479.44	150.094	25.741	427.07	531.81	240	958
	Total	96	700.72	360.397	36.783	627.70	773.74	88	1900

Tab. 5: Mean, standard deviation, standard error, 95% confidence interval, minimum and maximum colour change times (in seconds) for the Neuropad plaster in Group A (diabetics without neuropathy), Group B (diabetics with neuropathy) and in Group C (healthy).

4.4.2. Test for differences between the populations

To check whether the differences observed in the Neuropad change times were significant across the Groups, multiple comparisons were used. The differences between the test points at MTH I / II under the right and left foot were not significant in any of the three Groups. However, high significance emerged between the Groups. To be precise, the significance between the diabetics without sensory neuropathy (Group A) and the healthy individuals (Group C) on both feet was p=0.002; between Group C and diabetics with neuropathy (Group B) it was p<0.001, and between Group A and B also p<0.001 on the right, and p=0.004 on the left. Table 6 gives an overview of these results.

Tamhane

Dependent variable	GROUP (I)	GROUP (J)	Mean difference	Standard error	**Signifi-cance**	95% confidence interval	
						Lower	Upper
Neuropad Right	Diabetics w.o. NP (Group A)	with NP	-378.31(*)	90.457	**.000**	-602.81	-153.81
		healthy	200.42(*)	54.574	**.002**	65.81	335.04
	Diabetics with NP (Group B)	w.o. NP	378.31(*)	90.457	**.000**	153.81	602.81
		healthy	578.73(*)	80.393	**.000**	375.62	781.84
	Healthy (Group C)	w.o. NP	-200.42(*)	54.574	**.002**	-335.04	-65.81
		with NP	-578.73(*)	80.393	**.000**	-781.84	-375.62
Neuropad Left	Diabetics w.o. NP (Group A)	with NP	-322.21(*)	94.521	**.004**	-556.56	-87.85
		healthy	207.50(*)	57.938	**.002**	64.48	350.53
	Diabetics with NP (Group B)	w.o. NP	322.21(*)	94.521	**.004**	87.85	556.56
		healthy	529.71(*)	83.082	**.000**	319.78	739.65
	Healthy (Group C)	w.o. NP	-207.50(*)	57.938	**.002**	-350.53	-64.48
		with NP	-529.71(*)	83.082	**.000**	-739.65	-319.78

* The mean difference is significant at the .05 level.

Tab. 6: Multiple comparison to find significant differences in the results of colour change times between the diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and the healthy individuals (Group C) both for the right and left foot.

4.4.3. Cutpoint determination by means of a ROC curve

Of particular clinical and practical interest was the point in time at which the border between "pathological" and "normal" lay as regards the Neuropad. This threshold, at which the probability is high that in addition to sudomotor disturbance, sensory paralgesia also existed, was designated the so-called "cutpoint".

The Group of patients with reliably verified neuropathy (Group B) and the Group of healthy individuals (Group C) were tested with the new diagnostic test procedure, the indicator plaster. The results of the test can be presented in the form of a chi-square table (Table 7).

		Actual Situation		
		ill (positive)	healthy (neg.)	
Test result states	ill (positive)	right decision **a**	false positive **b**	positive predictive value **a/(a+b)**
	healthy (negative)	false negative **c**	right decision **d**	negative predictive value **d/(c+d)**
		Sensitivity a/(a+c)	**Specificity d/(d+b)**	

Tab. 7: Chi-square table for the definition of sensitivity, specificity and predictive values.

On the basis of arbitrarily set cutpoints, the time of colour change with the indicator plaster which involved the highest sensitivity and specificity was sought. To this end, the Group of diabetics with sensory neuropathy (Group B) was compared with the Group of healthy individuals (Group C).

This trial-based procedure can be systematised by consistently calculating the cutpoint from the smallest to the largest value in the universal set (Group B and Group C) in terms of sensitivity and specificity. For historical reasons the value "1-specificity" is used instead of specificity [53]. In the graph below (Fig. 13) one took sensitivity to be the Y-axis and 1-specificity as the X-axis. The resulting curve is called a "ROC curve" (Receiver Operator Characteristic).

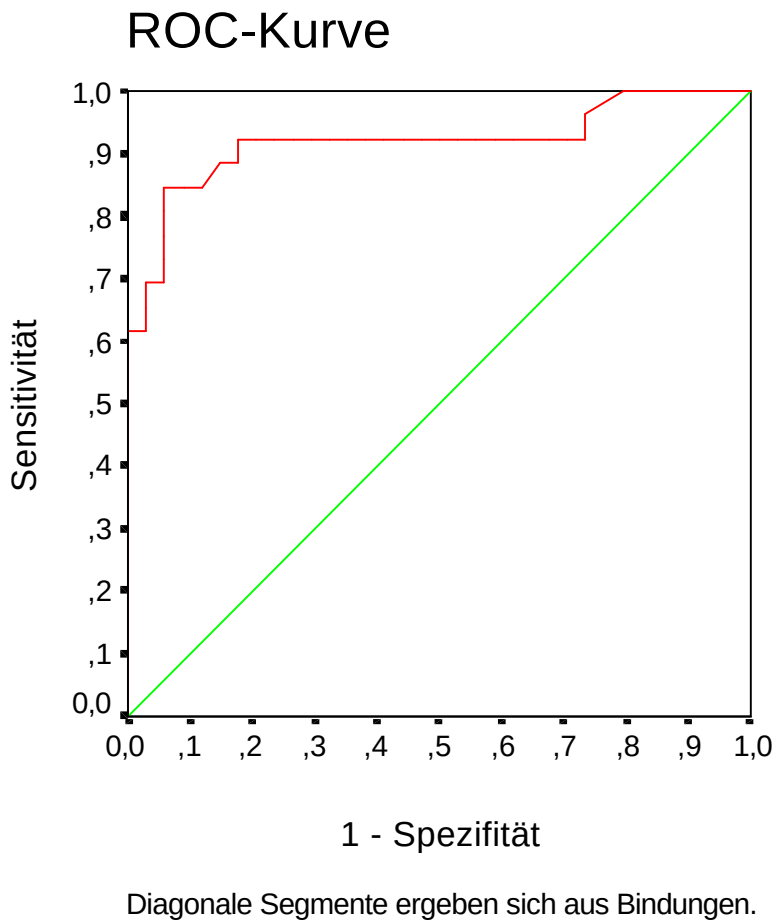


Fig. 13: ROC curve for the colour change times of the indicator plaster on the right foot.

The individual values of the cutpoints can no longer be made out in this curve. But one can see at a glance which combinations of sensitivity and specificity can be achieved. Since both parameters are equally important, one selects the combination at which the sum of sensitivity (Y) + specificity (1-X) is highest:

Y + (1-X) = 1 + (Y-X) = highest

One can do this graphically by shifting the Y=X diagonal parallel upwards (Y=X+C), and taking the largest constant C, such that Y=X+C still hits a point on the ROC curve.

In Table 8 below all cutpoints for the indicator plaster on the right foot are listed.

Curve coordinates

Variable(s) for test result: indicator plaster; right

Positive, if greater than or equal to(a)	Sensitivity	1-specificity
239.00	1.000	1.000
248.50	1.000	.971
263.50	1.000	.941
300.50	1.000	.912
345.50	1.000	.882
375.00	1.000	.853
395.00	1.000	.794
402.50	.962	.735
407.50	.923	.735
411.00	.923	.706
414.50	.923	.676
418.50	.923	.618
427.50	.923	.588
437.50	.923	.559
443.00	.923	.529
449.50	.923	.500
456.00	.923	.471
461.00	.923	.441
464.50	.923	.412
473.00	.923	.382
485.50	.923	.353
500.50	.923	.324
525.00	.923	.294
548.00	.923	.265
556.50	.923	.235
564.00	.923	.206
580.50	.923	.176
597.50	.885	.176
606.00	.885	.147
618.50	.846	.118
675.00	.846	.088
722.50	**.846**	**.059**
739.50	.808	.059
777.00	.769	.059
801.00	.731	.059
807.50	.692	.059
814.00	.692	.029
864.00	.654	.029
928.00	.615	.029
944.00	.615	.000
950.50	.577	.000
977.00	.538	.000
1032.00	.500	.000
1068.00	.462	.000
1107.00	.423	.000
1149.50	.385	.000
1177.50	.346	.000
1237.50	.308	.000
1302.50	.269	.000
1342.00	.231	.000
1399.50	.192	.000
1477.50	.154	.000
1585.00	.115	.000
1683.00	.077	.000
1796.00	.038	.000
1887.00	.000	.000

Tab. 8: Cutpoints and coordinates of the ROC curve for the colour change times of the indicator plaster on the right foot.

On the basis of Table 8 above one gets the value of 722.5 as the highest sum of the coordinates. At this cutpoint sensitivity is 84.6% and specificity is 94.1%. If one chooses a cutpoint at a colour change time of 606 seconds, the sensitivity shifts to 88.5%, and the specificity to 85.3%.

One also regards the ROC curve (Fig. 13) as a whole as a measure of the diagnostic value of the parameter that generated it. As a measure of the diagnostic quality one thus selects the area under the curve, or AUC for short. One obtains a value of AUC = 0.5, as it corresponds

to the diagonals, for instance, when the distribution of the parameter in question is identical in both Groups. The AUC should thus be at least considerably greater than 0.5, if the parameter is to be used as a diagnostic criterion.

In this ROC curve for the times in the indicator plaster test the AUC = 0.919. This AUC has a one-sided significance in relation to the value 0.5 at the 5% level, where $p<0.001$.

Area under the curve

Variable(s) for test result: Indicator plaster; right foot

Area	Standard error(a)	Asymptotic significance (b)	Asymptotic 95% confidence interval	
			Lower	Upper
.919	.042	.000	.838	1.001

a Under the nonparametric assumption
b Null hypothesis: true area = 0.5

The same calculations can be made for the colour change times of the indicator plaster on the left foot. The resulting ROC curve is depicted in Figure 14.

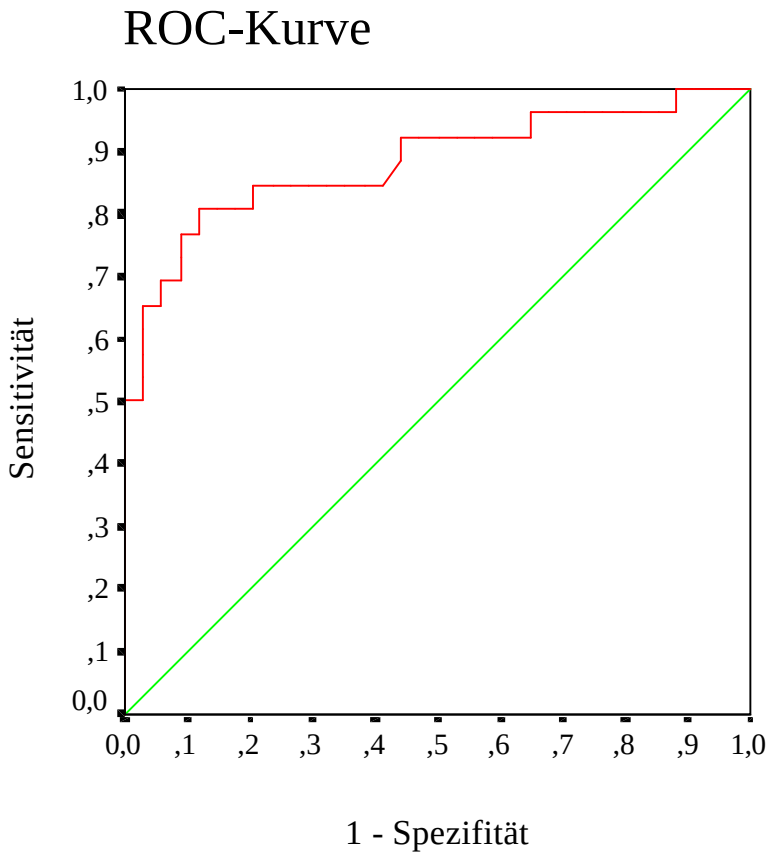


Fig. 14: ROC curve for the colour change times of the indicator plaster on the left foot.

One can see straight away that the curve form is similar to that of the right foot, but is less steep. The cutpoint for the left foot, at which sensitivity and 1-specificity is highest, can be read from Table 9 below.

The AUC in this ROC curve is 0.882 and somewhat poorer as regards diagnostic accuracy compared with the right foot, but nevertheless significant in relation to 0.5 at the 5% level, where $p<0.001$.

Area under the curve

Variable(s) for the test result: Indicator plaster; left foot

Area	Standard error(a)	Asymptotic significance(b)	Asymptotic 95% confidence interval	
			Lower	Upper
.882	.048	.000	.788	.976

Curve coordinates

Variable(s) for test result: Indicator plaster; left

Positive, if greater than or equal to(a)	Sensitivity	1-specificity
239.00	1.000	1.000
255.00	1.000	.971
284.00	1.000	.941
302.00	1.000	.912
310.50	1.000	.882
330.00	.962	.882
351.00	.962	.853
366.00	.962	.824
377.50	.962	.794
390.00	.962	.765
405.00	.962	.735
415.00	.962	.706
421.50	.962	.676
423.50	.962	.647
425.00	.923	.647
430.50	.923	.588
436.50	.923	.559
440.50	.923	.529
444.00	.923	.500
447.50	.923	.471
452.50	.923	.441
457.50	.885	.441
467.00	.846	.412
477.00	.846	.382
481.00	.846	.353
494.50	.846	.324
525.00	.846	.294
548.00	.846	.265
561.00	.846	.235
571.50	.846	.206
580.50	.808	.206
587.50	.808	.176
594.00	.808	.147
600.50	**.808**	**.118**
630.50	.769	.118
692.00	.769	.088
738.50	.731	.088
761.50	.692	.088
775.00	.692	.059
781.50	.654	.059
821.50	.654	.029
887.50	.615	.029
930.00	.577	.029
951.00	.538	.029
957.50	.500	.029
1059.00	.500	.000
1163.00	.462	.000
1179.00	.385	.000
1194.50	.346	.000
1207.50	.308	.000
1231.50	.269	.000
1283.50	.231	.000
1353.50	.192	.000
1408.50	.154	.000
1473.50	.115	.000
1546.00	.077	.000
1738.50	.038	.000
1901.00	.000	.000

Tab. 9: Cutpoints and coordinates of the ROC curve for the colour change times of the indicator plaster on the left foot.

The highest sum of the coordinates for colour change with the indicator plaster under the left foot is 600.5 seconds. At this cutpoint the sensitivity is 80.8% and the specificity is 88.2%.

In the following, tables 10 a) and 10 b) give an overview of the relationship between sensitivity and specificity at various different cutpoints, for both feet separately. The corresponding bar charts in Figure 15 illustrate the results.

Colour change: indicator plaster; right foot:

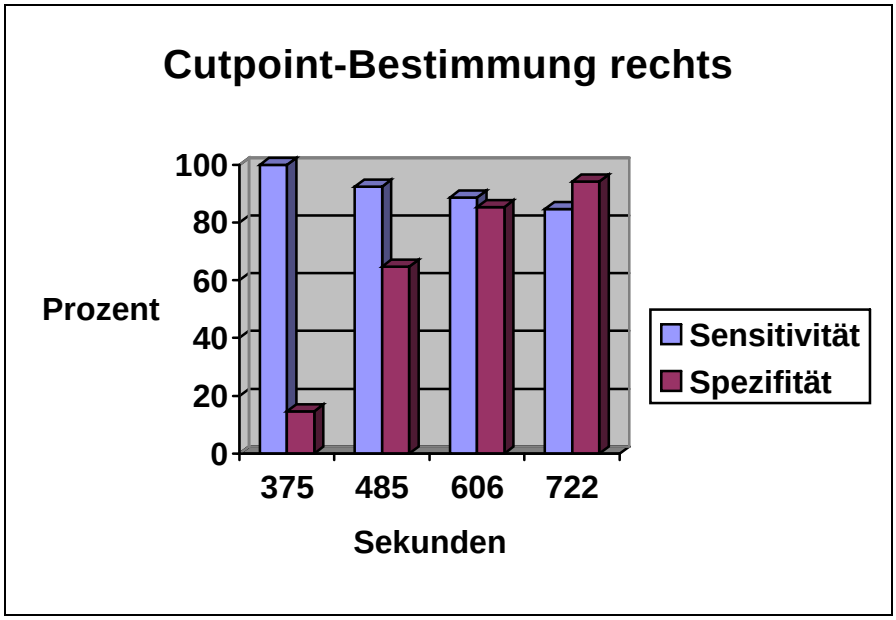
Cutpoint	Sensitivity (%)	Specificity (%)
375 seconds	100	14.7
485 seconds	92.3	64.7
606 seconds	88.5	85.3
722 seconds	84.6	94.1

Tab. 10 a): Sensitivity and specificity at various cutpoints of the indicator plaster on the right foot.

Colour change: indicator plaster; left foot:

Cutpoint	Sensitivity (%)	Specificity (%)
366 seconds	96.2	17.6
481 seconds	84.6	64.7
600 seconds	80.8	88.2
738 seconds	73.1	91.2

Tab. 10 b): Sensitivity and specificity at various cutpoints of the indicator plaster on the left foot.



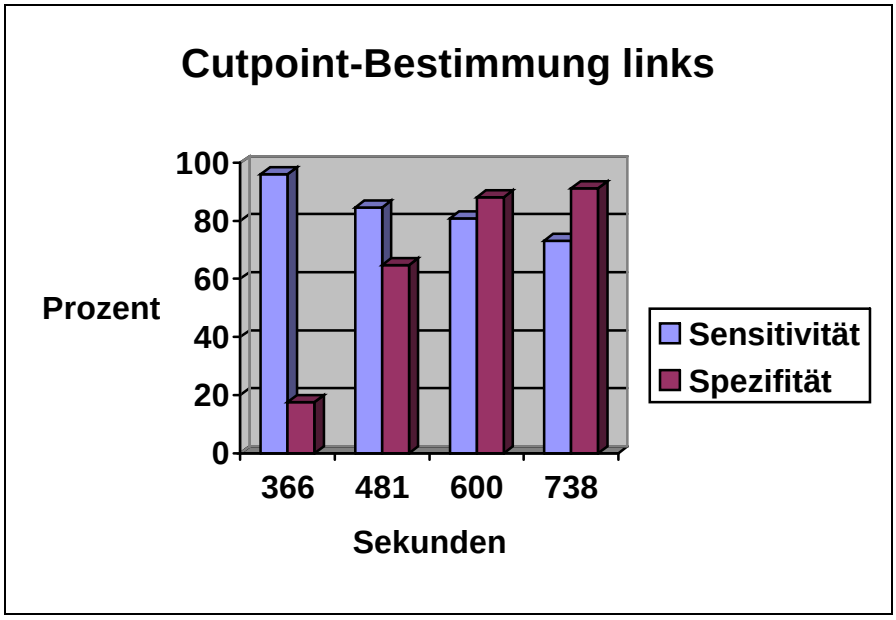


Fig. 15: Bar chart for sensitivity and specificity at various cutpoints of the indicator plaster on the right and left foot.

In the next step the cutpoints found were used to define a positive or negative test result.
For the indicator plaster method the optimum cutpoint under the right foot was established as 722 seconds. This cutpoint was laid down as the criterion as to whether the result is to be classified as "healthy" (colour change by 722 seconds) or as "ill" (colour change after 722 seconds). The result is shown in Table 11 in analogy to Table 7.

Cutpoint 722 sec.			**Group**		**Total**
			Diabetics with NP	Healthy	
Result of plaster test; right foot	ill	Number	22	2	24
		% of plaster test	91.7%	8.3%	100.0%
		% of Group	84.6%	5.9%	40.0%
	healthy	Number	4	32	36
		% of plaster test	11.1%	88.9%	100.0%
		% of Group	15.4%	94.1%	60.0%
Total		Number	26	34	60
		% of plaster test	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 11: Chi-square table for the cutpoint 722 seconds in the indicator test under the right foot for diabetics with neuropathy (Group B) and healthy individuals (Group C).

It became apparent that at the optimum cutpoint for the right foot, sensitivity was 84.6% and specificity was 94.1%. The number of false positive decisions at the selected cutpoint was 5.9% (two subjects), and the number of false negative decisions was found to be 15.4% (four subjects).
The positive predictive value was 91.7%; the negative predictive value was 88.9%.
Using the cutpoint determined for the indicator plaster under the right foot as an example, the Group of diabetics without sensory neuropathy (Group A) was analysed for positive, or negative, test results. Since the Group of diabetics without neuropathy should indeed be seen as healthy as regards neuropathy, this Group was compared with the "ill" diabetics with neuropathy. The results can be seen in Table 12.

Cutpoint 722 sec.			Group		Total
			Diabetics with NP	Diabetics without NP	
Result of plaster test; right foot	ill	Number	22	15	37
		% of plaster test	59.5%	40.5%	100.0%
		% of Group	84.6%	41.7%	59.7%
	healthy	Number	4	21	25
		% of plaster test	16.0%	84.0%	100.0%
		% of Group	15.4%	58.3%	40.3%
Total		Number	26	36	62
		% of plaster test	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 12: Chi-square table for the cutpoint 722 seconds in the indicator plaster test under the right foot for diabetics with neuropathy (Group B) and diabetics without neuropathy (Group A).

When the Group of diabetics without verified sensory neuropathy was tested with the indicator plaster at a cutpoint of 722 seconds, only 58.3% were diagnosed as healthy. However, 41.7% of this Group had a pathological test result and thus reduced skin moisture. A negative predictive value of 84% was calculated, while the positive predictive value was only 59.5%.

The cutpoint for colour change of the indicator plaster was 600 seconds for the left foot. A chi-square stable (Table 13) could also be drawn up for the Group of diabetics with neuropathy (Group B) and the healthy individuals (Group C). Subsequently, the Group of diabetics without neuropathy (Group A) were analysed applying the cutpoint.

Cutpoint 600 sec.			Group		Total
			Diabetics with NP	Healthy	
Result of plaster test; left foot	ill	Number	21	4	25
		% of plaster test	84.0%	16.0%	100.0%
		% of Group	80.8%	11.8%	41.7%
	healthy	Number	5	30	35
		% of plaster test	14.3%	85.7%	100.0%
		% of Group	19.2%	88.2%	58.3%
Total		Number	26	34	60
		% of plaster test	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 13: Chi-square table for the cutpoint 600 seconds in the indicator plaster test under the left foot for diabetics with neuropathy (Group B) and healthy individuals (Group C).

As is apparent from the table of coordinates (Tab. 13), sensitivity is 80.8%, and specificity is 88.2%. The number of false positive results is 11.8%, and false negatives is 19.2%. The positive predictive value is calculated as 84.0%, while the negative predictive value can be given as 85.7%.

The Group of diabetics without neuropathy (Group A) had the following result for the cutpoint of 600 seconds for the left foot: 44.4% were diagnosed as healthy using the indicator plaster measuring method, and 55.6% were diagnosed as ill. Compared to the diabetics with manifest neuropathy (Group B) the positive predictive value was only 51.2%; the negative predictive value was 76.2%. Table 14 gives an overview.

Cutpoint 600 sec.			Group		Total
			Diabetics with NP	Diabetics without NP	
Result of plaster test; right foot	ill	Number	22	20	41
		% of plaster test	51.2%	48.8%	100.0%
		% of Group	80.8%	55.6%	66.1%
	healthy	Number	5	16	21
		% of plaster test	23.8%	76.2%	100.0%
		% of Group	19.2%	44.4%	33.9%
Total		Number	26	36	62
		% of plaster test	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 14: Chi-square table for the cutpoint 600 seconds in the indicator plaster test under the left foot for the diabetics with neuropathy (Group B) and the diabetics without neuropathy (Group A).

In order to establish a common cutpoint for both feet for the indicator plaster measuring method, the mean of the values of the colour change times on the right and left feet was found. The results of the combinations of sensitivity and specificity for the cutpoints 600 seconds, 660 and 690 seconds were determined using chi-square tables. We defined a cutpoint of 600 seconds for the indicator plaster as optimum, since at this time a sensitivity of 84.6% and a specificity of 85.3% emerged. Shifting the cutpoint to longer colour change times led to a drop in sensitivity and an increase in specificity, as the following Table 15 illustrates.

At a cutpoint of 600 seconds the number of false positive (14.7%) and false negative (15.4%) results were nearly the same, and the number of false negative decisions was at its lowest compared with cutpoints 660 and 690 seconds.

Cutpoint 600 sec.			Group		Total
			Diabetics with NP	Healthy	
Result of plaster test; both feet	ill	Number	22	5	27
		% of plaster test	81.5%	18.5%	100.0%
		% of Group	84.6%	14.7%	45.0%
	healthy	Number	4	29	33
		% of plaster test	12.1%	87.9%	100.0%
		% of Group	15.4%	85.3%	55.0%
Total		Number	26	34	60
		% of plaster test	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Cutpoint 660 sec.			Group		Total
			Diabetics with NP	Healthy	
Result of plaster test; both feet	ill	Number	20	3	23
		% of plaster test	87.0%	13.0%	100.0%
		% of Group	76.9%	8.8%	38.3%
	healthy	Number	6	31	37
		% of plaster test	16.2%	83.8%	100.0%
		% of Group	23.1%	91.2%	61.7%
Total		Number	26	34	60
		% of plaster test	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Cutpoint 690 sec.			Group		Total
			Diabetics with NP	Healthy	
Result of Plaster test; both feet	ill	Number	20	2	22
		% of plaster test	90.9%	9.1%	100.0%
		% of Group	76.9%	5.9%	36.7%
	healthy	Number	6	32	38
		% of plaster test	15.8%	84.2%	100.0%
		% of Group	23.1%	94.1%	63.3%
Total		Number	26	34	60
		% of plaster test	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 15: Chi-square table with the values for sensitivity, specificity, positive and negative predictive value and false positive and false negative decisions for the cutpoints 600, 660 and 690 seconds in the indictor plaster test on both feet for diabetics with neuropathy (Group B) and healthy individuals (Group C).

Using the mean colour change times of the indicator plaster method the diabetics without neuropathy (Group A) were examined as regards the fixed cutpoint of 600 seconds. At a sensitivity of 84.6%, 41.7% were classified as “healthy”. The decision for “ill” was made in 58.3% of cases. The positive predictive value was determined as 51.2%; the negative predictive value as 78.9%, as Table 16 illustrates.

Cutpoint 600 sec.			**Group**		**Total**
			Diabetics with NP	Diabetics without NP	
Results of plaster test; both feet	ill	Number	22	21	43
		% of plaster test	51.2%	48.8%	100.0%
		% of Group	84.6%	58.3%	69.4%
	healthy	Number	4	15	19
		% of plaster test	21.1%	78.9%	100.0%
		% of Group	15.4%	41.7%	30.6%
Total		Number	26	36	62
		% of plaster test	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 16: Chi-square table for the cutpoint 600 seconds in the indicator plaster test under both feet for diabetics with neuropathy (Group B) and diabetics without neuropathy (Group A).

4.5. Flat-electrode hydrometry

Flat-electrode hydrometry constituted a further method of measuring skin moisture, as was confirmed by the work of Hilling [47]. We studied the moisture values for both hands and feet, left and right separately. Under the terms of this thesis only the measurements taken on the skin of the plantar region of the foot were of relevance, however. These were calculated in the unit µS (microSiemens) and included in statistical evaluation. The following statistical parameters were analysed.

4.5.1. Statistical parameters

The mean, the standard deviation, the standard error, the 95% confidence interval and the minimum and maximum value of flat-electrode hydrometry were calculated. All three Groups were evaluated.

The healthy population had a mean of 68.526 ± 27.156 µS. In Group A with the diabetics without peripheral sensory neuropathy the electrical conductance was lower, at 58.356 ± 34.856 µS. The lowest values were to be found in the Group of diabetics with verified peripheral neuropathy. Here, a mean conductance of 34.335 ± 37.693 µS was measured. In the healthy Group C the mean electrical conductance was twice as high as in Group B.

Figures 16 and 17 along with Table 17 present this clearly.

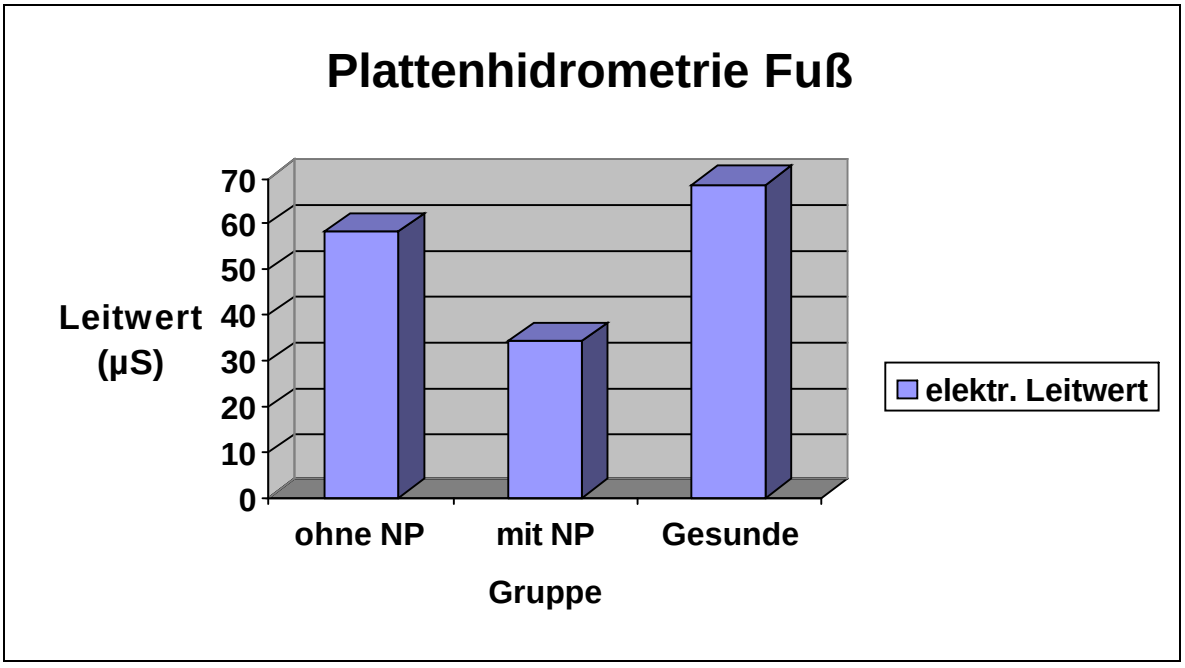


Fig. 16: Graph of the electrical conductance of flat-electrode hydrometry for diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C).

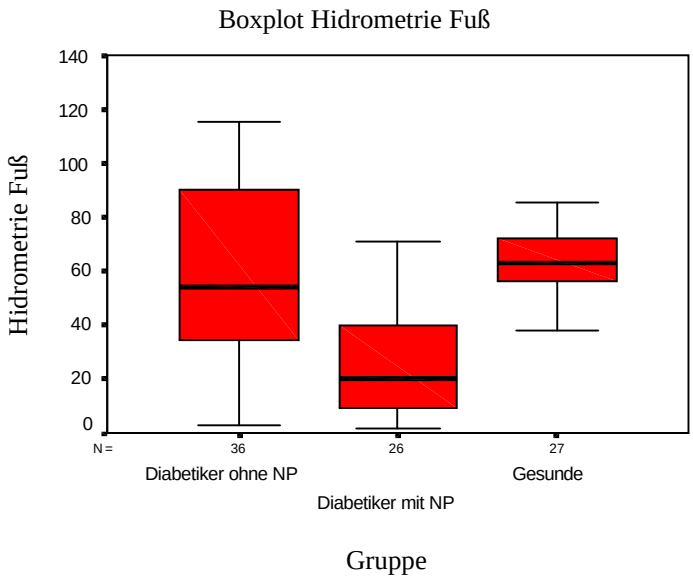


Fig. 17: Box-whisker plot of electrical conductance (µS) of flat-electrode hydrometry in the Groups: diabetics without neuropathy, diabetics with neuropathy and healthy individuals for the plantar region of the foot.

Flat-electrode hydrometry	N	Mean	Standard deviation	Standard error	95% confidence interval for the mean		Mini-mum	Maxi-mum
					Lower	Upper		
Diabetics w.o. NP	36	58.356	34.8565	5.8094	46.562	70.149	3.0	115.6
Diabetics with NP	26	34.335	37.6926	7.3921	19.110	49.559	1.6	146.0
Healthy	34	68.526	27.1561	4.6572	59.051	78.002	38.0	178.5
Total	96	55.452	35.5540	3.6287	48.248	62.656	1.6	178.5

Tab. 17: Mean, standard deviation, standard error, 95% confidence interval, minimum and maximum electrical conductance (µS) of flat-electrode hydrometry on the feet of diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C)

4.5.2. Test for differences between the populations

Distinct differences in the means of the three Groups were found. The diabetics with verified sensory neuropathy (Group B) had the driest feet, followed by the diabetics without neuropathy (Group A).

The difference between the healthy individuals and the diabetics with peripheral sensory neuropathy (Group B) was highly significant, at p=0.001. There was a significant difference, still within the bounds of discreteness, between the Groups of diabetics without and with neuropathy (Group A versus Group B), that is p=0.04. However, the diabetics without sensory neuropathy did not differ significantly from the healthy individuals in flat-electrode hydrometry: p=0.442.
Table 18 gives an overview of this.

(I) GROUP	(J) GROUP	Mean difference (I-J)	Standard error	Significance	95% confidence interval	
					Lower	Upper
Diabetics w.o. NP	Diabetics with NP	24.021(*)	9.4017	**.040**	.817	47.225
(Group A)	Healthy	-10.171	7.4457	**.442**	-28.414	8.072
Diabetics with NP	Diabetics without NP	-24.021(*)	9.4017	**.040**	-47.225	-.817
(Group B)	Healthy	-34.192(*)	8.7369	**.001**	-55.885	-12.498
Healthy	Diabetics without NP	10.171	7.4457	**.442**	-8.072	28.414
(Group C)	Diabetics with NP	34.192(*)	8.7369	**.001**	12.498	55.885

Tab. 18: Multiple comparison to find significant differences in the results of flat-electrode hydrometry on the feet of diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C).

4.6. Selective hydrometry

Selective hydrometry is a new method of selectively measuring sweat at individual points on the sole of the foot. It makes it possible to test the areas of the foot that elude measurement in conventional flat-electrode hydrometry.

Unlike flat-electrode hydrometry, which is based on measuring electrical conductance, selective hydrometry measures skin resistance. The lower the skin resistance measured, the higher the skin moisture. This method facilitates the specific testing of individual, selected areas, spread out over the toes, the forefoot, the metatarsus and the heel, for moisture content on each foot separately, regardless of the curvature of the foot. In addition, a separate result for sweat secretion on the left and right is possible, something which integral flat-electrode hydrometry does not achieve.

4.6.1. Statistical parameters

Twelve test points were defined on each sole. The resulting means of these twelve points on each foot were analysed using statistical methods, and compared.

Additionally, a study of the defined test points, which, when totalled, represent the sweat content of the toes, the forefoot, metatarsus and the heel, was undertaken.

Statistical analysis included the mean, the standard deviation, the standard error, the 95% confidence interval, as well as the minimum and maximum resistance measurement of selective hydrometry for each foot separately. A calculation was done for all three Groups.

The Group of healthy individuals (Group C) had a mean resistance of 12.69 ± 10.61 MOhm. The Group of diabetics without peripheral sensory neuropathy (Group A) presented a resistance almost four time this value; a mean resistance of 46.41 ± 70.53 MOhm was measured here. The highest resistances by far were found among the Group of patients with sensory neuropathy (Group B), with 127.08 ± 112.47 MOhm.

Table 19 and Figure 18 below present the results clearly.

Selective	**hydrometry**	N	Mean	Standard deviation	Standard error	95% confidence interval for the mean		Mini-mum	Maxi-mum
						Lower	Upper		
Selective hydrometry; right foot	Group A	36	46.4125	70.53080	11.7551	22.5483	70.2767	.66	300.0
	Group B	26	127.0831	112.47090	22.0573	81.6551	172.5111	3.40	380.0
	Group C	34	12.6902	10.61356	1.82021	8.9870	16.3935	1.62	41.48
	Total	96	56.3175	85.37954	8.71401	39.0180	73.6170	.66	380.0
Selective hydrometry; left foot	Group A	36	44.0439	70.90080	11.8168	20.0545	68.0333	1.84	331.1
	Group B	26	128.9188	112.17337	21.9990	83.6110	174.2267	5.37	380.0
	Group C	34	14.2670	13.29182	2.27953	9.6292	18.9047	1.81	51.15
	Total	96	56.4849	85.77386	8.75426	39.1055	73.8643	1.81	380.0

Tab. 19: Mean, standard deviation, standard error, 95% confidence interval, minimum and maximum skin resistance in MOhm with selective hydrometry on 12 points of the plantar skin of the foot for diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C)

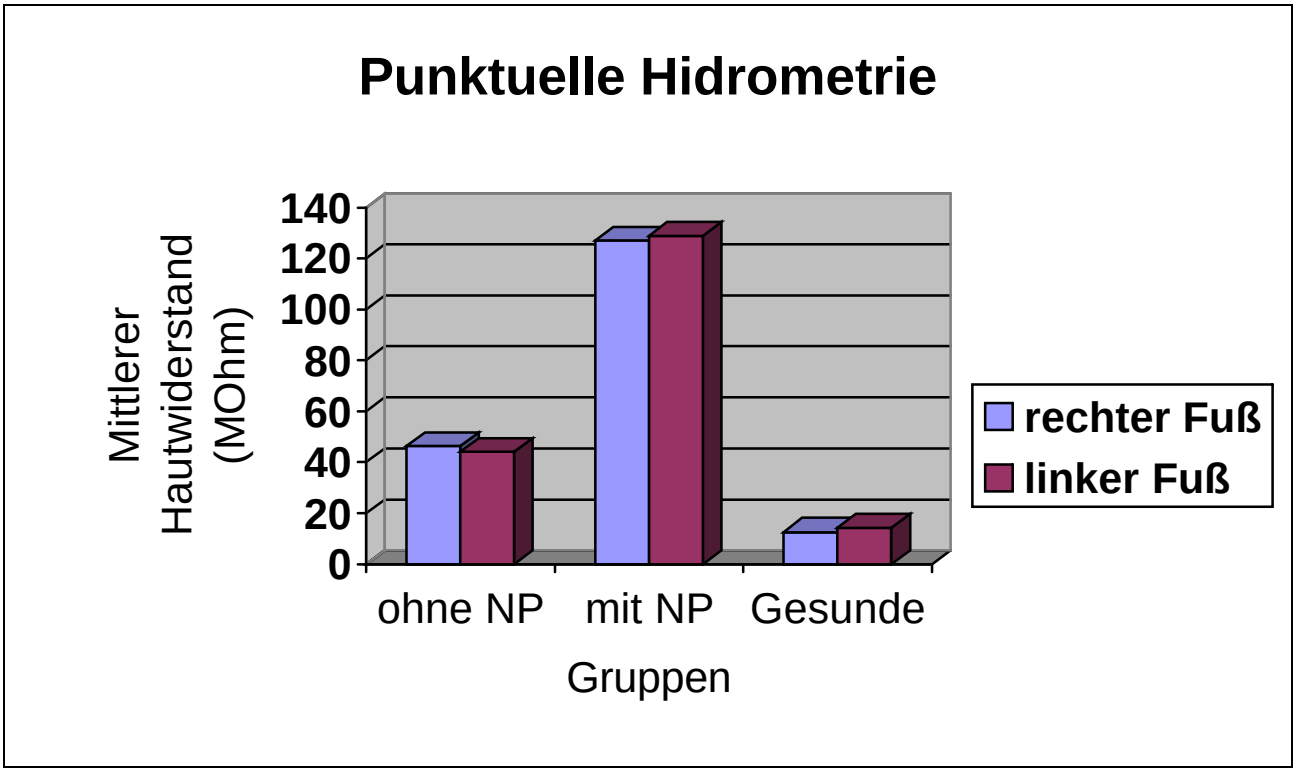


Fig. 18: Mean resistance in MOhm with selective hydrometry for diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C) for twelve test points on the plantar skin of both the right and left foot

4.6.2. Test for differences between the populations

It is evident that there are no significant differences between the selective measurements under the right and left foot, in relation to the means of all twelve measurements; however, there seem to be distinct differences between the three populations.

In order to be able to draw more precise conclusions about Group differences the means of the selective totals were analysed for each side separately using ANOVA. This revealed a significant difference between the Groups. The differences could be classified as significant for the skin of both the left and right foot. The results of the diabetics without neuropathy (Group A) differ considerably from the diabetics with neuropathy (Group B) ($p=0.008$ right and $p=0.005$ left). The differences between the healthy individuals (Group C) and the diabetics with neuropathy were in fact highly significant ($p<0.001$). The right foot presented an even more significant difference between Group C and Group A of $p=0.022$. As regards

the selective measurement on the left sole, there was no significant difference between the healthy individuals and diabetics without sensory neuropathy (p=0.053).

The individual Group differences were determined in detail using multiple comparison, which are shown in Table 20.

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Dependent variable	GROUP (I)	GROUP (J)	Mean difference (I-J)	Standard error	**Signifi-cance**	95% confidence interval	
						Lower	Upper
Selective	Diabetics w.o. NP	Diabet. with NP	-80.6706(*)	24.99420	**.008**	-143.024	-18.317
		Healthy	33.7223(*)	11.89522	**.022**	3.9638	63.4808
	Diabetics with NP	Diabet. w.o. NP	80.6706(*)	24.99420	**.008**	18.3170	143.024
		Healthy	114.3929(*)	22.13233	**.000**	57.8218	170.963
	Healthy	w.o. NP	-33.7223(*)	11.89522	**.022**	-63.4808	-3.9638
		with NP	-114.3929(*)	22.13233	**.000**	-170.963	-57.821
	Diabetics w.o. NP	Diabet. with NP	-84.8750(*)	24.97185	**.005**	-147.158	-22.591
		Healthy	29.7769	12.03466	.053	-.2953	59.8491
	Diabetics with NP	Diabet. w.o. NP	84.8750(*)	24.97185	**.005**	22.5918	147.158
		Healthy	114.6519(*)	22.11679	**.000**	58.1511	171.152
	Healthy	w.o. NP	-29.7769	12.03466	.053	-59.8491	.2953
		with NP	-114.6519(*)	22.11679	**.000**	-171.152	-58.151

* The mean difference is significant at the .05 level.

Tab. 20: Multiple comparison to find significant differences in the results of selective hydrometry on both soles in the populations: diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and the healthy individuals (Group C).

4.6.3. Cutpoint determination by means of a ROC curve

For the selective hydrometry method, determination by means of a ROC curve lent itself to calculating an optimum cutpoint and sensitivity and specificity. To this end, as already explained in Section 4.4.3., sensitivity and, for traditional reasons, 1-specificity of the calculated resistances in MOhm were plotted against each other in the form of a graph and a table. The value representing the highest sum of sensitivity and specificity could then be gauged from the ROC curve (Figure 22).

The area under the curve (AUC), as a measure of diagnostic quality of the measuring method, was 0.893. At the one-sided 5% level, a significance of $p<0.001$ in relation to the value of 0.5 was calculated.

Area under the curve

Variable(s) for test result: Selective hydrometry; right foot

Area	Standard error(a)	Asymptotic significance (b)	Asymptotic 95% confidence interval	
			Lower	Upper
.893	.049	.000	.797	.988

a Under the nonparametric assumption
b Null hypothesis: true area = 0.5

Taking around 30 megaohms as the optimum cutpoint, the sensitivity of selective hydrometry is 84.6%, and the specificity is 91.2%. If one uses this cutpoint to define a positive (resistance > 30 MOhm) or negative (resistance < 30 MOhm) test result, a chi-square table in analogy to Table 7 can be generated. Thus 8.8% are diagnosed falsely positive (3 subjects) and 15.4% (4 subjects) falsely negative.

The positive predictive value at this cutpoint of 30 MOhm is 88.0%, while the negative predictive value can be determined as 88.6% (Table 21).

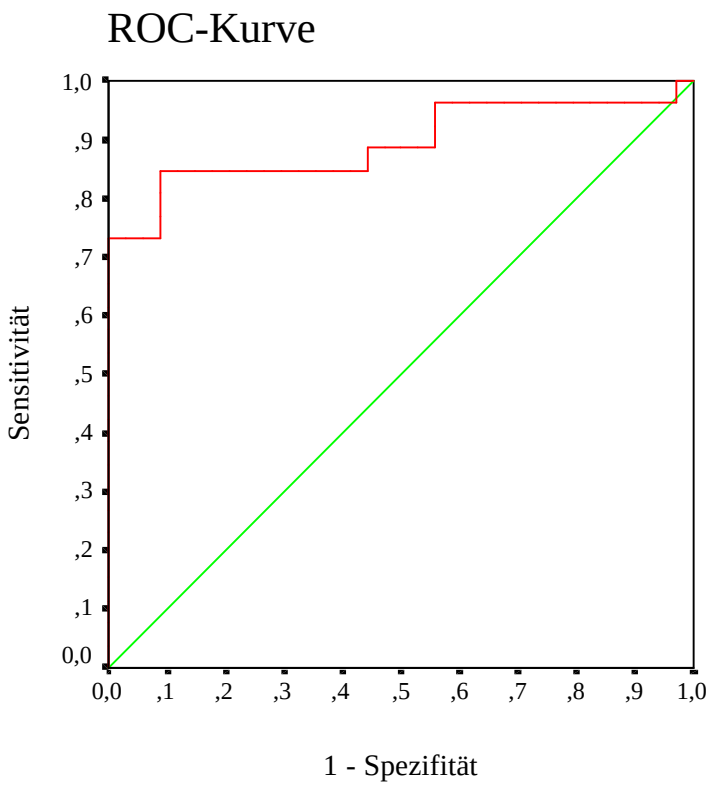

Fig. 19: ROC curve for the mean resistances in MOhm of selective hydrometry on twelve test points under the right foot.

Cutpoint 30 MOhm			**GROUP**		**Total**
			Diabetics with NP	Healthy	
Result of selective hydro-metry; right foot	Ill	Number	22	3	25
		% of test result	88.0%	12.0%	100.0%
		% of Group	84.6%	8.8%	41.7%
	Healthy	Number	4	31	35
		% of test result	11.4%	88.6%	100.0%
		% of Group	15.4%	91.2%	58.3%
Total		Number	26	34	60
		% of test result	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 21: Chi-square table for the cutpoint 30 MOhm with selective hydrometry at the mean of twelve test points under the right foot for diabetics with neuropathy (Group B) and healthy individuals (Group C)

Variable(s) for test result: Selective hydrometry; right foot

Positive, if greater than or equal to (a)	Sensitivity	1-specificity
.6200	1.000	1.000
2.5100	1.000	.971
3.4600	.962	.971
3.5900	.962	.941
3.7600	.962	.912
4.0400	.962	.882
4.3800	.962	.853
4.5900	.962	.824
4.6550	.962	.794
4.6900	.962	.765
4.8154	.962	.735
5.0204	.962	.706
5.1942	.962	.676
5.3992	.962	.647
5.5700	.962	.618
5.7050	.962	.588
6.5750	.962	.559
7.5450	.923	.559
7.8400	.885	.559
8.4950	.885	.529
9.1050	.885	.500
10.1942	.885	.471
11.5842	.885	.441
12.0500	.846	.441
13.0271	.846	.412
14.0071	.846	.382
14.3021	.846	.353
14.8221	.846	.324
15.2700	.846	.294
15.9600	.846	.265
16.7588	.846	.235
17.2687	.846	.206
18.0300	.846	.176
22.0050	.846	.147
26.8750	.846	.118
29.4350	**.846**	**.088**
31.1600	.808	.088
33.6150	.769	.088
36.5150	.731	.088
38.1970	.731	.059
40.1920	.731	.029
42.0450	.731	.000
44.1300	.692	.000
59.4750	.654	.000
77.0650	.615	.000
84.0150	.577	.000
89.7500	.538	.000
96.1900	.500	.000
108.6500	.462	.000
123.2650	.423	.000
130.6950	.385	.000
134.5500	.346	.000
155.8900	.308	.000
175.5950	.269	.000
188.9550	.231	.000
221.3300	.192	.000
242.2450	.154	.000
300.5450	.115	.000
360.5550	.077	.000
371.6650	.038	.000
381.0000	.000	.000

Tab. 22: Cutpoints and coordinates of the ROC curve for the mean resistances in MOhm of selective hydrometry on twelve test points under the right foot.

The Group of diabetics without neuropathy (Group A) were tested with the selective hydrometry measuring method and a cutpoint of 30 MOhm set for the mean resistance of the twelve test points. This resulted in 63.9% being correctly diagnosed as healthy, and 36.1% of the diabetics without neuropathy had reduced skin dryness. The positive predictive value was 62.9%; the negative predictive value was 85.2% (Table 23).

Cutpoint 30 MOhm			GROUP		Total
			Diabetics with NP	Diabetics w.o. NP	
Result of selective hydro-metry; right foot	Ill	Number	22	13	35
		% of test result	62.9%	37.1%	100.0%
		% of Group	84.6%	36.1%	56.5%
	Healthy	Number	4	23	27
		% of test result	14.8%	85.2%	100.0%
		% of Group	15.4%	63.9%	43.5%
Total		Number	26	36	62
		% of test result	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 23: Chi-square table for the cutpoint 30 MOhm with selective hydrometry at the mean of twelve test points under the right foot for diabetics with neuropathy (Group B) and diabetics without neuropathy (Group A).

The calculations for selective hydrometry on the right foot could, of course, also be carried out for the left foot. The mean resistances of the twelve test points under the left sole were calculated and a ROC curve generated to determine the cutpoint (Figure 20). As was the case with the right foot, the AUC was also calculated for the left foot results, the coordinates of possible cutpoints listed (Table 24) and a chi-square table generated for the Group of diabetics with neuropathy (Group B) and the healthy individuals (Group C) (Table 25).

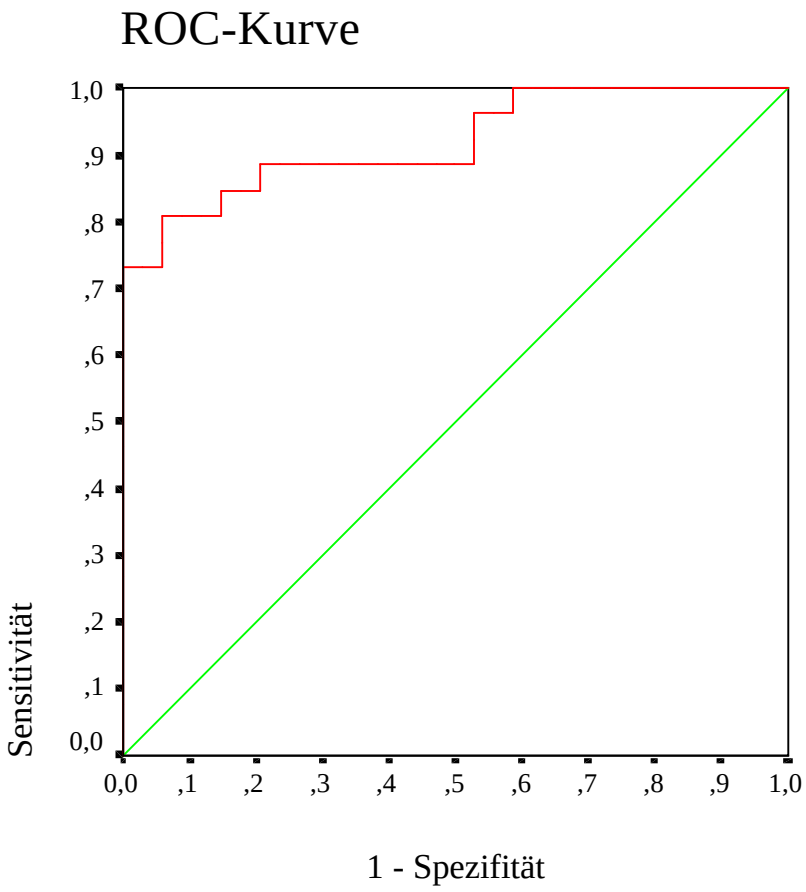

Fig. 20: ROC curve for the mean resistances in MOhm with selective hydrometry on twelve test points under the left foot.

The AUC as a quality criterion was determined; the value 0.919 is significant at the one-sided 5% level in relation to the value 0.5, with $p<0.001$.

Area under the curve

Variable(s) for test result: Selective hydrometry; left foot

Area	Standard error (a)	Asymptotic significance (b)	Asymptotic 95% confidence interval	
			Lower	Upper
.919	.038	.000	.844	.993

a Under the nonparametric assumption
b Null hypothesis: true area = 0.5

Curve coordinates

Variable(s) for test result: Selective hydrometry; left foot

Positive, if greater than or equal to (a)	Sensitivity	1-specificity
.8100	1.000	1.000
2.4400	1.000	.971
3.1550	1.000	.941
3.3700	1.000	.912
3.5450	1.000	.882
3.7000	1.000	.853
3.9358	1.000	.824
4.2908	1.000	.794
4.6563	1.000	.735
4.9063	1.000	.706
5.0850	1.000	.676
5.1600	1.000	.647
5.1750	1.000	.618
5.2750	1.000	.588
5.9000	.962	.588
6.8400	.962	.559
7.2750	.962	.529
8.0100	.923	.529
9.4050	.885	.529
10.4800	.885	.500
11.1879	.885	.471
11.5979	.885	.441
12.3500	.885	.412
13.4600	.885	.382
14.0400	.885	.353
14.8050	.885	.324
15.6417	.885	.294
17.7867	.885	.265
20.3317	.885	.235
22.3616	.885	.206
24.6029	.846	.206
25.4454	.846	.176
29.1325	.846	.147
33.1900	.808	.147
33.7900	.808	.118
36.3700	.808	.088
41.8150	**.808**	**.059**
45.2200	.769	.059
46.9800	.731	.059
49.7750	.731	.029
53.8550	.731	.000
60.0500	.692	.000
64.6850	.654	.000
66.3000	.615	.000
76.2450	.577	.000
91.0050	.538	.000
101.0800	.500	.000
112.9350	.462	.000
120.4750	.423	.000
123.1800	.385	.000
126.4750	.346	.000
132.4650	.308	.000
151.5750	.269	.000
193.7150	.231	.000
228.6000	.192	.000
272.6450	.154	.000
322.6600	.115	.000
349.4450	.077	.000
371.6650	.038	.000
381.0000	.000	.000

Tab. 24: Cutpoints and coordinates of the ROC curve for the mean resistances in MOhm with selective hydrometry at twelve test points under the left foot.

One can see that a mean resistance of 41.8 MOhm as the selected cutpoint on the left foot produces the highest possible sum of sensitivity and 1-specifity. At this point sensitivity is 80.8%, and specificity 94.1%.

Cutpoint 40 MOhm			GROUP		Total
			Diabetics with NP	Healthy	
Result of selective hydro-metry; left foot	Ill	Number	21	2	23
		% of test result	91.3%	8.7%	100.0%
		% of Group	80.8%	5.9%	38.3%
	Healthy	Number	5	32	37
		% of test result	13.5%	86.5%	100.0%
		% of Group	19.2%	94.1%	61.7%
Total		Number	26	34	60
		% of test result	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 25: Chi-square table for the cutpoint 40 MOhm with selective hydrometry at the mean of twelve test points under the left foot for diabetics with neuropathy (Group B) and healthy individuals (Group C).

Taking around 40 MOhm as the optimum cutpoint, the sensitivity of selective hydrometry under the left foot is 80.8%; the specificity 94.1%. If one defines a test result of <40 MOhm as healthy and a mean resistance of >40 MOhm as ill, 5.9% are diagnosed falsely positive (2 subjects) and 19.2% (5 subjects) falsely negative.
The positive predictive value is 91.3% at the cutpoint of 40 MOhm, while the negative predictive value can be determined as 86.5% (Table 25).

The cutpoint on the left foot established above was applied to the Group of diabetics without neuropathy (Group A). The results are presented in Table 26.

In Group A 69.4% of the diabetics without neuropathy were diagnosed correctly using selective hydrometry. However, 30.6% were classified as ill (“false positive”). The positive predictive value was 65.6%, while the negative predictive value was found to be 83.3%.

Cutpoint 40 MOhm			GROUP		Total
			Diabetics with NP	Diabetics w.o. NP	
Result of selective hydro-metry; left foot	Ill	Number	21	11	32
		% of test result	65.6%	34.4%	100.0%
		% of Group	80.8%	30.6%	51.6%
	Healthy	Number	5	25	30
		% of test result	16.7%	83.3%	100.0%
		% of Group	19.2%	69.4%	48.8%
Total		Number	26	36	62
		% of test result	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 26: Chi-square table for the cutpoint 40 MOhm with selective hydrometry at the mean of twelve test points under the left foot for diabetics with neuropathy (Group B) and diabetics without neuropathy (Group A).

Since no significant differences between the right and left foot were discovered in descriptive statistics, the results of resistance measurement taken by selective hydrometry were consolidated to define a common cutpoint for selective hydrometry.

The results of the combinations of sensitivity and specificity for the cutpoints 30 MOhm and 35 MOhm were determined by means of chi-square tables. We set a cutpoint of 35 MOhm, since it was at this point that the highest correlation of sensitivity and specificity was obtained for the mean resistances of both feet. The sensitivity was 84.6%, and specificity was 94.1%. The positive predictive value was 91.7%; the negative predictive value 88.9%. The number of false negatives was put at 15.4%, and the false positives at 5.9% (Table 27).

Cutpoint 35 MOhm			GROUP		Total
			Diabetics with NP	Healthy	
Result of selective hydro-metry; both feet	Ill	Number	22	2	24
		% of test result	91.7%	8.3%	100.0%
		% of Group	84.6%	5.9%	40.0%
	Healthy	Number	4	32	36
		% of test result	11.1%	88.9%	100.0%
		% of Group	15.4%	94.1%	60.0%
Total		Number	26	34	60
		% of test result	43.3%	56.7%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 27: Chi-square table for the cutpoint 35 MOhm with selective hydrometry at the mean of the right and left foot for diabetics with neuropathy (Group B) and healthy individuals (Group C).

The Group of diabetics without neuropathy (Group A) was examined with the newly defined cutpoint of 35 MOhm for the mean resistances of both feet. This resulted in 30.6% being diagnosed as “ill” and 69.4% as “healthy” (Table 28).

Cutpoint 35 MOhm			GROUP		Total
			Diabetics with NP	Diabetics w.o. NP	
Result of selective hydro-metry; both feet	Ill	Number	22	11	33
		% of test result	66.7%	33.3%	100.0%
		% of Group	84.6 %	30.6%	53.2%
	Healthy	Number	4	25	29
		% of test result	13.8%	86.2%	100.0%
		% of Group	15.4%	69.4%	46.8%
Total		Number	26	36	62
		% of test result	41.9%	58.1%	100.0%
		% of Group	100.0%	100.0%	100.0%

Tab. 28: Chi-square table for the cutpoint 35 MOhm with selective hydrometry at the mean of the right and left foot for diabetics with neuropathy (Group B) and diabetics without neuropathy (Group A).

4.6.4. Foot areas compared

Under the terms of this thesis the question arose as to whether the observed reduced sweat secretion on the skin of the plantar region of diabetics' feet was as equally as pronounced in all regions of the foot or whether certain areas had a greater and more frequent tendency to become affected by impaired sudomotor function.
Table 29 below shows the assignment of the individual test points [Fig. 8] to the areas of the foot:

Foot region	**Test points (n = 12)**
Toes	1, 9, 10, 11, 12
Forefoot	2, 3, 4
Metatarsus	5, 6
Heel	7, 8

Tab. 29: Test points on the sole for the relevant regions of the foot: toes, forefoot, metatarsus and heel.

A descriptive statistical evaluation established that the toe region in each Group represented the area of greatest sweat secretion. A progressive drop in sweat secretion from distal toward proximal emerged among all three populations, the heel region being the driest.

The right toe region of the healthy individuals had a mean of 4.5 ± 3.5 MOhm; by contrast the diabetics without peripheral sensory neuropathy (Group A) had 29.1 ± 54.2 and the diabetics with verified neuropathy (Group B) as much as 110.4 ± 1239.7 MOhm. Comparable results were observed in the left toe region.

The right forefoot region was dryer than the toes. Here the skin resistances measured in healthy individuals were 11.9 ± 8.7 MOhm; in Group A they were 44.5 ± 86.1, and in Group B 104.3 ± 132.9 MOhm.

A further increase in skin resistances was found by measurements in the metatarsus region. On the right foot a mean resistance of 20.4 ± 24.2 MOhm for the healthy individuals, 57.2 ± 97.8 for Group A and 160.3 ± 144.9 MOhm for Group B was measured.

The results for the heel, the driest region of the foot in all three Groups, were as follows: for the healthy individuals the skin resistance as measured by selective hydrometry in the right heel region was 27.9 ± 41.3 MOhm, for Group A 59.9 ± 101.4 and finally for Group B 169.8 ± 139.8 MOhm. Appendix 10 presents in the form of a table the results of the skin resistances determined in the relevant areas of the foot for all three Groups in terms of the mean and standard deviation.

Some of the differences between the Groups were highly significant; dryness of the toes in the Group of diabetics with sensory neuropathy (Group B) was almost 24 times more pronounced than in the healthy individuals (Group C). By comparison, diabetics without neuropathy (Group A) had dryness in the toe region no less than six times (6.46) that of the healthy individuals.
The mean resistances in the forefoot region for Group A showed a 2.5 to 3.5-times increase in skin dryness, a 2.8 to 3.3-times increase in the metatarsus region, and were twice that of the healthy individuals (Group C) in the heel region. Showing a marked difference from this were the changes in skin dryness of Group B in the same areas of the foot; the skin of the forefeet of diabetics with neuropathy is 6.3 to 8.7 times dryer than for Group C. In the metatarsus and heel regions the factor is 7.5 and 5.6 respectively.
Figure 21 shows a graphical presentation of the results on the right sole.

On the basis of statistical calculations, several significant differences between the three populations present themselves regarding skin moisture on the various different regions of the foot.
In the toe region of both the left and right foot all results of selective hydrometry are significantly different. The greatest differences are found between the diabetics with neuropathy and the healthy individuals ($p<0.001$). Only for the left toes is the difference between healthy individuals and diabetics without sensory neuropathy significant at the 0.05 level.

In the right forefoot region a significant difference can be identified between the healthy individuals and diabetics with neuropathy ($p=0.005$); this is also true of the left forefoot ($p=0.001$).

The metatarsus region, which cannot be measured by flat-electrode hydrometry, revealed significant differences in all Groups. However, no significant difference between healthy individuals and diabetics without neuropathy could be measured for the right foot.

Comparisons of the heel region come up with similar results; both on the right and the left no statistically significant difference between healthy individuals and diabetics without neuropathy is apparent.

Table 30 displays the overall results of the multiple comparisons.

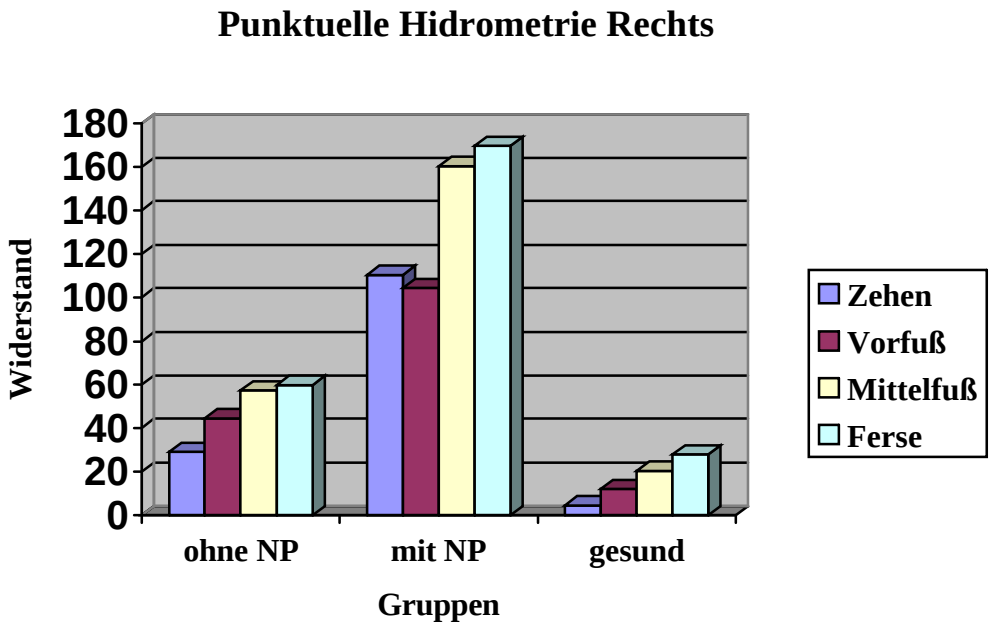

Fig. 21 a): Mean electrical skin resistances in MOhm for the right foot.

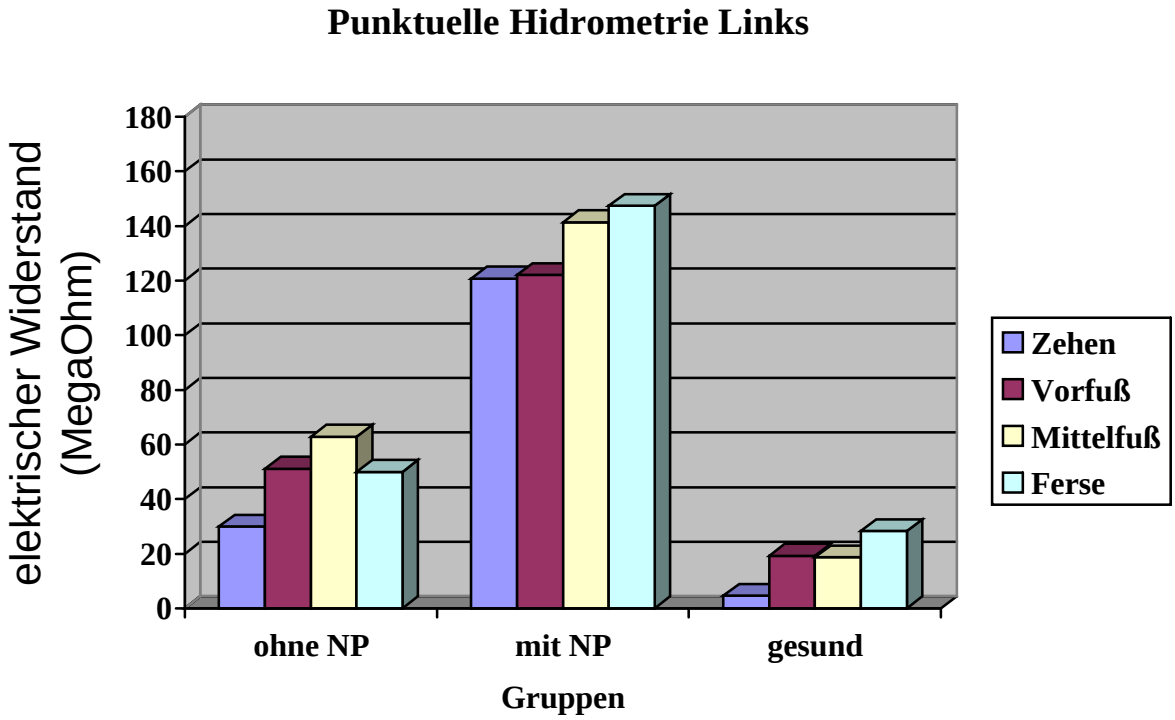

Fig. 21 b): Separate charts of the mean electrical skin resistances in MOhm for the four areas of the foot among diabetics without neuropathy (Group A), with neuropathy (Group B) and the healthy individuals (Group C) for the right foot (Fig. 21 a) and the left foot (Fig. 21 b) using selective hydrometry.

Multiple comparison of the means

Tamhane

Depend-ent variable	GROUP (I)	GROUP (J)	Mean difference (I-J)	Standard error	**Signifi-cance**	95% confidence interval	
						Lower	Upper
Toes right Means	Diabetics w.o. NP	Diabetics with NP	-81.2919(*)	27.02624	**.015**	-149.434	-13.149
		Healthy	24.5756(*)	9.17755	**.033**	1.5398	47.6114
	Diabetics with NP	Diabetics w.o. NP	81.2919(*)	27.02624	**.015**	13.1493	149.434
		Healthy	105.8675(*)	25.43452	**.001**	40.7989	170.93
	Healthy	Diabetics w.o. NP	-24.5756(*)	9.17755	**.033**	-47.6114	-1.5398
		Diabetics with NP	-105.8675(*)	25.43452	**.001**	-170.936	-40.798
Toes left Means	Diabetics w.o. NP	Diabetics with NP	-90.7979(*)	26.69776	**.005**	-157.909	-23.686
		Healthy	25.2645	10.08735	**.050**	-.0185	50.5474
	Diabetics with NP	Diabetics w.o. NP	90.7979(*)	26.69776	**.005**	23.6861	157.909
		Healthy	116.0624(*)	24.73567	**.000**	52.7828	179.342
	Healthy	Diabetics w.o. NP	-25.2645	10.08735	**.050**	-50.5474	.0185
		Diabetics with NP	-116.0624(*)	24.73567	**.000**	-179.342	-52.782
Forefoot right Means	Diabetics w.o. NP	Diabetics with NP	-59.8064	29.76358	.146	-133.987	14.3748
		Healthy	32.5057	14.42709	.089	-3.6300	68.6414
	Diabetics with NP	Diabetics w.o. NP	59.8064	29.76358	.146	-14.3748	133.987
		Healthy	92.3121(*)	26.12086	**.005**	25.5141	159.110
	Healthy	Diabetics w.o. NP	-32.5057	14.42709	.089	-68.6414	3.6300
		Diabetics with NP	-92.3121(*)	26.12086	**.005**	-159.110	-25.514
Forefoot left Means	Diabetics w.o. NP	Diabetics with NP	-70.8497	28.84875	.055	-142.816	1.1172
		Healthy	31.9173	14.32422	.091	-3.6780	67.5126
	Diabetics with NP	Diabetics w.o. NP	70.8497	28.84875	.055	-1.1172	142.816
		Healthy	102.7670(*)	25.85616	**.001**	36.8973	168.636
	Healthy	Diabetics w.o. NP	-31.9173	14.32422	.091	-67.5126	3.6780
		Diabetics with NP	-102.7670(*)	25.85616	**.001**	-168.636	-36.897
Metatarsus	Diabetics w.o. NP	Diabetics with NP	-103.0867(*)	32.76705	**.009**	-184.652	-21.520

Right Means		Healthy	36.7921	16.83419	.101	-5.1738	78.7580
	Diabetics with NP	Diabetics w.o. NP	103.0867(*)	32.76705	**.009**	21.5207	184.652
		Healthy	139.8788(*)	28.73418	**.000**	66.5820	213.175
	Healthy	Diabetics w.o. NP	-36.7921	16.83419	.101	-78.7580	5.1738
		Diabetics with NP	-139.8788(*)	28.73418	**.000**	-213.175	-66.582
Metatarsus left Means	Diabetics w.o. NP	Diabetics with NP	-78.5242(*)	31.60757	**.050**	-156.959	-.0890
		Healthy	44.1362(*)	17.24347	**.043**	1.0206	87.2518
	Diabetics with NP	Diabetics w.o. NP	78.5242(*)	31.60757	**.050**	.0890	156.959
		Healthy	122.6604(*)	26.81332	**.000**	54.1746	191.146
	Healthy	Diabetics w.o. NP	-44.1362(*)	17.24347	**.043**	-87.2518	-1.0206
		Diabetics with NP	-122.6604(*)	26.81332	**.000**	-191.146	-54.174
Heel right Means	Diabetics w.o. NP	Diabetics with NP	-109.9601(*)	32.19985	**.004**	-189.94	-29.97
		Healthy	31.9214	18.36705	.243	-13.5468	77.3896
	Diabetics with NP	Diabetics w.o. NP	109.9601(*)	32.19985	**.004**	29.9775	189.942
		Healthy	141.8815(*)	28.33812	**.000**	70.0010	213.761
	Healthy	Diabetics w.o. NP	-31.9214	18.36705	.243	-77.3896	13.5468
		Diabetics with NP	-141.8815(*)	28.33812	**.000**	-213.761	-70.001
Heel left Means	Diabetics w.o. NP	Diabetics with NP	-97.4516(*)	32.08926	**.012**	-177.326	-17.576
		Healthy	21.5877	17.84997	.547	-22.4773	65.6526
	Diabetics with NP	Diabetics w.o. NP	97.4516(*)	32.08926	**.012**	17.5767	177.326
		Healthy	119.0392(*)	28.93846	**.001**	45.7352	192.34
	Healthy	Diabetics w.o. NP	-21.5877	17.84997	.547	-65.6526	22.4773
		Diabetics with NP	-119.0392(*)	28.93846	**.001**	-192.34	-45.735

* The mean difference is significant at the .05 level.

Tab. 30: Multiple comparisons to find significant differences between diabetics without neuropathy (Group A), diabetics with neuropathy (Group B) and healthy individuals (Group C) in relation to the means of selective hydrometry in the following regions of the foot: toes, forefoot, metatarsus and heel.

4.7. Measuring methods compared

4.7.1. Comparison of indicator plaster / flat-electrode hydrometry

There is a strong and highly significant Pearson's correlation (0.953) between the results of the Neuropad indicator plaster for the right and left foot. We were further able to record a moderate, but significant, correlation between the results of the indicator plaster and those of flat-electrode hydrometry (Pearson's correlation coefficient 0.46 and 0.52 respectively), as shown in Table 31.

		Neuropad right	Neuropad left	Flat-electrode hydrometry
Neuropad right foot	Pearson's correlation	1	.953(**)	-.521(**)
	Significance (2-sided)	.	.000	.000
	N	96	96	96
Neuropad left foot	Pearson's correlation	.953(**)	1	-.461(**)
	Significance (2-sided)	.000	.	.000
	N	96	96	96
Flat-electrode hydrometry	Pearson's correlation	-.521(**)	-.461(**)	1
	Significance (2-sided)	.000	.000	.
	N	96	96	96

** The correlation is significant at the 0.01 (2-sided) level.

Tab. 31: Pearson's correlations for the measurements of flat-electrode hydrometry (electrical conductance in µS) and Neuropad (colour change in seconds) for both sides.

4.7.2. Comparison of indicator plaster / selective hydrometry

The distinctly different results with the selective hydrometry method for sweat secretion in the areas of skin described made a review for possible correlations with the Neuropad result necessary.

A high correlation between the results of selective hydrometry and the Neuropad indicator plaster was found. Pearson's correlation coefficient was 0.696 ($p<0.001$) between the right Neuropad and selective hydrometry carried out on the right side. Compared with hydrometry on the left foot the correlation was 0.689 ($p<0.001$). Table 32 presents this clearly.

		Neuropad right	Neuropad left	Sel. hydro-metry right	Sel. hydro-metry left
Neuropad right	Pearson's correlation	1	.953(**)	.696(**)	.689(**)
	Significance (2-sided)	.	.000	.000	.000
	N	96	96	96	96
Neuropad left	Pearson's correlation	.953(**)	1	.660(**)	.657(**)
	Significance (2-sided)	.000	.	.000	.000
	N	96	96	96	96
Selective hydrometry right	Pearson's correlation	.696(**)	.660(**)	1	.937(**)
	Significance (2-sided)	.000	.000	.	.000
	N	96	96	96	96
Selective hydrometry left	Pearson's correlation	.689(**)	.657(**)	.937(**)	1
	Significance (2-sided)	.000	.000	.000	.
	N	96	96	96	96

** The correlation is significant at the 0.01 (2-sided) level.

Tab. 32: Pearson's correlations for the measurements of selective hydrometry (electrical skin resistance in MOhm) right and left and the Neuropad (colour change in seconds) for both sides.

5. Discussion

Diabetic foot syndrome is a frequent complication in diabetics and still assumes an exceptional position among the secondary diseases of diabetes mellitus. The prevalence of the foot ulcus among the diabetic population in different countries ranges from between 2 and 10 percent [54]. A yearly incidence of 1 to 7% has been reported [17, 55]. It is estimated that around 15% of all diabetics will undergo a minor or major amputation in the feet over the course of the disease [56, 57]. Over 80% of all amputations of the lower limbs are caused by foot ulcers, and diabetes mellitus is the most common cause of non-traumatic amputations in western nations [56]. That said, sequelae of diabetic foot syndrome can generally be avoided, with a reduction of up to 80% in the amputation figures possible, by means of relatively simple interventions [57].

In Germany approx. 70% of all amputations are performed on diabetics. According to AOK figures from 2001 this amounts to more than 29,000 amputations on diabetics per year [58]. Compared to some European countries (e.g. Netherlands, Denmark, Spain) these figures are extremely high, and Germany has not seen a drop in amputation figures in the past few years either [48].

Diabetic foot ulcers are the most common reason for diabetics being hospitalised in western nations, and they are a significant factor in the morbidity and mortality of these patients [59, 60]. For health insurers this fact represents a primary cost factor, since American studies, based on the data of 7 million individuals, have put the total costs of a lower limb ulcer over a period of 2 years at 16 million US dollars [61]. For the patients affected, diabetic foot syndrome, together with its sequelae, means lower quality of life combined with disabilities, pain and social disadvantages [48].

The cause of diabetic foot syndrome is essentially diabetic neuropathy [62], in which one differentiates between sensory, motor and autonomic neuropathy. Autonomic neuropathy, which is accompanied in particular by reduced sweat secretion, has often been ignored until now for lack of appropriate examination methods.
The literature has up to now cited various different methods to examine sudomotor function as the manifestation of autonomic neuropathy in diabetes mellitus. Many measuring methods for recording sweat production are complicated or not reproducible. Low [63, 64] gives an

overview of the history of the various procedures. Significant results have, however, only been described for a few methods of testing skin dryness, which, modified according to Mathias et al. [65], can be distinguished as follows:

- Thermoregulatory Sweat Test (TST): The TST is a sensitive, qualitative, semi-quantitative test of sudomotor function, which indicates a pattern and the distribution of sweat loss. The first to describe it, Guttmann [66] used quinizarin as an indicator in 1947, which, in the presence of sweat, turned from brown to violet. Quinizarin is rarely available today and is highly allergenic. An optimisation of the test was described by Fealey et al. [67], who defined the test conditions and developed the "sweat booth".

- Quantitative Sudomotor Axon Reflex Test (QSART): This test is regarded as a routine test of autonomic function and is a component of autonomic reflex screening [68]. The test is very susceptible to many confounding variables. Furthermore, standardisation and patient-preparation are intensive. Acetylcholine is introduced into the skin using iontophoresis and the postganglionic sweat gland response measured using a chemical procedure. The QSART is sensitive; differentiation between the left and right has not been described [63].

- Skin Potential Recordings, Sympathic Skin Response (SSR): The electrical activity of the skin, generated by the sweat glands and the adjacent tissue, is known as electrodermal activity and is generally referred to as SSR. SSR measurement is regarded as a sensitive test of autonomic function, which is used in many EMG laboratories, as described by Shahani et al. [69]. After brief electrical stimulation of peripheral afferent nerves (e.g. median nerve), the change in electrodermal activity is recorded by electrodes on the palm of the hand or sole of the foot. This method is very error-prone, to everything from skin diseases to multiple sclerosis and axonal neuropathies. Its clinical application fails because of the high variability of results and bad specificity [70, 71].

None of these methods has yet become established in clinical routine. The most important objective of this thesis was thus to investigate new methods of measuring skin moisture as the manifestation of autonomic neuropathy in terms of their scientific cogency and suitability for routine application.

The measuring methods to be investigated were flat-electrode hydrometry, selective hydrometry and the Neuropad indicator plaster.

Three Groups were formed for testing. Firstly a healthy control population (Group C), secondly a Group of diabetics without (Group A) and a Group of diabetics with peripheral sensory neuropathy (Group B). A number of around 30 subjects was specified for the size of each Group. With this number of n = 30 subjects per Group the accuracy (defined as half the length of the 95% confidence interval) of the sensitivity and specificity was between 16% (at a sensitivity of 70%) and 10% (at a sensitivity of 90%). This accuracy was considered as sufficient and appropriate for this first investigation of the indicator plaster and selective hydrometry.

The control Group was slightly younger than the diabetics with and without sensory neuropathy. However, we consider this fact as irrelevant, as there was no significant age difference between the two Groups of diabetics and the literature does not report age as an influence on differences in the development of neuropathy (72).

Before clinical tests began a Total Symptom Score (TSS, Appendix 6) was found for all three Groups, in which the symptoms “smarting”, “tingling”, “numbness” and “pain” were recorded by intensity and frequency. This score originally served to divide up the severity of diabetic neuropathy [73].
It was interesting to find that the complaints and symptoms described, which were added up to give the Total Symptom Score (TSS), plainly contradicted the measured test results.

Subjective perceptions in the feet do not suffice as the sole criterion in detecting peripheral sensory neuropathy. We were able to demonstrate that while healthy individuals (Group C) were correctly identified as such by the TSS 91.2% of the time, 81% of the diabetics without neuropathy (Group A) were classified as healthy. This means that 19% of this Group were given a false positive diagnosis of neuropathy.

Among diabetics with verified sensory neuropathy (Group B) the cogency of the TSS was even more greatly reduced. On the basis of the TSS, 44% of this population were incorrectly assessed as being healthy. Only 56% of these diabetics with neuropathy were correctly identified by the TSS as having a sensory neuropathy.

Ultimately, case history on its own, as it is represented by the Total Symptom Score, can be assessed as inadequate and unreliable with respect to the actual existence of sensory neuropathy. For this reason at least one apparative test, e.g. with the Semmes-Weinstein 10g monofilament, should be done. The important conclusion, which has been published as the international consensus by Boulton and Gries [74], states that neuropathical symptoms only correlate with sensory loss to a certain degree and therefore their absence cannot be equated with a lower risk of foot ulcer. The authors endorse the statement that after a thorough examination of the bare foot and having obtained a comprehensive case history it is essential to carry out an apparative examination.

Other studies [74] show that symptoms are often incorrectly perceived; this includes in particular decreased sensitivity to pain (hypalgesia to analgesia), temperature (thermohypaesthesia to thermanaesthesia) and vibration (pallhypaesthesia). Not infrequently neuropathy is not diagnosed until the manifestation of a foot ulcus without a prior clinical examination or subjectively experienced symptoms having led to it. For these reasons, too, it is necessary to always complement case history with apparative examination.

Inspection of the feet alone finds its limits in assessment of skin dryness. Skin turgor and the integrity of the skin can certainly be examined by inspection and palpation; likewise muscular atrophies and foot deformities can be identified. The autonomic-neuropathic foot only seems dry in extreme cases, however. Only in the more advanced stages of neuropathy does the skin of the foot appear cracked, rough and dry. In the early stage the foot appears more pinkish, with good circulation, warm to the touch and has no bad odour. With increased blood flow when the arteriovenous shunts are enlarged circulation in the foot is very high and the foot does not look ill. The lack of cooling of the foot due to reduced sweat secretion enhances this effect.

As a method of testing skin dryness, the subjects were examined with flat-electrode hydrometry, selective hydrometry and the indicator plaster.
Flat-electrode hydrometry as a means of diagnosing skin moisture was optimised by Hilling and Zick and put into practice [45]. The measurement of conductivity, which is influenced by sweat production, is, in terms of concept, an old procedure. Back in 1927 Darrow [75]

described change in skin conductivity, with this also being the underlying principle of the lie detector.
The results of flat-electrode hydrometry did not fully coincide with those of Hilling. While Hilling et al. [45] established significant differences between each of the three Groups A, B and C in terms of the mean measurements, this study did not calculate any significant differences (p=0.442; Hilling p=0.003) between healthy individuals (Group C) and diabetics without sensory neuropathy (Group A).

It can be inferred from our data that flat-electrode hydrometry can only reliably differentiate between healthy individuals and patients with peripheral neuropathy (p=0.001). In addition, it succeeded in distinguishing between diabetics with and without neuropathy (p=0.04), but failed to differentiate with certainty between healthy individuals and diabetics without peripheral neuropathy. It would only be possible to explain the discrepancy between the data presented here and those of Hilling et al. [47] if this question were examined again with a larger population of healthy individuals and diabetics without sensory neuropathy.

Despite this just discovered and ultimately not conclusively clarified data discrepancy flat-electrode hydrometry is still used in many practices. The advantage of the method consists in its simple application and reliable detection of patients with actual, objectively speaking, dry feet. A further advantage is that patients can undergo effective therapy, such as rubbing in urea preparations. Flat-electrode hydrometry is currently the most commonly used method of screening skin dryness in the foot and hand regions in Germany.

The method of selective hydrometry was developed from flat-electrode hydrometry. While the principle of flat-electrode hydrometry is conductivity determination, with selective hydrometry the skin resistance is measured. Sweating leads to a reduction in skin resistance, which was described by Richter back in 1946 [76]. Using the apparatus from tip therm GmbH, Düsseldorf, twelve test points on the sole of each foot were tested for local skin resistance and the means evaluated for left and right separately.

The advantage of selective hydrometry over flat-electrode hydrometry is that the moisture content of every small area on the skin of the plantar region of the foot can be indirectly tested. With flat-electrode hydrometry not all regions of the foot are examined; only those areas of skin touching the measuring plate. Furthermore, flat-electrode hydrometry only

represents a conductance of both feet together; analysis of left and right separately is not possible. Using selective hydrometry, by contrast, each skin region and each foot can be examined individually. Measurement is quick and simple.

The ROC curve for selective hydrometry on the right foot gave an AUC of 0.893 as the measure of diagnostic quality of the measuring method. For the left foot the AUC was as much as 0.919. The measuring method of selective hydrometry delivered sensitivity of 84.6% and specificity of 91.2% (results for the right foot). We were able to show that there were no significant differences between the right and left foot in terms of the means of the selectively determined resistances.

Descriptive statistics on selective hydrometry produced more significant differences between all three Groups than flat-electrode hydrometry. The differences between the means of the right foot in healthy individuals (Group C) and in diabetics with neuropathy (Group B) were highly significant ($p<0.001$). There was also a highly significant difference between diabetics without (Group A) and with neuropathy (Group B) namely $p=0.008$.
While flat-electrode hydrometry could not detect any significant difference between the healthy individuals (Group C) and patients without neuropathy (Group A), a significant difference in the means of $p=0.022$ was identified by selective hydrometry.
For almost a third (30.6%) of diabetics without peripheral sensory neuropathy (Group A) an already striking skin dryness in the plantar region of the foot was diagnosed with the method of selective hydrometry. One can conclude from this result that autonomic neuropathy develops before the manifestation of sensory neuropathy. This conclusion finds a possible histomorphological explanation in what Risse [77] reports, who postulated that the earlier nerve damage occurs, the thinner the nerve is. This is especially true of autonomic nerve fibres, which are unmyelinated and thus considerably thinner than the sensory nerve fibres.

The formation and development of the sweat glands happens in the embryonic phase. After birth no new eccrine glands develop [78]. It is known from the literature that sweat gland density varies greatly over the surface of the body [79]. The greatest density of the in total two to five million sweat glands is found on the palms of the hand and soles of the feet, where there are around 400 per mm^2.

Analysis of the individual areas of the foot by means of selective hydrometry revealed large differences. In all three Groups the heel region was the driest point. A peculiarity presented itself in the reduced sweat secretion among the Group of diabetics with peripheral sensory neuropathy (Group B). Here, the drop in sweat secretion was most evident in the toes. We were able to show that dryness in this region is 24 times greater than among healthy individuals. By comparison, there was only a 6.5-times drop in skin moisture in the toes within the Group of diabetics without neuropathy (Group A). Reduction in sweat secretion was greater in the forefoot than in the metatarsus and in turn sweat secretion was greater in the metatarsus than in the heel region; in other words, a drop from distal toward proximal. These findings are in accord with Risse's remarks [58].
Due to glycation-induced changes in axons, myelin sheaths and the smallest vessels, the longer the nerve, the earlier nerve damage occurs, and when it does it begins at its distal end. This is also confirmed by the observation of Said et al. [80], that neuropathy normally starts in the distal nerve segment and appears to spread centripetally.

This gives a new pathophysiological explanation for the phenomenon that foot infections, fissures, mycosis and panaritia arise frequently in diabetics.
This would thus suggest the recommendation that diabetics change their foot care. Moisturising substances should therefore be applied on a daily basis primarily in the toe region. Urea-containing preparations proved to be better than oil-based creams and ointments in previous studies [45]. The case history data of this study showed that no less than a quarter (25%) of the Group of diabetics without neuropathy had an almost-daily foot care routine, almost as many patients with sensory neuropathy (23%) also had such a routine. It was discovered, however, that 46.2% of diabetics with neuropathy did not have any foot care routine whatsoever, compared with 38.9% of diabetics without neuropathy.

This poses the interesting question of how long the externally applied moisture actually remains in the skin. Is it sufficient to rub cream into the feet and toes once daily or should at risk patients engage in appropriate foot care several times a day? This is currently under investigation in our working Group.

The Neuropad indicator plaster is a new clinical diagnostic procedure for determining skin dryness. Unlike Guttmann's method [66], the indicator plaster has a skin-friendly adhesive, and the cobalt salt compound is risk free in its concentration and at the recommended reaction

time (Miro Verbandstoffe GmbH). Furthermore, with the Neuropad indicator plaster there is no soiling of clothing by dyes nor is the method inconvenient, as described by Fealey [67].

In order to be considered a valid clinical procedure the indicator plaster as a measuring method had to be subjected to the quality criteria of a diagnostic test.
The aim was to clarify whether the indicator plaster is actually able to correctly detect a reduction in sweat secretion as the manifestation of autonomic neuropathy. Sensitivity and specificity are properties that characterise the quality of a test in pure populations (consisting of either all-ill or all-healthy patients). To this end, ROC curves were generated from the results of the colour change times of the indicator test. Determination of the cutpoint for left and right separately resulted in high sensitivity (84.6% for the right and 80.8% for the left foot) and specificity (94.1% right and 88.2% left).

By calculating the mean colour change times of both feet a common cutpoint was set, which proved to be optimum at 600 seconds. At this cutpoint sensitivity was 84.6% and specificity was 85.3%. Shifting the cutpoint to longer colour change times led to a fall-off in sensitivity and an increase in specificity, which in turn led to an increase in false negatives. At a cutpoint of 600 seconds the number of false positive (14.7%) and false negative (15.4%) results were almost the same. All in all the quality criteria of a clinical test are thereby met.

Using the indicator plaster skin dryness in diabetics without neuropathy (Group A) could be shown more clearly in 58.3% of cases than with selective hydrometry. Only 41.7% of this Group actually presented no autonomic neuropathy. As with selective hydrometry, the earlier development of autonomic neuropathy compared to peripheral sensory neuropathy was confirmed.
The results of selective hydrometry showed that if the reaction location were changed different colour change times would be found for the indicator plaster. Therefore the determined colour change time (cutpoint 600 seconds) is only applicable to the plantar region of the foot at MTH I / II.

Using descriptive statistics the Groups were analysed with respect to the results of the indicator plaster method. Once again no significant differences between left and right could be identified, as was already observed with selective hydrometry. This confirms the theory that peripheral diabetic polyneuropathy develops symmetrically. It remains unclear, however,

why foot ulcers as a result of diabetic foot syndrome recurrently affect the same foot in many cases. Studies have not found any logical conclusion for this. An additional vascular component in conjunction with infections may possibly need to be considered.

It was important to answer the question as to whether the results of the different test procedures correlated as regards measurement of sudomotor function.
All three measuring methods gave coinciding results for skin moisture measurement, with the Neuropad indicator plaster, based on a chemical reaction, and selective hydrometry, which measures skin resistance, having the highest correlation.

The three populations tested with the indicator plaster method exhibited highly significant differences in the mean colour change times. The means of the healthy individuals (Group C) and the diabetics with neuropathy (Group B) were highly significantly different, at $p<0.001$. The exact same significance was found for selective hydrometry. Flat-electrode hydrometry revealed a significant difference of $p=0.001$ between Groups C and B.
The Group of diabetics without neuropathy (Group A) differed highly significantly from the diabetics with neuropathy (Group B) when the indicator plaster was used, with a value of $p<0.001$ on the right and $p=0.004$ on the left. Selective hydrometry showed a significant difference of $p=0.008$ on the right and $p=0.005$ on the left here, with differentiation by flat-electrode hydrometry being only $p=0.04$.
Compared to selective hydrometry and even more so to flat-electrode hydrometry, the indicator plaster method differentiated to a greater extent between healthy individuals (Group C) and diabetics without neuropathy (Group A) ($p=0.002$). With selective hydrometry the value was $p=0.022$. Flat-electrode hydrometry did not reveal any significant differences between Groups A and C ($p=0.442$).

This means that by using the Neuropad indicator plaster skin dryness could already be identified in 58.3% of the diabetics without neuropathy (Group A); selective hydrometry succeeded in doing so for 30.6% of this Group.

Application of both selective hydrometry and the indicator plaster is simple, fast and safe. Both procedures fulfil the criteria of a clinical test, as confirmed by plotting the ROC curves.

Calculating the Pearson's correlation coefficients gave a result of 0.696 ($p<0.001$) between the Neuropad and selective hydrometry. This confirms our statements on the close correlation and cogency of both methods.
It must be said in conclusion that selective hydrometry is bound to replace flat-electrode hydrometry as a screening method to identify manifest skin dryness. The principle of skin resistance measurement has been proven for decades [76]. Selective hydrometry has the benefits of early detection of affected patients, fast, simple and very practical application, giving high sensitivity and specificity with additional local diagnostics.

Another important objective of this thesis was to find out whether the indicator plaster could help prevent diabetic foot syndrome. Despite considerable endeavours the number of amputations in recent years in Germany is not coming down and the main targets of the St. Vincent declaration [81] have still not been met.
The diabetic foot still does not command enough attention in the minds of the affected. Other authors [77, 82, 83] emphasise that this amounts to "suicide" in the case of peripheral sensory neuropathy. Other sources show that even patients with a loss of peripheral sensibility, but without neuropathic symptoms, are hard to convince that they are at risk of developing diabetic foot syndrome. It is precisely this patient Group that is difficult to motivate to take their health into their own hands and develop a "foot care routine" [84, 85]. Patients attached inadequate to no importance to their feet. This problem of diabetic foot syndrome, thought to be rather unimportant, is all in the head of the person affected; in a way the amputation has already happened mentally.

All the existing test procedures for neuropathy measurement that are used in practice as the gold standard [15] are based on perceptual-physiological test principles and are not standardised [86, 87], not readily reproducible and are highly variable, as the Diabetes Control and Complications Trial [88] showed.
Different nerve fibres can fail in varying degrees at different times in various Groups of people. For this reason there is no standard screening test for diagnosing diabetic peripheral neuropathy [15].
Test methods such as vibration measurement using a tuning fork are flawed by a relatively high rate of error. The only prospective studies that relate a measurement of neurological function to the development of a foot ulcer and consequent amputation, utilise quantitative

sensory procedures such as vibration measurement using the tuning fork and monofilament [17, 89].
Since the Neuropad indicator plaster does not depend on the patient's cooperation, it provides a good alternative to the existing perceptual-physiological test procedures.

With the indicator plaster the patient can see for himself and understand whether the skin moisture is reduced or not. The procedure is thus "comprehensible" in the truest sense of the word. Seeing a colour change, with which a diabetic will be familiar from conventional colorimetric test strips, boosts his faith in this new method. The resulting acceptance can lead to a higher level of compliance, possibly with a change for the better in attitude to the desired foot care, and also function as a check of progress. The patient obtains a result after only ten minutes without having to visit the doctor. Afterwards, depending on the result, he can go to a specialised treatment centre, such as a special diabetological unit with adjoining out-patient podiatry department.
It remains to be seen how acceptance and practicability will develop in the coming years, and this is something which must be proved by further scientific studies which also involve a greater number of cases.

Up-to-date studies show that identifying high-risk patients in time can bring about a reduction in the number of amputations of over 80% [74, 90, 91]. With that, the essential preventive significance of the indicator plaster lies in its application as a screening procedure. Patients and GPs can detect earlier the skin dryness resulting from autonomic neuropathy and take the right precautions or start specialist diabetological treatment. The indicator plaster succeeds in reliably identifying patients at high risk of developing diabetic foot syndrome by means of screening.
For reasons of method this thesis was not based on a division of the degrees of severity of manifest sensory neuropathy. The question arises, however, as to whether the colour change times of the indicator plaster may depend on the degree of sensory neuropathy. In our opinion further studies are warranted, which employ a different classification. The diabetics with verified peripheral sensory neuropathy ought to be divided into varying degrees of severity according to the suggestions of Dyck et al. [29, 92]. The colour change times would then have to be compared for these alternatively composed populations. The hypothesis as to whether the colour change times lengthen in proportion to the degree of sensory neuropathy can then be verified. Subsequent studies could confirm this.

This thesis deals exclusively with the sudomotor function of the foot as a component of diabetic autonomic neuropathy. However, other organ systems are also affected; essential to mention here are diabetic cardiopathy, gastroparesis and erectile dysfunction. Impaired erection especially is all too often concealed out of misunderstood shame. It is vital to discuss whether a correlation exists between impairment of sudomotor function and erectile dysfunction. If this is the case, if a patient has peripheral autonomic neuropathy, he can be spoken to directly about possible impaired erection. Studies must be done on this topic.
Similar considerations arise for autonomic diabetic cardiopathy. If there is a close and temporal correlation between the onset of peripheral autonomic neuropathy and autonomic diabetic cardiopathy, further cardiological diagnostics can be undertaken if impaired sudomotor function has been verified.

Finally, let it be said prospectively that in view of the comments on the straightforward handling and reliable cogency of the indicator plaster, it seems by all means conceivable that health insurers allow their patients avail of this test free of charge and for the GP to be informed. This concept seems to lend itself to countries with Disease Management Programmes, where the existing measures to prevent diabetic foot syndrome are not effective.
Ultimately, even global application as an “early detection plaster” is conceivable, something which must be verified in large-scale studies.

6. Summary

Diabetic neuropathy is the main cause of diabetic foot syndrome (DFS). The autonomic part of this nervous disturbance is accompanied by a change in sudomotor function and a reduction in plantar sweat secretion, and for reasons of method has been up to now a minor concern in DFS. The objective of this study was to find out whether the Neuropad indicator plaster, as a novel diagnostic product, is able to detect changed sweat secretion as the manifestation of a loss of sudomotor function and to identify possible differences between diabetics with and without verified sensory neuropathy. The same applied to the novel test method of selective hydrometry. A further objective was to discover, also by means of selective hydrometry, whether there is a difference in plantar sweat secretion between the forefoot and heel region.

62 diabetics were examined; the control Group consisted of 34 healthy persons. Peripheral sensory neuropathy was verified in 26 diabetics, determined by NDS, 10g monofilament, tuning fork and qualitative and quantitative thermoreception. The colour change times of the indicator plaster in seconds (standardised colour scale from HSK 46 K 55% to HSK 17 K 30%) were determined in the plantar region of both feet at MTH I/II. Parallel to this, selective hydrometry was performed on twelve defined test points on the plantar skin of the foot.

This thesis shows that the difference in the mean change time of the Neuropad indicator plaster as well as the selective measurement of moisture on the plantar skin between the left and right foot within the control Group and diabetics with and without sensory neuropathy was not significant. The mean time taken for colour change was 499 sec. ± 155 for the healthy individuals, 680 sec. ± 290 for the diabetics without neuropathy, and 1058 sec. ± 389 for the diabetics with neuropathy. The differences between the three Groups were significant for both the right and left foot ($p<0.001$). A just as significant difference was observed between the Groups in relation to selective skin moisture measurement. The Group of diabetics with and without sensory NP additionally presented a high correlation between the Neuropad and selective measurement of moisture at MTH II, that is, 0.639 (significant at the 0.01 level); similar results are found in the correlation between Neuropad and forefoot moisture. Finally, there was a highly significant difference in the distribution of the selective measurements both within the three Groups and between the populations ($p<0.001$), in particular between the healthy individuals and the diabetics with NP and between the diabetics with and without NP.

Using a ROC curve a cutpoint of 600 seconds was found for the indicator plaster. Determining sensitivity (84.6%), specificity (85.3%), positive predictive value (81.5%) and negative predictive value (87.9%) verified it as a valid clinical test for diagnosing autonomic neuropathy.
Selective hydrometry also fulfilled the criteria of a clinical test upon generation of a ROC curve and calculation of sensitivity (84.6%) and specificity (94.1%), with a cutpoint of 35 MOhm being established.

Both the indicator plaster and selective hydrometry were able to diagnose the existence of autonomic neuropathy in the diabetics without verified sensory neuropathy. The percentage was, depending on the method, 30.6% (selective hydrometry) to 58.3% (indicator plaster). Therefore loss of autonomic nerve function precedes sensory neuropathy.

It could finally be shown that loss of sweat secretion was far more pronounced in the toe region than in the heel region. This confirms the theory that neuropathy manifests itself distally and then spreads centripetally. The results of this thesis suggest that a care routine must be modified to more frequent moisturising of the toes in particular, and with higher concentrations of urea. This furthermore provides a new explanation for the nail mycosis and felon that are regularly observed in diabetics.

It can be said to sum up that selective hydrometry as an enhancement of flat-electrode hydrometry will replace the latter as a diagnostic product in practice. The Neuropad indicator plaster facilitates the simple, reliable and safe verification of reduced sweat secretion as a manifestation of autonomic neuropathy, meaning that this method is ideal for self-checking and as a rapid screening procedure. The two methods are highly correlated.
Further studies are warranted to confirm the results.

7. Appendices

Appendix 1

Information sheet

Dear Patient,

You have been diagnosed with diabetes (diabetes mellitus).

As is the case with every diabetic, you, too, are at risk of developing so-called "secondary illnesses" of the eyes, kidneys and nerves as a result of the diabetic metabolic condition.
Changes in the nerve cells of a diabetic can, alongside other factors, lead to the development of "diabetic foot". In order to prevent the often tragic amputation of toes or even the entire foot, early examination methods and appropriate treatment is necessary.

One precursor to "diabetic foot syndrome" is dry skin on the feet.
This is why we would like, as part of a scientific study, to investigate whether the nerve cells of your feet have already been damaged and how moist the skin on the soles of your feet is.

To find this out, we test the skin of your feet for sweat secretion and skin moisture. In addition we measure nerve function regarding sensitivity to temperature, vibration and touch. These tests are completely painless and pose no health risk. No side-effects are expected. Besides taking one blood sample, we will ask you about your diabetes and its treatment. The therapy begun by your GP will remain unaffected by all this, so any other accompanying illness (such as high blood pressure) will not change as a result.

You will probably be examined only once by us; in some cases a second, additional examination may be necessary.

If you take part in the study you may benefit from the findings of the examination. It is possible that individual care adapted specially to your feet can prevent serious consequences of diabetes.

Not least, by taking part in the study, you can help improve early detection of diabetic foot syndrome. The results of the study will ultimately be available to all our patients.

On completion of this study the data gathered will be scientifically evaluated. Your personal data will of course remain anonymous. Your name will be changed to a serial number, making identification by outsiders impossible.

Patient no.: _____

Appendix 2

Informed consent

I have been informed of the objective and conditions of examination to test skin moisture of the feet and what this requires of me adequately and in a form I was able to understand.
I was given the opportunity to ask questions and enough time to make my decision about whether or not to participate in the study. In making it, I was not influenced by the doctor attending me or any other persons affiliated with the hospital.

I am participating voluntarily and without payment.

I feel fully informed and am willing to participate in this study to examine skin dryness and foot nerves. I will follow all doctor's instructions necessary to carry out the examination.
I reserve the right, however, to withdraw from the study at any time and without giving reasons. This will not result in any disadvantages for me.

I also consent to the data gathered from my examination being handled confidentially and being stored and evaluated anonymously for scientific purposes and not passed on.

_____ _____

Place, date Place, date

_____ _____

Signature (doctor) Signature (participant)

Patient no.: _____

Appendix 3

Patient record – Case history

Patient number: _____

Surname, first name: _____ Initials: ____/____

Date of birth: ___.___._____ Age: _____ years old

Gender: _____

Height: _____ cm Weight: _____ kg BMI: _____ kg/m^2

Type of diabetes: 1 ◯ 2 ◯ Not diabetic ◯

Duration of diabetes: _____ years

Therapy: Diet: _____ bread units/d ◯ flexible ◯ no diet

Oral antidiabetics:
Glibenclamide_____ mg/d,
Acarbose _____ mg/d,
Metformin _____ mg/d,
Repaglinide _____ mg/d,
Glitazone _____ mg/d,

Insulin therapy:
CT _____ insulin units/d
ICT _____ insulin units/d
CSII _____ insulin units/d

Sequelae:

Retinopathy	Known	No	Unknown
	Laser therapy	Yes	No
Nephropathy	Known	No	Unknown
	Micral test	Negative	Positive
Angiopathy	PAOD	CHD	Unknown
Neuropathy	Known	No	Unknown
	Smarting	Right	Left
	Pain	Right	Left
	Tingling	Right	Left
	Paraesthesia	Right	Left

Therapy:
None
Alpha-lipoic acid
Carbamazepine
Other _____

Patient no.: _____

Foot defects None
Hyperkeratoses: Toes: _____
MTH: _____
Lateral border of foot right left
Heel right left

Foot deformity: _____
Ulcus: _____ Wagner's stage: _____

Allergies: Yes No Unknown

Atopy: Yes No Unknown

Skin type: Dry Oily Combination

UV sensitivity: I (never tan, always erythema)
II (sometimes tan, always erythema)
III (always tan, sometimes erythema)
IV (always tan, no erythema)
V (dark-skinned race)
VI (black)

Foot care: Never Less than 1x/d More than 2x/d
Evening Morning Evening and morning

Footwear: Normal
Trainers
Sandals
Orthopaed. shoes
Others

Medication:

Laboratory: HbA1c _____ %
Creatinine _____ mg/dl
Urea _____ mg/dl
TSH _____ pg/dl
GOT _____ U/l
GPT _____ U/l
Albuminuria _____ g/l

Blood pressure: ____/____ mmHg Heart rate: _____ /min

Appendix 4

Exclusion criteria

	Not present
1. Age < 18 or > 75	○
2. Manifest PAOD (tibiobrachial index > 0.9	○
Mediasclerosis	○
3. Non-diabetic neuropathy	
Renal insufficiency requiring dialysis	○
Uraemia	○
Alcoholism	○
Severe hepatopathy	○
Paraneoplasia	○
4. Drugs	
Corticosteroids	○
Antihistamines	○
Psycho-active drugs	○
5. Peripheral nerve lesion	
Traumatic lesion	○
Plexus paresis	○
Spinal root compression syndrome	○
Herpes zoster	○
Polyradiculopathies	○
Others	○
6. Dermatological illnesses	
Neurodermatitis	○
Psoriasis	○
Raynaud's syndrome	○
Hyperhidrosis	○
Acrocyanosis	○
Allergies	○
Sclerodermatitis	○
Others	○

Appendix 5

Neuropathy Disability Score (NDS)

Patient no.: _____

1.) Achilles tendon reflex right	
Normal	0
Increased	1
Absent	2
2.) Achilles tendon reflex left	
Normal	0
Increased	1
Absent	2
3.) Vibratory perception metatarsophalangeal joint right dorsal	
Values > 6/8 (< 40 y) and >5/8 (> 40 y) are normal	
Present	0
Reduced, absent	1
4.) Vibratory perception metatarsophalangeal joint left dorsal	
Values > 6/8 (< 40 y) and >5/8 (> 40 y) are normal	
Present	0
Reduced, absent	1
5.) Pain sensation big toe right (toothpick)	
Present	0
Absent	1
6.) Pain sensation big toe left (toothpick)	
Present	0
Absent	1
7.) Sensation of temperature instep, Tip-Therm, right	
Present	0
Absent	1
8.) Sensation of temperature instep, Tip-Therm, left	
Present	0
Absent	1

Total: _____

Appendix 6

Total Symptom Score (TSS)

Patient no.:_____

1.) Symptom "smarting"

Absent	0
Slight	1
Moderate	2
Severe	3

2.) Frequency of the symptom "smarting"

Occasional	1
Frequent	2
Constant / almost always	3

3.) Symptom "tingling"

Absent	0
Slight	1
Moderate	2
Severe	3

4.) Frequency of the symptom "tingling"

Occasional	1
Frequent	2
Constant / almost always	3

5.) Symptom "numbness"

Absent	0
Slight	1
Moderate	2
Severe	3

6.) Frequency of the symptom "numbness"

Occasional	1
Frequent	2
Constant / almost always	3

7.) Symptom "pain"

Absent	0
Slight	1
Moderate	2
Severe	3

8.) Frequency of the symptom "pain"

Occasional	1
Frequent	2
Constant / almost always	3

Total: _____

Appendix 7

Neuropathy measurement

Patient no.: _____

Semmes-Weinstein monofilament (10g):

Right:	MTH I	Left:	MTH I
	MTH II		MTH II
	MTH V		MTH V
	Heel		Heel
	Dorsum pedis		Dorsum pedis

Tuning fork (C 128):

Right:	Malleolus med.: _____/8	Left:	Malleolus med.: _____/8
	Dorsum pedis: _____/8		Dorsum pedis: _____/8

Normal: <40 years > 6/8 >40 years 5/8

Tip-Therm:

Right:	Dorsum pedis	Left:	Dorsum pedis
	Lower leg		Lower leg

Quantitative thermoreception:

	Measurement 1(°C)	Measurement 2(°C)	Measurement 3(°C)	Mean(°C)
Right				
Left				

Doppler (mmHg):

Right:	Dorsalis pedis art. _____	Left:	Dorsalis pedis art. _____
	Tibialis post. art. _____		Tibialis post. art. _____
	Radialis art. _____		Radialis art. _____

Normal : Ankle pressure index/radialis artery > 1.0

Appendix 8

Skin moisture measurement

Patient no.: _____ Pat. initials: ___/____

Hydrometer test:

- Hands: _____ µS
- Feet: _____ µS

Neuropad plaster:

- Right foot: Big toe: _____ min
- Left foot: Big toe: _____ min

Selective Thio-Test:

Right foot:
1 _____
2 _____
3 _____
4 _____
5 _____
6 _____
7 _____
8 _____
9 _____
10 _____
11 _____
12 _____

Left foot:
1 _____
2 _____
3 _____
4 _____
5 _____
6 _____
7 _____
8 _____
9 _____
10 _____
11 _____
12 _____

Appendix 9

	Group A		**Group B**		**Group C**		**Total**	
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Number n	36	37.5	26	27.1	34	35.4	96	100
Female	17	47.2	10	38.5	24	70.6	51	53.1
Male	19	52.8	16	61.5	10	29.4	45	46.9
18-45 years old	7	19.4	5	19.2	14	41.2	26	27.1
46-75 years old	29	80.6	21	80.8	20	58.8	70	72.9
Mean age	54.3		58.9		47.6		53.6	
SD age	± 11.5		± 11.0		± 11.7			
Mean diabetes duration (years)	11.61		16.08		/	/		
SD diab. duration	± 8.9		± 8.8		/	/		
Type 1 diabetes	13	36.1	2	7.7	/	/	15	24.2
Type 2 diabetes	23	63.9	24	92.3	/	/	47	75.8
Mean HbA1c value (%)	8.1		8.7		5.2			
SD HbA1c	± 1.4		± 1.8		± 0.4			
No foot care	14	38.9	12	46.2	14	41.2	40	41.7
Weekly foot care	13	36.1	8	30.8	12	35.3	33	34.3
Daily foot care	9	25.0	6	23.0	8	23.5	23	24.0

Appendix 10

		N	Mean	Standard deviation
Toes right	Diabetics w.o. NP	35	29.0805	54.17816
	Diabetics with NP	26	110.3723	129.65480
	Healthy	33	4.5048	3.45859
	Total	94	42.9378	86.39363
Toes left	Diabetics w.o. NP	36	29.9109	60.39943
	Diabetics with NP	26	120.7088	126.08449
	Healthy	33	4.6465	3.71769
	Total	95	45.9848	88.51176
Forefoot right	Diabetics w.o. NP	36	44.4841	86.08615
	Diabetics with NP	26	104.2905	132.96760
	Healthy	33	11.9784	8.68320
	Total	95	49.5607	93.92269
Forefoot left	Diabetics w.o. NP	36	51.1649	81.48657
	Diabetics with NP	26	122.0146	129.78021
	Healthy	33	19.2476	26.15959
	Total	95	59.4683	94.13393
Metatars. right	Diabetics w.o. NP	36	57.2254	97.80339
	Diabetics with NP	26	160.3121	144.93880
	Healthy	33	20.4333	24.15562
	Total	95	72.6582	111.89798
Metatars. left	Diabetics w.o. NP	36	62.8218	101.94895
	Diabetics with NP	26	141.3460	135.89892
	Healthy	33	18.6856	16.87230
	Total	95	68.9811	105.97046
Heel right	Diabetics w.o. NP	36	59.8582	101.39203
	Diabetics with NP	26	169.8183	139.76017
	Healthy	33	27.9368	41.33835
	Total	95	78.8641	113.76066
Heel left	Diabetics w.o. NP	36	49.9156	95.89753
	Diabetics with NP	26	147.3671	141.88354
	Healthy	33	28.3279	45.65670
	Total	95	69.0876	109.11770

Results of mean, standard deviation for skin resistances measured using selective hydrometry in the relevant areas of the foot for all three Groups

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9. Curriculum vitae

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Marital status:		Single
Nationality:		German
Religion:		Roman Catholic

Education:	School:	1982-1991	Johannes-Kepler School Ibbenbüren
		08.06.1991	Abitur (A-levels) with an average grade of "1.9"
	Studies:	Human Medicine from 1992 to 1998 at the Johannes-Gutenberg University of Mainz:	
		24.03.1994	Intermediate examination in medicine
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		26.08.1997	2nd part of the state examination in medicine
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Interns:	1994	Internal Medicine, v.-Bodelschwingh-Krankenhaus Ibbenbüren
	1996	General Surgery, St.-Elisabeth-Hospital, Ibbenbüren
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Practical year:	1997 to 1998 at Idar-Oberstein Municipal Hospital, Academic Teaching Hospital of the Johannes-Gutenberg University of Mainz, elective: neurology
Practical activities:	1991 Work placement in nursing service 1992 Voluntary social year, Red Cross, district of Unna
Professional activities:	11/1998 to 05/2000 houseman in the Department of Internal Medicine at St. Bonifatius Hospitals Lingen headed by Head Physician Prof. Dr. med. R. Zick since 05/2000 medical assistant in advanced training in the Department of Internal Medicine of St. Bonifatius Hospital Lingen since 07/2003 emergency physician Voluntary medical support of Lingen Optifast programme as well as supervising the cardiac sports group in Lingen "Eisenbahn" sports club
Scientific work:	03/1996 to 01/1997 Experimental molecular genetics laboratory work using PCR technology within the framework of a dissertation, funded by the German Research Foundation, SFB "Immunopathogenesis" 09/1996 to 02/1997 Molecular genetics study of predisposing, genetic factors in a population of SLE patients, Mainz Institute for Legal Medicine

Publications: Co-author of the following publications:

12/1997: "No primary association between LMP2 polymorphisms and extraspinal manifestations in spondyloarthropathies" *Annals of the rheumatic diseases*

"TNF-alpha promoter alleles TNF308.2 and TNF238.2 protect against the development of ankylosing spondylitis in HLA-B27 positive individuals" *Arthritis and Rheumatism*

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Reproducibility of the new indicator test for sudomotor function (neuropad®) in patients with Type 2 diabetes mellitus

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Background and aims:
Sudomotor neuropathy contributes to the pathogenesis of diabetic foot ulcers. The aim of the present study was to examine the reproducibility of the new indicator test for sudomotor function (Neuropad®) in type 2 diabetic patients.

Patients and methods:
This study included 142 type 2 diabetic patients (70 men, 72 women) with a mean age of 67.3±7.6 years and a mean diabetes duration of 14.2±6.3 years. Sudomotor function was assessed by means of colour change in the indicator test, applied to both soles at the level of the 1st-2nd metatarsal heads. Each patient was examined twice, with an interval of two months.

Results:
In the right foot, a highly significant (r=0.998, p=0.001) correlation was observed between time until complete colour change of the test on the first (15.33±7.63 minutes) and second examination (15.34±7.59 minutes). Difference in time until complete colour change of the test between the two examinations was 0.007±0.43 minutes. In the left foot, a highly significant (r=0.998, p=0.001) correlation was observed between time until complete colour change of the test on the first (15.33±7.59 minutes) and second examination (15.32±7.60 minutes). Difference in time until complete colour change of the test between the two examinations was 0.007±0.45 minutes. In the right foot, reproducibility of the test was excellent both in patients with sudomotor neuropathy (longer than 10 minutes time until complete colour change, r=0.990, p=0.001) and in those without sudomotor neuropathy (time until complete colour change not exceeding 10 minutes, r=0.996, p=0.001). Similar results were obtained on the left foot. Patients diagnosed as having sudomotor dysfunction on the first examination were 100% identical with those diagnosed on the second examination.

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 neuropathy.

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

Article

Abstract

The aim of this study was to examine the reproducibility of the new indicator test for sudomotor function (Neuropad®) in type 2 diabetic patients. The study included 142 type 2 diabetic patients (70 men) with a mean age of 67.3 ± 7.6 years and a mean diabetes duration of 14.2 ± 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 ± 431.6 seconds) and second examination (935.8 ± 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 ± 430.3 seconds) and second examination (940.5 ± 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 Upender, 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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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 ± 7.6 years and a mean diabetes duration of 14.2 ± 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 °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: CV % = SD/ $\bar{x}$ × 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_{12} depletion, malignancy), thyroid disease, drugs (corticosteroids, antihistaminic and psychoactive drugs, which may affect sweating), peripheral nerve lesions (traumatic lesions, plexus paresis, spinal root compression, herpes zoster, polyradiculopathy), skin diseases (neurodermatitis, psoriasis, scleroderma, allergy to metals, Raynaud syndrome, hyperhidrosia, acrocyanosis).

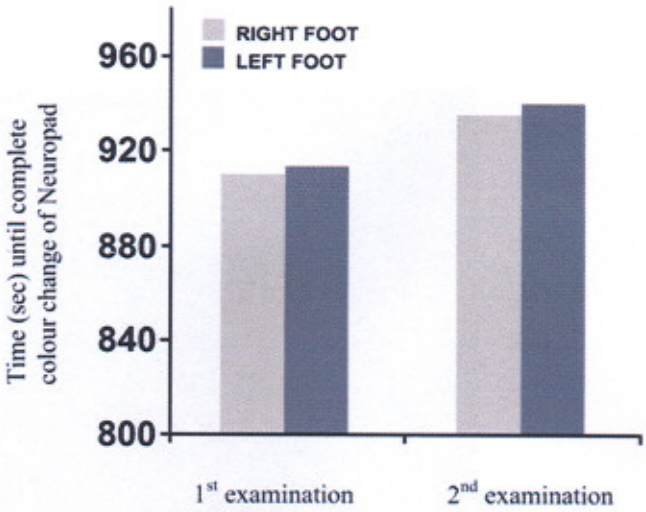

Fig. 1 Time until complete colour change of the test in the right foot (1st examination: 910.7 ± 431.6 sec, 2nd examination: 935.8 ± 440.1 sec, r = 0.91, p = 0.001) and in the left foot (1st examination: 911.6 ± 430.3 sec, 2nd examination: 940.5 ± 441.2 sec, r = 0.89, p = 0.001). On each examination,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

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 ± Standard Deviation ($\bar{x}$ ± 1SD). 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 ± 431.6 seconds) and second examination (935.8 ± 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 ± 430.3 seconds) and second examination (940.5 ± 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	intra-observer		inter-observer
	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 becomes 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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9th Annual Greek Diabetological Congress, Rhodes, Greece
17.-20. March 2005

Evaluation of new indicator test neuropad® for the diagnosis of peripheral neuropathy (PN) and for the diagnosis of autonomic neuropathy (AN) in diabetic patients (SUMMARY)

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Introduction

The indicator test neuropad reckoning the moisture of the surface that it's in contact. Neuropad's ingredient is Cobaltium chloride solution that changes its color from blue to pink. The aim of the present study was the evaluation of neuropad's sensitivity and specificity for the diagnosis of peripheral neuropathy and autonomic neuropathy in diabetic patients.

Patients and Method

75 patients with Diabetes mellitus were selected with accidental choice at diabetological clinic of Laiko General Hospital. Mean know duration of diabetes was at least 5 years.
The indicator test neuropad was applied on the foot sole, in the area corresponding to the head of the first metatarsal bone. The diagnosis of Peripheral neuropathy was established with Neuropathy Symptom Score, Neuropathy Disability Score and Vibrameter. The diagnosis of Autonomic neuropathy (Cardiac Autonomic Neuropathy) was established with classical Ewing test.

Results

The incidence of Peripheral neuropathy with classical methods was 42,7%.
The neuropad's sensitivity for the diagnosis of Peripheral neuropathy was 90,6% and neuropad's specificity was 69,7%.
The incidence of Autonomic neuropathy with Ewing test was 36,0%.
The neuropad's sensitivity for the diagnosis of Autonomic neuropathy was 66,7% and neuropad's specificity was 44,4%.
The neuropad's sensitivity in combination for Peripheral neuropathy and Autonomic neuropathy was 100% (17/17 patients).

Conclusion

The indicator test neuropad have high sensitivity and satisfactory specificity as diagnostic tool of Peripheral neuropathy in diabetic patients. The above in combination with the easy way of use and easy assessment make's neuropad a satisfactory screening test for Peripheral neuropathy in Diabetic patients.

9th Annual Greek Diabetological Congress, Rhodes, Greece, March 2005

Evaluation of Clinical Examination and Neuropad for the Assessment of Peripheral Nervous Dysfunction in Patients with Diabetes Mellitus type II

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Background and aims

Peripheral neuropathy is of crucial importance in the pathogenesis of foot ulcers in patients with diabetes mellitus.
The nerve dysfunctions appertains small nervous fiber dysfunction (sensation of pain, light touch, cold) and large nervous fiber dysfunction (sensation of vibration).
The aim of the present study was to evaluate a) The usual clinical examination for the assessment small and large nervous fiber function. b) The indicator test neuropad (as diagnostic tool for peripheral autonomic nervous fiber dysfunction – small fiber) to identify peripheral diabetic neuropathy in patients with diabetes mellitus.

Materials and methods

This study included 103 type 2 patients with diabetes mellitus (49 men) with a mean age 65,37±9,9 and a mean diabetes duration of 15,63±9,4 years. Small and large fiber dysfunction were assessed in both legs and scored using a modified scoring system of this proposed by P. J. Dyck – Neuropathy Disability Score NDS –NDS1 0-15, NDS2- 0-10.

a) For the diagnosis of small fiber dysfunction (e.g. reduced pain, touch and cold sensation) the NDS1 was used as the sum of these scored sensory deficits.
b) For the diagnosis of large fiber dysfunction NDS2 the score of reduced vibration sensation
c) Neuropad applied in both soles of patients. Stability or partial change of the color evaluated as small fiber dysfunction.

The NDS1, ND2, Neuropad evaluated as individual parameter for the diagnosis of DN. For the statistical analysis the chi – square test and the multiple regression stepwise model were used. We assessment the sensitivity of each parameter for the diagnosis of DN.

Results

a) Mildly small fiber dysfunction NDS1≥2 have significant correlation with DN. (P<0.001)
b) The large fiber dysfunction have significant correlation with DN (P<0,001).
c) Neuropad results have also significant correlation with DN (P<0,05)
d) The sensitivity of neuropad was 74%
e) The sensitivity of sensation of vibration was 60%.

Conclusions

The assessment of small fiber dysfunction (clinical examination, neuropad) identify biggest part of diabetic patients with D.N than the clinical examination with the sensation of vibration.

41st Annual Meeting of the EASD 2005, Athens, Greece

Usefullness of the indicator plaster neuropad for the diagnosis of peripheral and autonomic neuropathy in patients with diabetes mellitus

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Background and Aims

The indicator plaster neuropad (IPN) is based on the color change of a cobalt II compound (placed on a commercially available sticker) from blue to pink, after exposure to dermal foot perspiration. Lack of perspiration (which results in non-change of the neuropad sticker color), is considered as a sign of peripheral neuropathy (PN) which in turn is a major risk factor for the development of diabetic foot syndrome. Perspiration is autonomic nervous system dependent. Autonomic neuropathy (AN) is relatively common in diabetic patients and lack of perspiration is often one of its clinical features. Aim of this study was to evaluate the sensitivity and specificity of IPN for the detection of sensory PN and cardiac AN, in patients with diabetes mellitus (DM).

Materials and Methods

The study population consisted of 116 patients (64 men and 52 women, mean age 61.6 years) with DM (9 with type 1 and 107 with type 2 diabetes) of at least 5 years duration, randomly recruited from the diabetologic outpatient clinic of our hospital. IPN was placed at the plantar surface of the first metatarsal of both feet. Patients were examined for PN by using a the neuropathy symptoms score (NSS), the neuropathy disability score (NDS) and the vibration sensitivity threshold. Cardiac AN was examined by using the classical battery of the Ewing tests.

Results

PN was documented in 50 out of 116 patients (43.1%). The sensitivity of IPN in diagnosing PN was found 86% (43/50 patients) while its specificity was 68.2% (45/66 patients). Positive predictive value was 67.2% (43/64 patients) and negative predictive value was 86.5% (45/52 patients). Cardiac AN was documented in 43 out of 112 patients (38.4%). The sensitivity of IPN in diagnosing cardiac AN was found 58.1% (25/43 patients) and its specificity was 44.9% (31/69 patients). The sensitivity of IPN in detecting those patients with combined PN and cardiac AN was 80.7% (21/26 patients).

Conclusion

IPN has a high sensitivity and a rather low specificity for the detection of PN in patients with diabetes mellitus, while both sensitivity and specificity concerning the detection of cardiac AN by this system are low. This finding along with the simplicity of the technique

41. Annual Meeting of the EASD 2005, Athens, Greece

Assessment of diabetic autonomic neuropathy in type 2 diabetic patients using neuropad: A new indicator plaster for detection of disturbed sweat secretion

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Background and Aims

The presence of autonomic dysfunction in diabetic patients predicts a poor prognosis. Clinical tests are limited to heart rate variability and blood pressure measurement. Moreover, it is difficult and needs long time to carry out. Autonomic sudomotor neuropathy is associated with reduction of plantar sweating. Early diagnosis of the sudomotor component of autonomic neuropathy may be helpful to detect diabetic autonomic dysfunction. Therefore, the aim of this study was to evaluate whether the new indicator plaster (neuropad®) was suitable screening test for diabetic autonomic neuropathy.

Materials and Methods

This study included 185 type 2 diabetic patients (78 men and 107 women) with a mean age of 57.4 ± 10.2 years. The average duration of the diabetes was 8.5 ± 5.0 years (median 5 years). The control group comprised 19 healthy young volunteers (< 30 yrs old). We carried out four autonomic function tests (E/I ratio, Valsalva, 30:15 ratio, Orthostatic-BP) as conventional standard tests. Indicator plasters were applied to both soles of patients. Autonomic neuropathy was assessed by means of color change in the indicator plasters (normal response: full color change within 10 minutes). And then we compared the results of both tests.

Results

Autonomic neuropathy was diagnosed in 163 patients (79.9 %) with conventional tests and 137 patients (67.2%) were positive with indicator plaster. Color change of the plaster in the right sole was associated with color change in the left sole ($p=0.0001$). We calculated kappa value to estimate the agreement between neuropad® and conventional tests for DAN. The weighted kappa value was 0.38. The sensitivity of the indicator plaster for diagnosis of autonomic neuropathy was 76.7% and specificity was 70.7%. In the logistic regression analysis, the following parameters; duration of diabetes, sex, HbA1c, serum total cholesterol and blood pressure were not significant factors for neuropad® results, whereas the age of patients could influence.

Conclusion

These results suggest that the indicator plaster is suitable of use in the screening test for diabetic autonomic neuropathy.

DISAUTONOMIA NELLA NEUROPATIA CRIOGLOBULINEMICA: STUDIO PRELIMINARE

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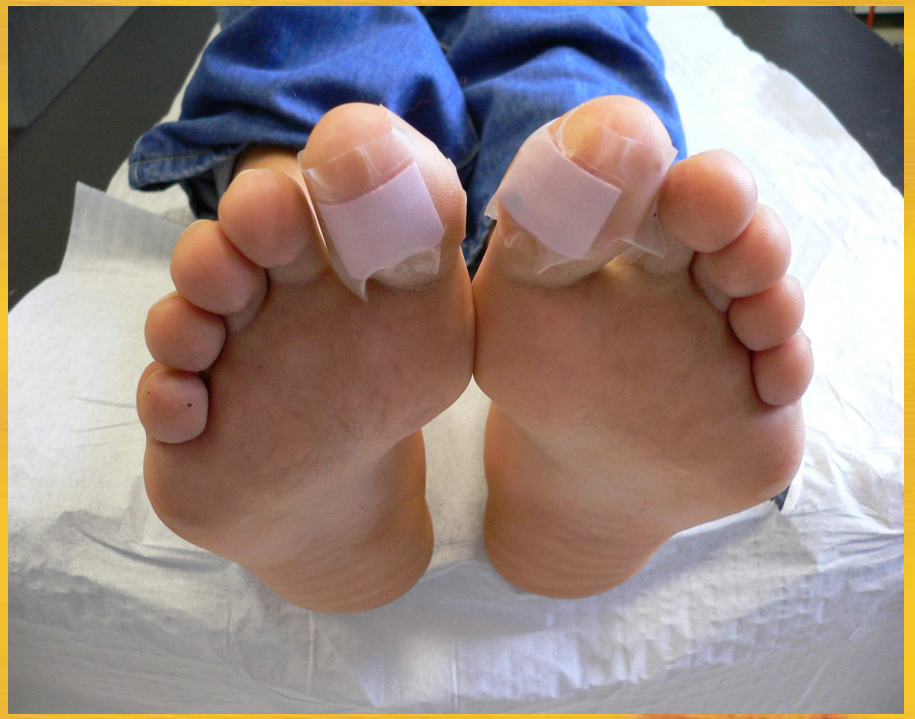
INTRODUZIONE La neuropatia periferica associata alla sindrome crioglobulinemica interessa circa il 60% dei pazienti. Si manifesta prevalentemente con un coinvolgimento periferico e ha componente mista sensitivo-motoria con prevalenza del deficit di sensibilità. Ad essa si associano parestesie talvolta dolorose, disestesia, allodinia e "restless leg syndrome" con conseguenze invalidanti. La sua eziologia non è certa. La presentazione clinica e strumentale è sovrapponibile a quanto si osserva nella neuropatia diabetica, dove è evidente una compromissione del sistema nervoso autonomo. Per questo abbiamo cercato la presenza di disautonomia in soggetti crioglobulinemici.
MATERIALI E METODI Sono stati arruolati 10 pazienti consecutivi affetti da sindrome crioglobulinemica (in accordo ai criteri del GISC) e per confronto 10 soggetti sani di pari sesso ed età, che non presentassero ipertensione, vasculopatia, cardiopatia ischemica, diabete, aritmie, ipotensione ortostatica. Ogni soggetto veniva sottoposto a due test: Neuropad® e Anscore. Neuropad® consiste nell'applicazione sulla pianta dei piedi, in regione metatarsale, per dieci minuti di un cerotto reattivo contenente soluzione cloridrica di cobalto divalente, che vira di colore in presenza di sudorazione. L'Anscore Test consiste nel valutare le variazioni di PA e FC (misurata come distanze R-R all'ECG) dopo manovra di Valsalva e al passaggio da posizione supina ad eretta.
RISULTATI Al test con Neuropad®, su 10 pazienti esaminati 9 mostravano positività per disautonomia (3 in modo completo). L'Anscore Test (effettuato su 8 casi, per l'impossibilità di due pazienti a compiere la manovra di ortostatismo rapido) ha evidenziato disautonomia solo negli stessi soggetti positivi al Neuropad®. I livelli di neuropatia emersi dai due test (score arbitrario per Neuropad® e Anscore) risultano correlati al test di Pearson con rho di 0.738, con una p di 0.0366 (significatività < 0.05). Nessuno dei controlli normali ha dato risultati positivi per disautonomia. Non sono state riscontrate correlazioni con le principali variabili di laboratorio e cliniche, se non tra lo score arbitrario per la neuropatia e l'Anscore (rho 0.915, $p < 0.002$).
DISCUSSIONE Il test Anscore utilizzato è ritenuto il gold standard per la neuropatia; nella nostra casistica si riscontra una buona correlazione col Neuropad® nella valutazione della neuropatia diabetica. In base ai nostri dati possiamo affermare che la neuropatia disautonomica è presente nel quadro clinico della sindrome crioglobulinemica. I nostri dati preliminari possono suggerire una correlazione tra presenza di quest'ultima e compromissione cutanea grave.

C.F. (03/04/81),
DM 1, DD 2 anni, fumo si
HbA1c 7,9

- MNSI: 1/15
- I.W. dx 1,08 - sx 1,08
- VPT
  - dx alluce 9, malleolo 13
  - sx alluce 11, malleolo 12
- Riflessi achillei e rotulei evocabili
- Monofilamento, sensibilità normali
- Forza normale
- Piede con deformità.

Classificazione rischio assente

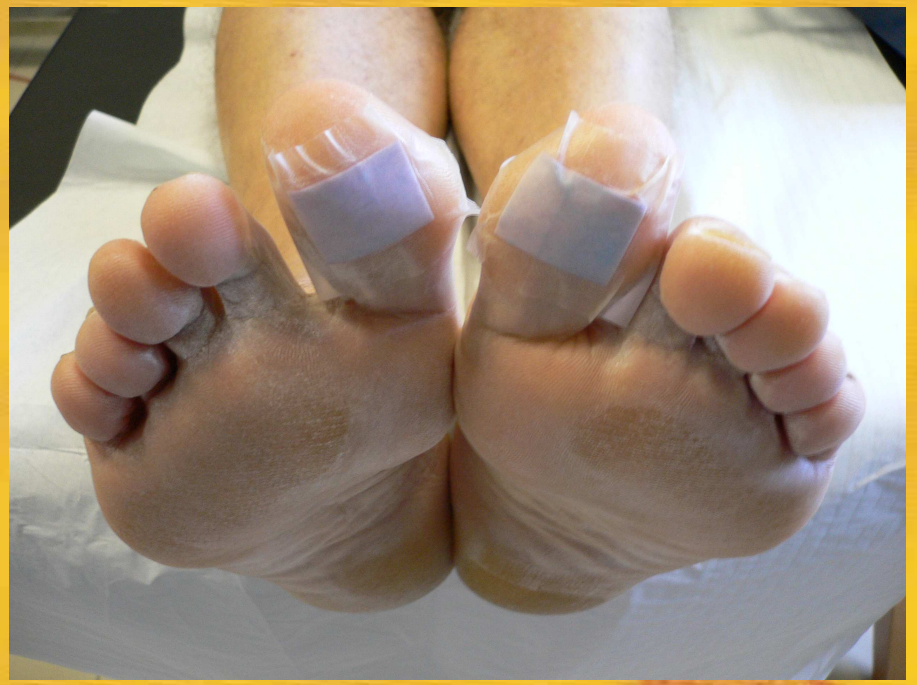



Neuropad® di colore rosa/blu
Piede a rischio

M.F. (13/05/40),
DM 2, DD 3 anni, fumo no
HbA1c 6.5

- MNSI: 0/15
- I.W. dx 1,24 - sx 1,12
- VPT
  - dx alluce 22, malleolo 24
  - sx alluce 12, malleolo 24
- Riflessi achillei e rotulei evocabili
- Monofilamento, sensibilità normali
- Forza normale
- Piede con valgismo dell'alluce bilaterale, secco.

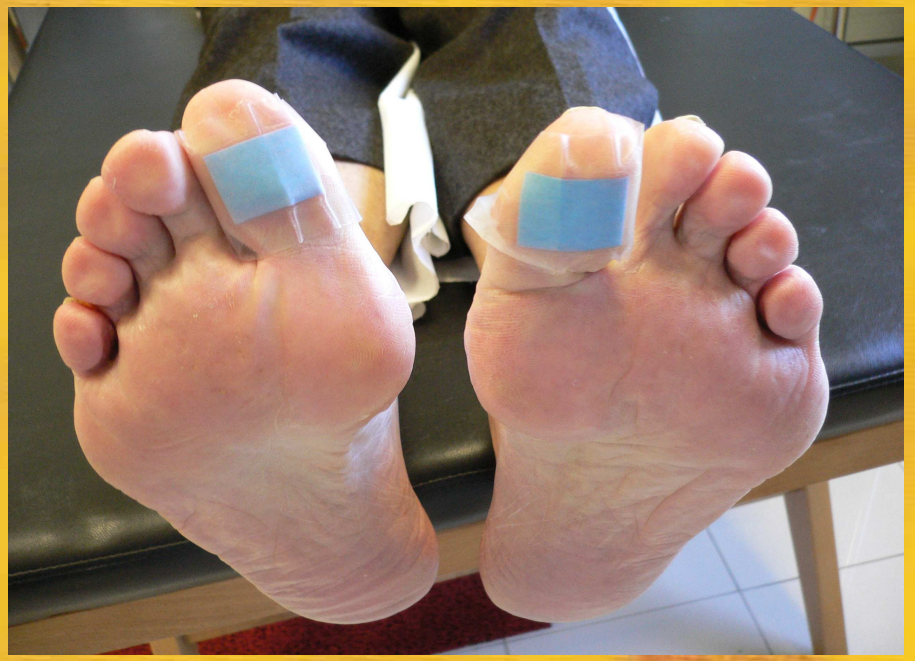
Classificazione rischio medio.




Neuropad® di colore blu
Piede neuropatico

M.V. (28/06/35),
DM2, DD 13 anni, fumo ex
HbA1c 9.4

- MNSI: 0/15
- I.W. dx 1,00 - sx 1,00
- VPT
  - dx alluce 33, malleolo 25
  - sx alluce 25, malleolo 31
- Riflessi achillei e rotulei non evocabili.
- Monofilamento, sensibilità normali
- Forza normale
- Piede cavo e valgismo dell'alluce.



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The New Indicator Test (Neuropad®): A Valuable Diagnostic Tool for Small-Fiber Impairment in Patients With Type 2 Diabetes

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The New Indicator Test (Neuropad®)

A Valuable Diagnostic Tool for Small-Fiber Impairment in Patients With Type 2 Diabetes

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Purpose

The purpose of this study was to evaluate the new indicator test for sudomotor function (Neuropad®) in the diagnosis of small-fiber impairment in patients with type 2 diabetes.

Methods

This study included 123 patients with type 2 diabetes (59 men; mean age, 64.3 ± 8.6 years; mean diabetes duration, 12 ± 6.1 years). Sudomotor dysfunction was assessed by means of the new indicator test. Neuropathy was diagnosed by the Neuropathy Disability Score and small-fiber impairment by temperature perception (Tiptherm device) and pain perception (Neurotip).

Results

The frequency of sudomotor dysfunction was significantly ($P = .001$) higher in patients with neuropathy (95%) than in those without neuropathy (30.2%). Sensitivity of the indicator test for neuropathy was 95%, and specificity was 69.8%. Frequency of neuropathy was significantly ($P = .018$) higher with the indicator test (74.8%) than with conventional clinical examination (65.4%). Sudomotor dysfunction was significantly ($P = .001$) more frequent in patients with small-fiber impairment (99%) than in those without small-fiber impairment (21.7%). Sensitivity for small-fiber impairment was 99%, and specificity was 78.3%. There was no difference ($P = .999$) in the frequency of small-fiber

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impairment as diagnosed with the indicator test (80.5%) and with clinical examination (81.3%).

Conclusions

The indicator test has a very high sensitivity and specificity for small-fiber impairment in patients with type 2 diabetes.

Neuropathy is one of the main chronic diabetes-related complications leading to increased morbidity and susceptibility to foot ulceration.[1-6] Diabetic neuropathy may affect the large myelinated nerve fibers, the small (myelinated and unmyelinated) nerve fibers, or both.[7] To date, scientific interest has mainly focused on large-fiber damage in diabetes, while small-fiber impairment has received less attention.[7,8] Indeed, the most popular part of clinical examination (ankle reflexes and tuning fork) and nerve conduction study may easily assess large-fiber function.[3-5] Conversely, the diagnosis of small-fiber function requires more sophisticated tests.[7-10]

The distinction between types of nerve fibers is based on fiber size and presence or absence of myelin sheath.[7,9,10] All large fibers (with a diameter of 6-12 μm) have a myelin sheath and mediate ankle reflexes, touch, pressure, vibration, and proprioception. Small fibers are either myelinated (A δ fibers with a diameter of 1-5 μm) or unmyelinated (C fibers with a diameter of 0.2-1.5 μm).[7,9,10] Small fibers mediate sensation of temperature and pain as well as the spectrum of autonomic functions.[7,9,10] Small-fiber neuropathy (or small-fiber impairment) is a subtype of neuropathy, characterized by impairment of small-fiber function and sparing or minimal involvement of large fibers.[7,9,10]

In diabetes, tests of small-fiber impairment rely on examination of somatic and autonomic functions subserved by these fibers.[7-11] Somatic functions include pain sensation and temperature sensation.[7,10] Pain sensation is examined by means of a pinprick that stimulates C fibers. Qualitative evaluation of temperature sensation is performed by using hot and cold tubes to examine sensation of hot (C) and cold (A δ) fibers, respectively.[7] Quantitative evaluation of temperature sensation relies on the measurement of thermal perception threshold.[7,10] This is assessed by application of an automatically heated or cooled probe on the patients' skin.[7,10] Computer-assisted, operator-independent systems have been developed, enabling the administration of repeatable thermal stimuli and recording of patients' response.[12] Autonomic functions mainly include cardiac autonomic testing and sweat tests.[7,9,10] Cardiac autonomic testing is conducted by recording heart rate variability and changes in blood pressure in response to simple and well-standardized maneuvers, such as deep breathing or standing up from the supine position.[7,10,13] Normal sweat production, known as sudomotor function, may be evaluated by a number of established tests, notably the quantitative sudomotor axon reflex test, the sweat imprint, the thermoregulatory test, and the sympathetic skin response.[7,10,13-15] Regrettably, these tests are not widely applicable because they require expensive equipment and trained personnel.[13-15] A minimally invasive skin biopsy assessing intraepidermal skin nerve fibers is a more modern technique that enables the evaluation of small-fiber function.[7,16]

More recently, a new indicator test for sudomotor function (Neuropad®; miro Verbandstoffe GmbH, Wiehl-Drabenderhöhe, Germany) has been introduced.[17,18] This is an easy-to-use patch that assesses plantar sweat production by means of a color change from blue to pink.[17,18] The indicator test contains the complex salt anhydrous cobalt-II-chloride. In the presence of water, this salt absorbs water molecules, changing its color from blue to pink, the time required for complete color change being negatively related to humidity.[19] The new test has been reported to yield results that show good correlation with severity of peripheral neuropathy.[18] Furthermore, the new test has been reported to have excellent reproducibility.[20]

To date, there is a limited number of studies of the new indicator test focused on the contribution of the indicator test to the diagnosis of large- rather than small-fiber impairment.[17,18,21] However, sweat tests traditionally belong to the modalities evaluating small fibers.[7,9,10] Thus, the aim of the present study was to investigate whether the new indicator test enables the diagnosis of small-fiber impairment in patients with type 2 diabetes.

Methods

This study included 123 patients with type 2 diabetes (59 men, 64 women; mean age, 64.3 ± 8.6 years; mean diabetes

duration, 12 ± 6.1 years). Subjects were recruited from the Second Department of Internal Medicine at 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 informed consent.

Diabetic neuropathy was diagnosed by the Neuropathy Disability Score (NDS).[22] This is a standardized examination of ankle reflexes, as well as 128-Hz tuning fork, pinprick, and temperature sensation at the hallux, as described earlier.[22] Patients with an NDS score ≥6 were considered to have neuropathy.[22,23]

Small-fiber function was assessed by means of temperature and pain perception. Temperature perception was assessed with the Tiptherm device.[24,25] This is a pen-like device comprising a plastic cylinder on one end and a metal cylinder on the other end, with a diameter of 14 mm each. The Tiptherm device was applied 3 times on the dorsum of each foot. An abnormal test result was defined as at least 2 incorrect responses out of 3 readings on the dorsum of each foot.[24,25] Pain sensation (pinprick) was assessed with a calibrated Neurotip (Owen Mumford, Oxford, UK) attached to a Neuropen (Owen Mumford) device.[23] In a random order, the sharp or blunt edge of the Neurotip was pressed against the plantar aspect of the hallux until the guiding markers of the Neuropen were aligned. Patients were asked to distinguish between the painful and painless stimuli. An abnormal test result was defined as at least 2 incorrect responses out of 3 readings on the hallux of each foot.[23] Small-fiber impairment was defined as abnormal temperature and pain sensation.

Peripheral arterial occlusive disease was evaluated by means of the Ankle-Brachial Index (ABI) measurement with a Doppler apparatus. Peripheral arterial disease was diagnosed in patients with ABI <0.9.[26]

Exclusion criteria were as follows: aged <17 years or >75 years, peripheral arterial occlusive disease, other potential causes of neuropathy (end-stage renal failure, alcohol abuse, malignancy), drugs (corticosteroids, antihistaminic and psychoactive drugs, which may impede sweating), peripheral nerve lesions (traumatic lesions, plexus paresis, spinal root compression, herpes zoster, polyradiculopathy), thyroid disease, and skin diseases (neurodermatitis, psoriasis, scleroderma, allergy to metals, Raynaud syndrome, hyperhidrosia, acrocyanosis).

Sudomotor dysfunction was assessed by means of the newly introduced indicator test (Neuropad®).[17,18] All measurements were performed in constant room temperature and humidity, with a 10-minute period allowed for patient acclimatization after having taken off shoes and socks. The indicator test was applied between the first and the second metatarsal head on the plantar surface of both feet, a common site of neuropathic ulcers. Time until complete color change from blue to pink was recorded. Sudomotor dysfunction was defined as the time until complete color change exceeding 10 minutes in at least 1 foot.[17,18]

Statistical analysis was performed using the Statistical Package for Social Sciences SPSS version 11.0. Significance was assessed by χ^2 test (with Yates correction for 2×2 contingency tables) and by Fisher exact test where appropriate for qualitative variables. Significance was defined at the 5% level ($P < .05$). Sensitivity was defined as the ratio of true positives/(true positives and false negatives). Specificity was defined as the ratio of true negatives/(true negatives and false positives). Positive prognostic value was defined as the ratio of true positives/(true positives and false positives). Negative prognostic value was defined as the ratio of true negatives/(true negatives and false negatives).

Results and Clinical Implications

Neuropathy was diagnosed in 80 patients (65.4%). Sudomotor dysfunction was diagnosed in 76 patients (95%) with neuropathy and in 16 patients (30.2%) without neuropathy, with a significant difference at $P = .001$ (Table 1). Sensitivity for neuropathy was 95%, and specificity was 69.8%. Positive prognostic value was 82.6%, and negative prognostic value was 90.2%. Sensitivity was only 69.8% because sudomotor dysfunction was also diagnosed in a substantial part (30.2%) of patients without neuropathy. Presumably, this may be ascribed to the early development of sudomotor dysfunction in diabetes.[27] Indeed, there is evidence to suggest that sudomotor dysfunction may even be detected in patients with normal clinical findings and nerve conduction study.[28,29] Sudomotor dysfunction has been shown to be mediated by small-fiber injury.[15,30] In this context, it is of interest that pathological studies have also been able to show that small-fiber injury may occur early in diabetic patients with normal clinical or electrophysiological findings[31] or even earlier in patients with impaired glucose tolerance.[32,33]

Frequency of neuropathy was significantly ($P = .018$) higher with the indicator test (92 patients, 74.8%) than

Table 1

Sudomotor Dysfunction in Patients With Diabetes According to Clinical Status (Presence or Absence of Neuropathy and Small-Fiber Impairment)

Sudomotor Dysfunction According to Neuropathy Status					
	With Neuropathy		Without Neuropathy		
Patients	n	%	n	%	Statistical Evaluation*
With sudomotor dysfunction	76	95	16	30.2	$P = .001$
Without sudomotor dysfunction	4	5	37	69.8	
Total (n = 123)	80		53		
Sudomotor Dysfunction According to Status of Small-Fiber Impairment					
	With Small-Fiber Impairment		Without Small-Fiber Impairment		
Patients	n	%	n	%	Statistical Evaluation†
With sudomotor dysfunction	99	99	5	21.7	$P = .001$
Without sudomotor dysfunction	1	1	18	78.3	
Total (n = 123)	100		23		

*P value refers to the difference between patients with neuropathy and those without neuropathy.
†P value refers to the difference between patients with and those without small-fiber impairment.

with conventional clinical examination (80 patients, 65.4%). A higher prevalence of neuropathy as diagnosed with Neuropad® has been reported previously, but this difference did not attain statistical significance.[17,18] The implication of the findings is that the indicator test might prove to be more sensitive in the detection of patients at risk for diabetic foot ulceration.

Small-fiber dysfunction was diagnosed in 100 patients (81.3%). Sudomotor dysfunction was diagnosed in 99 patients with small-fiber impairment (99%) and in 5 patients without small-fiber impairment (21.7%), with a significant difference at $P = .001$ (Table 1). Sensitivity was 99%, and specificity was 78.3%. Positive prognostic value was 95.2%, and negative prognostic value was 94.7%. There was no difference ($P = .999$) in frequency of small-fiber impairment as diagnosed with the indicator test (99 patients, 80.5%) and with clinical examination (100 patients, 81.3%). Obviously, sensitivity and specificity, as well as positive and negative predictive values for small-fiber impairment were excellent, higher than for neuropathy diagnosed by clinical examination. Essentially, there was no difference in the diagnosis of small-fiber impairment with the indicator test and with clinical examination. The close correlation between sudomotor dysfunction and small-fiber impairment is not surprising given that impaired sweat production is due to small-fiber dysfunction.[15,30] From a practical point of view, it should be emphasized that this ability of Neuropad® to diagnose small-fiber impairment may permit timely detection of neuropathy and so prevent underdiagnosis of this serious complication.[25,31,33]

Interestingly, the reduced color change of Neuropad® was an impressive finding for the patients themselves. Patients who took part in the study showed a keen interest in the diagnosis of neuropathy, in self-examination, and in the use of appropriate footwear. Accordingly, an additional advantage of the indicator test was its ability to promote patient education, which has been recognized as an important aspect in overall foot care.[4,6]

The strengths of the indicator test are as follows. The new test has a high sensitivity for the diagnosis of neuropathy. More important, it has excellent sensitivity and specificity for the diagnosis of small-fiber impairment and hence appears to enable early diagnosis of neuropathy. Moreover,

the test is based on an unequivocal color change, which does not require patient cooperation. It also lends itself to self-examination and promotes patient education. Finally, it is an easily applicable diagnostic tool, which may be used as a screening test by all health care providers (including general practitioners, podiatrists, and diabetes nurses) in primary health care. The weakness of the indicator test is that there are, so far, no prospective studies investigating its utility as a potential marker of the risk for foot ulceration. Such studies are eagerly awaited. It also remains to be determined if there is an association between results obtained with Neuropad® and the severity of small-fiber impairment (assessed, for instance, by quantifiable thermal perception threshold), given that such an association of Neuropad® has been shown for severity of peripheral neuropathy.[18]

In conclusion, the indicator test is very sensitive for neuropathy, and, more important, it has a very high sensitivity and specificity for small-fiber impairment in particular. Interestingly, it enables detection of sudomotor impairment in a considerable part of patients without clinical evidence of neuropathy. In view of these encouraging results and of its easy applicability as a simple, noninvasive diagnostic tool, it appears that the indicator test may prove useful as a screening test of early nerve fiber injury in the diabetic population.

Implications for Diabetes Educators

This study has shown that the new indicator test (Neuropad®) has an excellent sensitivity and specificity as a screening test for small-fiber impairment, thus facilitating early diagnosis of neuropathy in patients with type 2 diabetes. The indicator test may easily be used by all health care providers (including general practitioners, podiatrists, and diabetes nurses) in primary health care. More important, it may be used by the diabetes educator to illustrate the impaired nerve function to the patient as well as to encourage self-examination, and it has been found to promote patient education about foot care.

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SENSITIVITY AND SPECIFICITY OF THE NEW INDICATOR TEST (NEUROPAD) FOR THE DIAGNOSIS OF PERIPHERAL NEUROPATHY IN TYPE 2 DIABETIC PATIENTS: A COMPARISON WITH CLINICAL EXAMINATION AND NERVE CONDUCTION STUDY

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Abstract

Objective: To evaluate the sensitivity and specificity of the new indicator test (Neuropad®) for the diagnosis of peripheral neuropathy in type 2 diabetic patients as compared with clinical examination and nerve conduction study.

Patients and methods: This study included 120 type 2 diabetic patients (58 men) with a mean age of 67.3±5.9 years and a mean diabetes duration of 13.1±3.2 years. Diabetic neuropathy was diagnosed by the Neuropathy Disability Score (NDS). Nerve conduction study (NCS) was performed on radial, ulnar, sural and common and deep peroneal nerves. Patients were also examined with the new indicator test. Time until complete color change of the test from blue to pink was recorded. The test was considered abnormal in patients who exhibited a time until complete color change of the test exceeding 600 seconds in at least one foot.

Results: Neuropathy was diagnosed by clinical examination in 83 patients (69.2%). Sensitivity of the indicator test for clinical neuropathy was 95.2% and specificity was 67.6%. Sensitivity of NCS for clinical neuropathy was 94% and specificity was 62.1%. Sensitivity of the indicator test for abnormal NCS was 97.8% and specificity was 96.4%.

Conclusions: The new indicator test has a very high sensitivity not only for clinical, but also for neurophysiological diagnosis of neuropathy. Specificity is moderately high for clinical, while it is particularly high for neurophysiological diagnosis of neuropathy. The indicator test has comparable with NCS validity for the diagnosis of diabetic neuropathy. Finally, time until complete color change of the test is associated with severity of nerve conduction impairment.

Key words: Diabetes mellitus, Diabetic peripheral neuropathy, Nerve conduction study.

Introduction

Foot ulceration and amputation belong to the most common chronic complications of diabetes mellitus, with a considerable adverse impact on morbidity (Boulton et al, 2005; Jeffcoate, 2005). Neuropathy is of crucial importance in the pathogenesis of foot ulcers (Boulton, 2004; Reiber et al, 1999). In everyday practice, clinical examination is the mainstay of the diagnosis of neuropathy (Boulton, 2004; Boulton et al, 2005b; Valk et al, 1992). Nerve conduction study (NCS) significantly contributes to the diagnosis of neuropathy, enabling early diagnosis of nerve injury (Krarup, 2003; Olaleye et al, 2001; Rota et al, 2005). Nonetheless, it is not generally available and cannot, therefore, be widely used as a screening test (Boulton, 2004; Boulton et al, 2005b).

More recently, a new indicator test (Neuropad®) that measures sweat production has been proposed as a new test of neuropathy (Manes et al, 2004; Marinou et al, 2005; Papanas et al, 2005; Zick et al, 2003). Interestingly, the indicator test enables diagnosis of neuropathy in a substantial part of patients with normal clinical examination (Papanas et al, 2005). Previous work from our group has also shown an association between the indicator test and clinical severity of neuropathy (Papanas et al, 2005). More importantly, an excellent reproducibility of the test has been reported (Papanas et al, 2005b).

However, no study has so far examined the validity of the indicator test as compared with NCS. Therefore, the aim of the present study was to evaluate the sensitivity and specificity of the new indicator test (Neuropad®) for the diagnosis of peripheral neuropathy in type 2 diabetic patients in as compared with clinical examination and nerve conduction study.

Patients and methods

This study included 120 patients (58 men, 62 women) with type 2 diabetes mellitus. Mean age was 67.3±5.9 years and mean diabetes duration was 13.1±3.2 years. Patients were recruited from the outpatient department of Obesity, Diabetes and Metabolism of the Second Department of Internal Medicine at Democritus University of Thrace, Greece and from the Diabetic Department of the General Hospital of Alexandroupolis, Greece. Recruitment was consecutive and performed in a tertiary care setting. The control group comprised 30 healthy volunteers (15 men, mean age 63.8±4.6 years). The study was approved by the institutional ethics committee and all patients gave their informed consent.

Exclusion criteria were peripheral arterial occlusive disease, as well as chronic alcohol abuse, thyroid disease, Vitamin B_{12} depletion, lumbar spine disorders or any other cause of peripheral neuropathy.

Diabetic neuropathy was diagnosed by the Neuropathy Disability Score (NDS) (Young et al, 1993). This is a standardized examination of ankle reflexes as well as 128 Hz tuning fork, pin-prick and temperature (cold tuning fork) sensation at the hallux, as described earlier (Young et al, 1993). Sensory modalities (tuning fork, pin-prick and temperature sensation) were scored as follows: present= 0 and reduced/absent= 1 for each side (Young et al, 1993). Reflexes were scored as follows: normal= 0, present with reinforcement= 1, absent= 2 for each side (Young et al, 1993). Clinical neuropathy was defined as an NDS ≥ 6 (Paisley et al, 2002; Young et al, 1993).

Examination with the new indicator test (Neuropad®) was performed as follows (Papanas et al, 2005; Zick et al, 2003). Patients were allowed to rest in

constant room temperature (25°C) for 10 minutes after they had taken off their socks and shoes. Indicator tests were applied to a free from callus area on the plantar surface of the feet at the level of the 1^{st}-2^{nd} metatarsal heads bilaterally. Time until complete color change of the test from blue to pink was recorded (Papanas et al, 2005). Complete color change of the test in both feet within 600 seconds was considered normal response. The test was considered abnormal in patients who exhibited a time until complete color change of the test exceeding 600 seconds in at least one foot (Papanas et al, 2005; Zick et al, 2003).

Nerve conduction study (NCS) comprising conduction velocities, latencies and action potential amplitudes was carried out with a Nihon Kohden Neuropack Four Mini using temperature control and fixed distances for motor conduction. Motor conduction of the radial, ulnar and common and deep peroneal nerves, as well as sensory conduction of the radial, ulnar and sural nerves were recorded at non-dominant limbs. Motor conduction was studied at the radial nerve by recording at extensor digitorum communis and stimulation a) 6 cm centrally, b) between brachioradialis and tendon of biceps, c) between coracobrachialis and medial edge of the triceps. Motor conduction was studied at the ulnar nerve by recording at abductor digiti minimi and stimulation a) 8cm centrally, at wrist b) below and c) above elbow. Motor conduction was assessed at the common and deep peroneal nerve by recording at extensor digitorum brevis and stimulation a) 7cm centrally b) below and c) above the head of fibula. Motor conduction in the aforementioned nerves was studied both centrally and distally, in order to exclude entrapment neuropathies. After exclusion of these conditions, distal motor nerve conduction was used for the assessment of diabetic neuropathy (Olaleye et al, 2001). Sensory conduction was studied at the radial nerve by antidromic stimulation at the lateral edge of the radius in the distal

forearm and recording at the back of the hand, between the first and second metacarpals. Sensory conduction was studied at the ulnar nerve by orthodromic stimulation at the fifth digit and recording at the wrist. Sensory conduction was studied at the sural nerve by antidromic stimulation along the posterior surface of the distal leg and recording behind the lateral malleolus.

All conduction velocities and action potential amplitudes were scored as 0 for normal and 1 for abnormal. The normal range used was the mean reference values ±2 Standard Deviations, measurements outside these values being classified as abnormal. The aforementioned normal reference values were obtained by examination of age-matched subjects from the population of the same area. The sum of the abnormal scores was used to define the total NCS score (range: 0-14). Neuropathy was defined as a total NCS score ≥ 3 (Olaleye et al, 2001). Nerve conduction impairment was considered moderate in patients with an NCS score of 3-5 and severe in those with an NCS score ≥ 6. Patients with nerve conduction impairment in whom the number of abnormal sensory nerve attributes was higher than the number of abnormal motor nerve attributes were considered to have primarily sensory nerve conduction impairment. Conversely, those in whom the number of abnormal motor nerve attributes was higher than the number of abnormal sensory nerve attributes were considered to have primarily motor nerve conduction impairment. The concurrence of both abnormal clinical examination and NCS impairment was defined as confirmed clinical neuropathy (The Diabetes Control and Complications Trial Research Group, 1995). Each diagnostic test (clinical examination, examination with Neuropad, NCS) was conducted by an operator blinded to the results of the other tests.

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) 11.0. Significance of qualitative variables was assessed by chi-square

test (with Yates' correction for 2x2 contingency tables). Normally distributed quantitative variables were analysed by ANOVA and unpaired t-test. Data were expressed as mean ± 1 Standard Deviation ($\bar{x} \pm 1SD$). Significance was defined at a level of 5% ($p<0.05$).

Results

Neuropathy was diagnosed by clinical examination in 83 patients (40 men and 43 women; 69.2%). Abnormal Neuropad examination was observed in 79 patients (95.2%) with clinical neuropathy and 12 patients (32.4%) without clinical neuropathy (Table 1). Sensitivity of the indicator test for neuropathy was 95.2% and specificity was 67.6%. Positive prognostic value was 86.8% and negative prognostic value was 86.2%.

Abnormal NCS was observed in 78 patients (94%) with clinical neuropathy and 14 patients (37.8%) without clinical neuropathy (Table 1). Sensitivity of NCS for clinical neuropathy was 94% and specificity was 62.1%. Positive and negative prognostic values were 84.8% and 82.1% respectively.

Neuropad examination was abnormal in 90 patients (97.8%) with abnormal NCS and in one patient (1.1%) with normal NCS (p=0.001). Sensitivity of the indicator test for abnormal NCS was 97.8% and specificity was 96.4%. Positive prognostic value was 98.9% and negative prognostic value was 93.1%.

Among patients with abnormal NCS (n= 92), nerve conduction impairment was primarily sensory in 62 patients and primarily motor in 30 patients. Neuropad examination was abnormal in 61 patients (98.4%) with the former and 29 patients (96.7%) with the latter. There was no difference (p= NS) in sudomotor impairment assessed by Neuropad between these two conditions.

Confirmed clinical neuropathy was diagnosed in 78 patients (37 men and 41 women; 65%). Abnormal Neuropad examination was observed in 78 patients (100%) with confirmed clinical neuropathy and 13 patients (31%) without confirmed clinical neuropathy (Table 1). Sensitivity of Neuropad for confirmed clinical neuropathy was

100% and specificity was 69%. Positive prognostic value was 85.7% and negative prognostic value was 100%.

Time until color change of the indicator test in patients according to their neuropathy status (with and without clinical neuropathy, with and without confirmed clinical neuropathy), in patients according to NCS findings (normal or abnormal NCS), as well as in healthy controls is shown in Table 2. Differences were significant between the groups, as summarized in the same table.

In patients with clinical neuropathy, Neuropad examination was abnormal in 38/40 men and 41/43 women. Sensitivity was 95% in men and 95.3% in women. Specificity was 66.7% in men and 68.4% in women. Positive prognostic value was 86.4% in men and 87.2% in women. Negative prognostic value was 85.7% in men and 86.7% in women. In patients with confirmed clinical neuropathy, Neuropad examination was abnormal in 37/37 men and 41/41 women. Sensitivity was 100% both in men and in women. Specificity was in 71.4% in men and 66.7% in women. Positive prognostic value was 86% in men and 85.4% in women. Negative prognostic value was 100% both in men and in women.

Among patients with abnormal NCS, a further significant difference ($p=0.01$) was demonstrated in time until complete color change in relation to the severity of nerve conduction impairment. Indeed, this time was significantly higher in patients with severe (892±179 seconds) than in those with moderate nerve conduction impairment (1983±386 seconds).

Discussion

The present study showed that the new indicator test has a very high sensitivity (95.2%) for the diagnosis of neuropathy, while its specificity is less high (67.6%). These results are in agreement with previous studies (Marinou et al, 2005; Papanas et al, 2005; Zick et al, 2003). It has been proposed that specificity of the indicator test cannot be higher, since the test permits the diagnosis of neuropathy in a considerable part of patients with normal clinical findings (Papanas et al, 2005). This was also the case in the present study, neuropathy being diagnosed by the indicator test in 32.4% of patients without clinical evidence of neuropathy. The ability of the test to diagnose neuropathy even in patients with normal clinical findings has been attributed to the fact that the test assesses sudomotor function (Papanas et al, 2005). Indeed, there is evidence to suggest that sudomotor dysfunction may develop early in diabetes and thus be detected even in patients with normal clinical examination (Braune and Horter, 1996; Caccia et al, 1991; Hoeldtke et al, 2001; Kennedy and Navarro, 1989, Shimada et al, 2001). Given that sudomotor dysfunction has been shown to be mediated by small-fiber injury (Abdel-Rahman et al, 1992; Low, 2004), this argument is reinforced by the recent pathological studies which have been able to show that small-fiber injury may occur early in diabetic patients with normal clinical examination (Malik et al, 2005), or even earlier in patients with impaired glucose tolerance (Sumner et al, 2003).

NCS also enabled the diagnosis of neuropathy in 37.8% of patients without clinical signs. This is not unexpected, since NCS permits early diagnosis of subclinical neuropathy (Krarup, 2003; Olaleye et al, 2001; Rota et al, 2005). Consequently, NCS had a specificity of 62.1% in the diagnosis of clinical neuropathy,

similar to the indicator test. As anticipated, sensitivity of NCS for clinical neuropathy was very high, in keeping with the findings of Valk and associates (Valk et al, 1992). Interestingly, sensitivity, specificity and prognostic values of NCS were comparable with those of Neuropad.

Abnormal Neuropad examination was significantly more frequent in patients with nerve conduction impairment than in those with normal neurophysiological examination. More importantly, it was demonstrated that both sensitivity and specificity of the indicator test for abnormal NCS were particularly high (97.8% and 96.4% respectively). Although the indicator test evaluates sudomotor function (Manes et al, 2004; Papanas et al, 2005; Zick et al, 2003) and NCS is a measure of large fiber function (Krarup, 2003; Olaleye et al, 2001), the indicator test managed to identify all but two patients with abnormal NCS score. Arguably, this may be explained by the fact that diabetic neuropathy involves both small- and large fibers (Duby et al, 2004; Sima, 2003). Of note, sensitivity and specificity of Neuropad for NCS were higher than those for clinical neuropathy. This may be ascribed to the fact that both Neuropad and NCS are more objective than clinical examination, which requires patient cooperation. From a practical point of view, the indicator test had comparable with NCS validity for the diagnosis of diabetic neuropathy. It is, therefore, plausible that an inquiry into the utility of the indicator test in the detection of subclinical neuropathy is warranted. Additionally, it would be alluring to investigate whether the indicator test enables assessment of the risk for developing a foot complication, as has been shown for NCS (Carrington et al, 2002).

As might be expected, the majority of patients (67.4%) with abnormal NCS had primarily sensory nerve impairment (Pastore et al, 1999; Rota et al, 2005; Valk et al, 1992). There was no difference in abnormal Neuropad examination between

primarily sensory and motor nerve impairment. This may be attributable to the very high frequency of abnormal Neuropad examination in patients with impaired NCS. Indeed, abnormal Neuropad examination was very frequent both in patients with sensory (98.4%) and in those with motor nerve involvement (96.7%). In practice, it appears that the indicator test is not helpful in differentiating between primarily sensory and motor nerve involvement.

Furthermore, the combination of clinical examination and NCS was used to provide a more robust diagnosis of neuropathy, in accordance with the San Antonio consensus statement that diagnosis of neuropathy should incorporate various diagnostic tests (American Diabetes Association and American Academy of Neurology, 1988). The concurrence of abnormal clinical examination and NCS impairment was defined as confirmed clinical neuropathy, a term borrowed from the DCCT (The Diabetes Control and Complications Trial Research Group, 1995). Neuropad showed a particularly high sensitivity (100%) for confirmed clinical neuropathy, while its specificity was similar to that for clinical neuropathy. These findings confirm the validity of the indicator test in the diagnosis of neuropathy.

Analysis according to gender showed that sensitivity and specificity of the indicator test for clinical neuropathy were similar in men and women. This was also the case for confirmed clinical neuropathy. Consequently, no difference was identified between men and women in the diagnostic validity of the indicator test. This new finding suggests that the indicator test is independent of potential minor skin differences between males and females, and enhances its utility as a diagnostic modality.

Time until complete color change of the test was significantly higher in patients with clinical neuropathy than in those without clinical neuropathy. This result

is in accord with previous findings (Papanas et al, 2005; Zick et al, 2003). The same difference was also observed between patients with and without confirmed clinical neuropathy. Moreover, it was found that time until complete color change of the test was significantly higher in patients with abnormal than in those with normal NCS. Hence, prolonged time until complete color change of Neuropad is associated not only with clinical, but also with neurophysiological diagnosis of neuropathy.

We have previously reported a significant association between time until complete color change of the test and severity of clinical neuropathy (Papanas et al, 2005). The present investigation extended this association to the severity of nerve conduction impairment. Time until color change was significantly longer in patients with severe as compared to those with moderate nerve conduction impairment. Accordingly, until complete color change was an index of the severity of nerve conduction impairment.

The implications of our findings for clinical practice are as follows. The indicator test may be used as a highly sensitive tool for the diagnosis of both clinical and neurophysiological neuropathy. It should be noted that the indicator test has comparable with NCS validity for the diagnosis of diabetic neuropathy. However, NCS is not universally available, in contrast to the indicator test, which is a widely applicable, reproducible and easy to use diagnostic tool (Papanas et al, 2005; Papanas et al, 2005b). These findings imply a potential role for the indicator test in increasing the sensitivity of the diagnosis of neuropathy in the vulnerable diabetic population. In this respect, the sensitivity and high reproducibility of the test satisfy the recommendations for a diagnostic procedure formulated as early as in the San Antonio consensus statement (American Diabetes Association and American Academy of Neurology, 1988). However, there is no evidence that the indicator test

may replace the validated NCS, and further research is warranted before the encouraging results of the present study are applied to the general diabetic population.

In conclusion, the new indicator test has a very high sensitivity not only for clinical, but also for neurophysiological diagnosis of neuropathy. Specificity is moderately high for clinical, while it is particularly high for neurophysiological diagnosis of neuropathy. Moreover, the indicator test has comparable with NCS validity for the diagnosis of diabetic neuropathy. Finally, time until complete color change of the test is associated with severity of nerve conduction impairment. These results provide further evidence for the clinical utility of the indicator test in the timely diagnosis of neuropathy. Therefore, the new test may prove to be of value in the detection of patients at high risk for foot complications.

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CLINICAL NEUROPATHY STATUS			
PATIENTS	***With clinical neuropathy (n=83)***	***Without clinical neuropathy (n=37)***	***Statistical evaluation****
Abnormal NEUROPAD	79 (95.2%)	12 (32.4%)	p=0.001
Normal NEUROPAD	4 (4.8%)	25 (67.6%)	
Abnormal NCS	78 (94%)	14 (37.8%)	p=0.001
Normal NCS	5 (6%)	23 (62.2%)	
CONFIRMED CLINICAL NEUROPATHY STATUS			
PATIENTS	***With confirmed clinical neuropathy (n= 78)***	***Without confirmed clinical neuropathy (n=42)***	***Statistical evaluation****
Abnormal NEUROPAD	78 (100%)	13 (31%)	p=0.001
Normal NEUROPAD	0 (0%)	29 (69%)	

*****Patients with neuropathy vs. patients without neuropathy

Table 1. Examination with Neuropad and NCS in diabetic patients with or without neuropathy.

TIME TO COLOR CHANGE IN RELATION TO CLINICAL NEUROPATHY STATUS*				
Time (mean±SD, sec)	**Patients with clinical neuropathy**	**Patients without clinical neuropathy**	**Healthy controls**	**Statistical evaluation**
	1450±320	462±70	242±36	Patients with vs. without clinical neuropathy: p= 0.002 Patients with clinical neuropathy vs. controls: p= 0.001 Patients without clinical neuropathy vs. controls: p= 0.01
TIME TO COLOR CHANGE IN RELATION TO CONFIRMED CLINICAL NEUROPATHY STATUS**				
Time (mean±SD, sec)	**Patients with confirmed clinical neuropathy**	**Patients without confirmed clinical neuropathy**	**Healthy controls**	Patients with vs. without confirmed clinical neuropathy: p= 0.001 Patients with confirmed clinical neuropathy vs. controls: p= 0.001 Patients without confirmed clinical neuropathy vs. controls: p= 0.01
	1570±380	481±80	242±36	
TIME TO COLOR CHANGE IN RELATION TO NERVE CONDUCTION STUDY (NCS)***				
Time (mean±SD, sec)	**Patients with normal NCS**	**Patients with abnormal NCS**	**Healthy controls**	**Statistical evaluation**
	1830±328	490±85	242±36	Patients with normal vs. abnormal NCS: p= 0.001 Patients with abnormal NCS vs. controls: p= 0.001 Patients with normal NCS vs. controls: p= 0.02

*Significant difference (p= 0.001) between the three groups.

**Significant difference (p= 0.001) between the three groups.

***Significant difference (p= 0.002) between the three groups.

LEGEND FOR TABLE 2

Table 2. Time to color change of the indicator test in relation to neuropathy status and NCS, as well as in healthy controls.

O19

Neuropad: Validation of a new indicator plaster as a screening tool in identifying patients at risk of foot ulceration – a multicenter study

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Sensory loss is considered as one of the most important factors predisposing to foot ulceration (FU). Small fiber neuropathy contributing to sensory loss and anhydrosis as a consequence of sweet glands denervation can facilitate the process of ulceration. Since a new indicator (Neuropad) changes its color if moisture is present, this study was undertaken to validate this plaster as a simple screening tool to identify high risk patients for FU in a large population.
Patients – Methods: 506 diabetic patients (type 2) were included (47,5% males) mean age 64,9±12,1 and mean known duration of diabetes (yrs) 10,4±8,4. All the patients underwent a detailed clinical examination (testing ankle and knee reflexes, sensory signs for pain, thermal, light touch and vibration perception). In all the patients this indicator (Neuropad) was applied in the planter surfaces and partial change and/or the stability of its color (Neuropad positive) were recorded.
Results: a) 190 (37,5%) patients were recorded as Neuropad positive (Group A) and the remain were classified as Group B-neuropad negative). b) Bivariate analysis: Group A compared to group B has longer duration of diabetes and were older (12,11±8,41 vs 10,44±7,7 $p<0,05$ and 64,9±10,47 vs 62,42±10,73 $p<0,05$ respectively). More severe neuropathy was detected in Group A (Neuropathy disability score-NDS 5,8±4,18 vs 1,92±1,32 $p<0,05$) c) Multivariate analysis: Neuropathy score for small fiber dysfunction (NDS_1), overall nerve dysfunction (small and large fiber – NDS were the most powerful variables - $p<0,05$ - logistic regression stepwise model) for the neuropad positive results. d) Overall predictive value of the new indicator to identify neuropathic patients was 79,3%.
Conclusion: The present multicenter study clearly showed that the new indicator could be useful as a screening tool to detect patients at risk of foot ulceration.

O20

The New Indicator Test (NEUROPAD) in the Assessment of the Staged Severity of Diabetic Neuropathy

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Background and aims: The new indicator test for sudomotor function (Neuropad®) has been shown to be a highly sensitive and reproducible tool for the diagnosis of diabetic peripheral neuropathy. The aim of this study was to examine the utility of the indicator test in the assessment of the staged severity of diabetic neuropathy**. Patients and methods:** This study included 120 type 2 diabetic patients (58 men) with a mean age of 67.3±5.9 years and a mean diabetes duration of 13.1±3.2 years. Neuropathy was diagnosed and staged by clinical examination and nerve conduction study, according to the Michigan classification system (Feldman et al, 1994). Patients were also examined with the indicator test, applied on the plantar aspect of the feet. Time until complete colour change of the test was recorded and stratified into deciles according to the spread of measurements in the study population. **Results:** Neuropathy was staged as class 0 in 37 patients, class 1 in 44 patients, class 2 in 28 patients and class 3 in 11 patients. Time until complete colour change was 436.5±62.9, 740±88.1, 1192.5±161 and 1817.3±127.4 seconds in patients staged as class 0, 1, 2 and 3 respectively (p=0.001). Use of a threshold lower than 530 seconds until complete colour change had 97.3% sensitivity and 100% specificity for diagnosis of class 0. Use of a threshold lower than 1000 seconds until complete colour change had 100% sensitivity and 97.4% specificity for class 1 neuropathy. A threshold lower than 1440 seconds had 92.9% sensitivity and 100% specificity for class 2 neuropathy. A threshold above 1440 seconds had 100% sensitivity and 99% specificity for class 3 neuropathy. A highly significant (Kendall's tau-b= 0.848, p=0.001) correlation was shown between time until complete colour change of the test and Michigan class of neuropathy. **Conclusions:** It appears that the indicator test contributes substantially to the assessment of the staged severity of neuropathy. There is excellent agreement between the indicator test and the Michigan classification system. These results suggest a role for the indicator test in the assessment of diabetic neuropathy.

Validation of a new diabetic autonomic neuropathy bedside test (plaster) versus the cardiovascular reflex tests.

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Purpose: Autonomic neuropathy is a serious and fatal complication of Diabetes Mellitus (DM). Perspiration is a feature of Diabetic Autonomic Neuropathy **(DAN)** and the Indicator Plaster **(IP)** could provide an easy and accurate way to diagnose DAN especially for general practitioners. The aim of the present study was to evaluate indicator's specificity, sensitivity and accuracy in detecting DAN.

Patients-Methods: We studied 174 patients (79 with type 1 DM, 88 women), with mean age 49.8 $\pm$ 16.1 years and mean duration of DM. 17.3 $\pm$ 7.7 (range 4-45 years). We performed the following four Cardiovascular Reflex Tests **(CRT)**: R-R variation during deep breathing [assessed by Expiration/Inspiration ratio (E/I), Mean Circular Resultant (MCR, vector analysis) and Standard Deviation (SD)], Valsalva maneuver, 30:15 ratio and postural hypotension in all patients. The presence of DAN was established if 2 or more 4 CRT were abnormal. One test abnormal considered as early DAN According to the change of color of the sticker IP or not patients divided in two groups: Group A (n=82, change in color from pink to blue, normal perspiration), and Group B (n=92, no change, abnormal perspiration).

Results: According to the number of abnormal tests, established DAN detected in 81 patients. E/I index was abnormal in 118 patients and postural hypotension in 111.

Measured Parameter	Sensitivity	Specificity	Accuracy
Established DAN	79%	89%	84%
E/I index	94%	61%	85%
Postural Hypot	75%	95%	85%

Conclusions: Indicator has a high sensitivity, specificity and accuracy in detecting established DAN. Neuropathy of parasympathetic origin was observed with IP with high sensitivity but low specificity. Neuropathy of sympathetic origin was detected with IP with high specificity and sensitivity. Accuracy of the IP test was high in all measurements.

The Neuropad: a highly sensitive test to evaluate small and large fibre neuropathy in diabetic patients.

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OBJECTIVE

Distal symmetric neuropathy is characterised by loss of sensation and autonomic denervation of the sweat glands in the feet. The Neuropad is a simple test to semi-quantify sudomotor function and predicts risk of foot ulceration. The present studies aimed to validate it against more sophisticated and established measures of somatic and autonomic neuropathy.

METHODS

45 diabetic patients aged 58.9±10.0 years underwent detailed assessment of somatic and autonomic neuropathy and a 3 mm punch skin biopsy to evaluate intraepidermal nerve fibre (IENF) pathology.

RESULTS

Neuropad responses were normal in 24%, partial in 40% and absent in 36%. Presence of neuropathy (NDS<3) was strongly associated with a pathological Neuropad response (P=0.0006) and correlated with VPT (r=0.399, p=0.009). The sensitivity of Neuropad in detecting clinical neuropathy (NDS>3) was 86% and specificity was 60%. Neuropad results correlated with the cooling detection threshold (r=0.534, p<0.001), heat as pain minimal threshold (HPVAS0.5)(r=0.31, p<0.05), heat-as-pain tolerance 5.0-0.5(r=0.425, p=0.011) and autonomic function (deep breathing) (r=-0.557, p<0.001) producing a sensitivity of 100% and specificity of 41% for detecting small fibre damage. Comparing diabetic patients with normal and pathological Neuropad responses the IENF density (6.2±3.6 v 5.1± 3.7 fibre/mm; 85±50 v 78±77 fibre/mm^2), IENF branch density (40±38 v 36±40 no/mm^2), IENF length (33±7 μm v 29±20) did not show a significant difference although IENF correlated with an abnormal response (r=-0.291, p=0.059).

CONCLUSIONS

The Neuropad is highly sensitive in detecting large and in particularly small fibre damage. It is less sensitive in detecting intraepidermal nerve fibre loss but this may reflect the fact that the biopsy was performed on the dorsum of the foot.

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Validation of a new diabetic autonomic neuropathy bedside test (plaster) vs. the MNSI, monofilament of 10 gr test and biothesiometer

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Purpose: Absence of perspiration is a feature of diabetic autonomic neuropathy (DAN) and the indicator plaster (IP) could provide an easy and accurate way to diagnose diabetic peripheral neuropathy (DPN), which often coexists with DAN. The aim of the present study was to evaluate indicator's specificity, sensitivity and accuracy in detecting DPN in patients with diabetes mellitus (DM).

Patients-methods: We studied 174 patients (79 type 1 DM, 88 women), with mean age 49.8 ± 16.1 and mean duration of DM. 17.3 ± 7.7 years. We used the following methods for detecting DPN: the michigan neuropathy screening instrument questionnaire and examination (MNSIQ, MNSIE), application of monofilament of 10 gr (MONO) and measurement of vibration perception threshold with biothesiometer (BIO). According to the change of color of IP or not patients divided in two groups: group A (n = 82, completed change in color from pink to blue, normal perspiration), and group B (n = 92, uncompleted or no change, abnormal perspiration).

Results: MNSIQ and MNSIE were positive for DPN in 111 and 119 patients, respectively. BIO was abnormal in 109 and MONO in 59 patients. The sensitivity, specificity and accuracy of IP vs. all measured parameters appear in the following table.

Measured Parameter	Sensitivity	Specificity	Accuracy
MNSIQ	78%	92%	83%
MNSIE	73%	90%	78%
Biothesiometer	73%	81%	76%
Monofilament	95%	69%	78%

Conclusions: Indicator has a high sensitivity, specificity in detecting DPN vs. MNSIQ, MNSIE and biothesiometer. IP has a high sensitivity but moderate specificity vs. monofilament. Accuracy of the IP was high in all measurements.

欧米诺诊断膏贴（neuropad）在糖尿病周围神经病变早期诊断中的应用价值

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【摘要】 目的 探讨欧米诺诊断膏贴在糖尿病周围神经病变早期诊断中的应用价值。方法 对20例初诊糖尿病患者(DM组)、20例糖耐量减低者(IGT组）及10例糖耐量正常者（NGT组）进行欧米诺检测，并与神经传导速度检查、心脏自主神经功能检查对比。结果 DM组、IGT组、NGT组欧米诺平均变色时间分别为 14.6±2.3min、8.7±1.1min、5.5±0.8min，异常检出率分别为55%、25%、10%。在DM组及IGT组，欧米诺异常检出率高于神经传导速度及心脏自主神经功能检查。结论 初诊糖尿病患者及糖耐量异常者神经病变发生率高，欧米诺用于检测糖尿病周围神经病变具有敏感性及特异性，其在糖尿病周围神经病变早期诊断中的应用优于神经传导速度检查及心脏自主神经检查。

【关键词】糖尿病周围神经病变 欧米诺 神经传导 自主神经系统

Evaluation of neuropad in early detection of diabetic peripheral neuropathy

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【Abstract】 **Objective** To evaluate a new indicator test (Neuropad) in the early detection of diabetic peripheral neuropathy. **Methods** This study included 20 type 2 diabetic patients in the time of diagnosis (DM group), 20 impaired glucose tolerance subjects (IGT group) and 10 normal glucose tolerance subjects (NGT group). All were undergone Neuropad test. These results were compared with those obtained from nerve conduction study and cardiac autonomic function tests. **Results** The mean time until complete colour change of the neuropad test of DM group, IGT group and NGT group was 14.6±2.3min, 8.7±1.1min, 5.5±0.8min, and abnormal rate of each was 55%, 25%, 10%. In DM group and IGT group, the abnormal rate used with neuropad were higher than those used

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with nerve conduction study and cardiac autonomic function tests. **Conclusions** A high incidence of Neuropathy is ~~present in~~ found in early stage of DM and IGT. Neuropad is a sensitive and specific method, and is prior to nerve conduction study and cardiac autonomic function tests in the early detection of diabetic peripheral neuropathy. Neuropad is a test of high sensitivity and specificity.

【Key word】 diabetic peripheral neuropathy, Neuropad, Never conduction, Autonomic nervous system

神经病变是糖尿病早期临床症状之一，有文献报道用神经传导速度进行检测在初诊糖尿病者其发病率已达 18%[1]，在糖耐量减低者中亦有一定的检出率[2]。糖尿病血管病变和神经病变是引起糖尿病足合并症的基本原因，糖尿病人由于神经病变往往导致下肢远端感觉丧失或减低而易受外伤，迅速导致溃疡，感染和坏疽，以至最终不得不截肢。控制糖尿病足关键在于早期诊断、早期预防。我们用糖尿病神经病变早期诊断膏贴——欧米诺（neuropad），对 20 例初诊 2 型糖尿病（DM）患者、20 例糖耐量减低（IGT）患者及 10 例糖耐量正常(NGT)的健康体检者进行检测，并与其他检测方法进行比较，以探讨欧米诺在糖尿病周围神经病变早期诊断及糖尿病足早期预防中的应用价值。

对象和方法

一、研究对象及分组

选择我院 2005 年 7 月至 2006 年 5 月门诊及住院的初诊 2 型糖尿病患者 20 例为 DM 组，糖耐量减低者 20 例为 IGT 组，所有病例符合 1999 年 WHO 公布的关于 2 型糖尿病及糖耐量减低的诊断标准。另选择 10 例年龄、性别匹配的口服葡萄糖耐量试验正常的门诊健康体检者作为 NGT 组。排除标准：年龄大于 80 岁小于 18 岁、一般状态差、酗酒、甲状腺机能亢进或减低、有精神或心理疾患、有其他原因所致的中枢或周围神经病变、有肢体畸形、外伤、水肿、过敏性皮肤病、湿疹者。

二、方法

（一）“欧米诺”诊断膏贴试验

检测时室温维持在 20～25℃。患者平卧休息 15 分钟以上，并在除去袜子 5 分钟以后，将欧米诺膏贴贴在双侧大脚趾下球状部位的皮肤上，如该处皮肤较硬，则贴在小脚趾下球状部位的皮肤上。记录 10 分钟时膏贴的颜色变化情况及膏贴完全变为粉红色所需时间（min）。结果判断：10 分钟时膏贴完全变为粉红色为正常，10 分钟时至少一侧的膏贴保持蓝色或仅部分变为粉

红色为异常。

（二）神经电生理检查

神经传导速度采用日本 NIHON KOHDEN 公司的 MEB-5304K 型肌电－诱发电位仪测定，仪器置于温度为 20～25℃室内，由专人操作，在屏蔽条件下进行。分别测试每个受试者的双侧正中神经、尺神经、腓肠神经的运动传导速度（MCV）和双侧正中神经、尺神经、胫后神经感觉传导速度（SCV）。测定 MCV 时，刺激与记录均采用针电极，刺激电极分别在神经干的近端与远端刺激，从该神经支配的肌肉记录，即正中神经（肘－腕）、尺神经（肘—腕）、腓肠神经（腓骨小头－踝）。SCV 刺激与记录采用表面电极，用逆向法取得，即正中神经（腕－指）、尺神经（腕－小指）胫后神经（踝－拇趾）。正常值参照汤晓芙标准，异常诊断标准为传导速度低于健康人组平均值减去 2 倍的标准差[3]。3 条不同肢体不同神经或双下肢两条不同肢体不同神经异常为多发性神经损害[4] 。

（三）心脏自主神经功能检查

由专人进行，参照 Ewing 法[5]进行下列 5 项测定：(1)呼吸差：患者取平卧位深呼吸记录 II 导联心电图，分别计算深呼、深吸每分钟心率差。阳性值为≦10 次分，临界值为 11 ～ 14 次/分，阴性值为≧15 次/分。(2)立卧差：记录平卧位 II 导联心电图后，于 5 秒钟迅速起立，继续记录心电图 30 次心搏，计算立位与卧位心率之差。阳性值为≦10 次/分，临界值为 11 ～ 14 次/分，阴性值为≧15 次/分。(3)乏氏指数：病人深吸气后掩鼻闭口作呼气动作，放松后自然呼吸，同时记录心电图，测定乏氏动作后最大 R-R 间期与乏氏动作时最小 R-R 间期比值。阳性值为≦1.10 次/分，临界值为 1.11 ～ 1.20 次/分，阴性值为≧1. 21 次/分。(4)30/15 比值：记录患者由卧位到立位心电图，对比站立后第 30 次与第 15 次心搏 R-R 间期比值。阳性值为≦1.00 次分，临界值为 1.01 ～ 1.03 次/分，阴性值为≧1. 04 次/分。(5)卧立位血压差：测安静卧位和站立后的即刻血压差。阳性值为≧30 mm Hg，临界值为 11~29 mm Hg，阴性值为≦10mmHg。 (1)~(5)项每项正常各计“0”分，每项临界各计“0. 5 分”，每项异常各计“1 分”。总分最高 5 分，总积分≧1 为糖尿病合并心脏自主神经病变，总积<1 为不合并者。

（四）统计学处理

计数资料用均数±标准差($\overline{X} \pm s$)表示，均数间比较采用单因素方差分析，计数资料比较用 2×3 表 x^2 检验。统计分析在 SPSS 10.0 软件上进行，$P<0.05$ 有统计学意义。

结果

DM 组、IGT 组、NGT 组欧米诺膏贴平均完全变色时间为 14.6±2.3min、8.7±1.1min、5.5±0.8min，异常检出率分别为 55%、25%、10%，统计分析显示完全变色时间 DM 组与 IGT 组、DM 组与 NGT 组、IGT 组与 NGT 组的差异均有统计学意义（$P<0.05$）；DM 组异常率与 IGT 组、NGT 组比较，差异有统计学意义（$P<0.05$），IGT 组与 NGT 组比较，差异无统计学意义（$P>0.05$）；见表 1。

DM 组及 IGT 组各方法检查异常率比较见表 2。DM 组中，欧米诺检查异常率（55%）与神经传导速度检查的异常率（20%）比较，差异有统计学意义（$P<0.05$），与心脏自主神经检查的异常率（30%）相比，差异无统计学意义（$P>0.05$）。IGT 组中，欧米诺检查异常率（25%）与神经传导速度检查异常率（5%）、心脏自主神经功能检查异常率（20%）相比，差异均无统计学意义（$P>0.05$）。

讨论

糖尿病周围神经病变包括运动神经病变、感觉神经病变及自主神经病变。欧米诺是最新推出的一种检查方法，它通过膏贴颜色从蓝色到粉红色的变化来判断汗液分泌情况，从而检测自主神经中支配汗腺的交感神经功能。有很多研究表明周围神经病变在糖尿病的早期甚至糖耐量减低阶段即已发生[1,2,6,7]。糖尿病神经病变早期主要是小纤维神经如支配痛、温觉的神经及自主神经纤维受损。在我们的欧米诺检测结果中，NGT 组 90%的患者在 10 分钟内完成了颜色的变化，平均完全变色时间与 DM 组、IGT 组相比差异显著，表明欧米诺是检测周围神经病变的一个敏感指标。最近 Papanas 的两项研究也证实，欧米诺检查周围神经病变具有很高敏感性及特异性，而且还有很好的重复性，其完全变色时间还可以作为量化指标对神经病变的严重程度进行判断[8,9]。因此，如果膏贴完全变色时间偏长，则提示有周围神经病变的存在，则要采取足部保湿等措施预防糖尿病足的发展。欧米诺在 DM 组及 IGT 组的异常检出率分别为 55%和 25%，说明神经病变在初诊糖尿病者及糖耐量异常者中发生率较高。DM 组的异常率较 IGT 组高，考虑这是两组患者代谢障碍存在的时间长短不同所致，因为高血糖和高脂血症的代谢损害及直接的毒性作用是 DM 及 IGT 并发神经病变的主要原因，神经病变的发生与病程相关，也即与代谢障碍持续时间相关。

我们在欧米诺与其他检查方法的对比研究中，发现在初诊糖尿病的病人，欧米诺神经病变的阳性检出率高于神经传导速度及心脏自主神经功能检查，且与前者的差异显著，与后者的差异则无统计学意义。这可能是因为糖尿病神经病变早期主要是小纤维神经受累，而运动、感觉等大纤维神经往往到病变后期才受累，神经传导速度检测的是大纤维神经——运动神经、感觉神经功能，心脏自主神经功能检查检测的是小纤维神经——支配心血管的交感、副交感神经功

能，故在初诊糖尿病病人中，主要针对小纤维神经的欧米诺异常检出率较神经传导速度高，而与心脏自主神经功能检查差别不明显。

在糖耐量减低的病人中，欧米诺检出率亦高于其他两种检查方法，但未具统计学意义，分析其原因是 IGT 周围神经病变发生率尚较低，可能需更大样本才能突显各检查方法的差别。

糖尿病周围神经病变及糖尿病足必须进行早期诊断、早期预防。神经传导速度及心脏自主神经功能检查是目前诊断糖尿病周围神经病变的常用方法。神经传导速度检查开展的最早，应用也最为广泛，检测结果客观准确，但由于其检测对象为运动、感觉等大纤维神经，而大纤维神经往往到病变后期才受累，当神经传导速度发生改变时，多已为疾病的晚期，此时已失去了早期预防的意义。心脏自主神经功能检查一直以来都受到临床医生的重视，它不需特别的仪器，简单易行，但其结果易受老年、有否冠状动脉病变等的影响，因而敏感性及特异性差。支配汗腺的神经病变在早期糖尿病人中发生率很高，新近发展的一些用于检测汗腺功能的检查如定量轴索放射性排汗试验（QSART），排汗印记法（Sweat imprint）及交感神经皮肤反应（SSR）等均需要昂贵的仪器和专业人员进行操作，通常不容易进行。欧米诺诊断膏贴是德国最新研制的一种诊断支配汗腺神经病变的方法，与其他方法相比，该检测方法最为简单，仅需一张试纸，不需病人配合，不受病人的主观影响。另外，汗液的分泌最主要影响因素如室内温度、湿度等易于控制，故检测结果客观准确，重复性高，在一定程度上还有定量的意义。因此，欧米诺诊断膏贴在糖尿病周围神经病变的早期诊断中优于神经传导速度及心脏自主神经功能检查，适合于在门诊及病房对糖耐量异常及初诊糖尿病患者进行神经病变筛查及以后的随访观察。

综上所述，糖尿病周围神经病变在糖尿病早期阶段及糖耐量异常阶段有较高的发生率，主要用于诊断支配汗腺神经功能的欧米诺膏贴灵敏、客观、准确，是糖尿病周围神经病变早期诊断的理想方法。

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line IIEF-OS domain, scores of the IIEF-EF domain and the proportion of successful attempts were numerically increased, but not necessarily statistically significant. Years since, bother, age, aetiology, BMI and smoking status did not impact the extent of response to sildenafil. Although BMI did not influence the response to sildenafil, a strong correlation between ED severity at baseline and BMI was found in this patient population: men with a BMI >25 at baseline were much more likely to have severe ED (odds ratio: 2.67) as opposed to a lesser degree of ED severity. This odds ratio was further increased in men >65 years.
Conclusion: This retrospective analysis shows that several variables, including age and years since diagnosis, did not affect the extent of response to sildenafil. However, men with a BMI >25 were more likely to have severe ED at study entry.
Policy of full disclosure: S. Carrier: Investigator and advisory board member for Pfizer Canada, Eli Lilly Canada, Bayer Canada, Janssen-Ortho Canada.

P-02-016

Prevalence of neuropathic disturbances and their predictive value for erectile dysfunction in diabetic men

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Objective: Diabetic erectile dysfunction (DED) is of complex origin and diabetic neuropathy (DN) plays most important role in its development. The aim of this study was to evaluate the prevalence of neuropathy disturbances, determined with some neurologic tests, and their prognostic value for DED.
Methods: 150 consecutive men with mean age ± SD 53.0 ± 12.5 years, DM1/2 = 39/111 and diabetes duration of 4.9 ± 3.8 years were included in this study. ED was diagnosed with a questionnaire, about having erectile problems. For determination of somatic DN the Modified Neuropathy Disability Score (NDS): vibration (VP), thermal (TP) and tactile (MF) perceptions and Achilles reflexes (AR) were used. The presence of autonomic DN was evaluated with a new test for sudomotor dysfunction—Neuropad.
Results: Positive (>0) results for DN by different tests demonstrated for Neuropad –117 of all men(78.0%), VP-115(76.7%), TP-76(50.7%), MF-62(41.3%), AR-13(87.3%) and NDS-139(92.7%). ED was present in 44.7% of men (ED+). Compared to men without ED (ED–) the ED+ group had higher prevalence of abnormal results for VP ($p < 0.001$), TP ($p < 0.01$), MF ($p < 0.01$), Achilles reflexes ($p < 0.001$), NDS ≥ 6 points ($p < 0.001$), NDS ≥ 1 p. ($p < 0.001$) and Neuropad ($p < 0.01$). All these tests (but MF) were predictors (OR) for ED. After adjustment for age remained VP ≥ 6/8 [OR(CI;p)] – 3.350(1.193–9.410;$p < 0.05$) and NDS ≥ 6p. –2.957(1.348–6.485;$p < 0.01$). ED had prognostic power for abnormal neurological tests and for presence of DN respectively.
Conclusion: Presence of DN is a strong predictor for ED and vice versa. Simple neuropathy tests like NDS and Neuropad, used in the general practice for diagnosing DN are indirect indicators for ED.

P-02-017

In vivo electrophysiology in the paraventricular nucleus: Correlations with erectile activity

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Objective: The paraventricular nucleus of the hypothalamus (PVN) plays an important role in the control of male sexual function. However, PVN neuronal firing has not been demonstrated to change in response to sexual arousal inducing stimuli. The aim of this study was to record PVN neuronal activity in rats while measuring genital endpoints indicative of sexual arousal. Apomorphine, a non-selective dopamine agonist, was used in this study as a sexual arousal inducing stimuli.
Methods: All experiments were conducted in compliance with UK legislation. Under terminal anaesthesia, a glass electrode is placed into the PVN of the hypothalamus and lowered until a single unit is isolated. Pressure measurements are made from the corpus spongiosum of the penis, which indicates rising pressure within the penis during an erectile response. A metal recording electrode is then placed within the bulbospongiosum (BS) muscle. The BS muscle is involved in seminal emission and ejaculation; hence this measure provides an indication of muscular contractions involved in these processes.
Results: PVN neurones showed both increases (n = 10, mean 1394% of baseline values at 100 ug/kg) and decreases (n = 8, mean 54% of baseline values at 100 ug/kg) in firing rate. Animals simultaneously showed increases in ICP, and seminal emissions. Neuronal firing and ICP demonstrated dose-responsiveness in a range of 1.0 200 ug/kg apomorphine administered as an intravenous bolus. PVN local field potential recordings demonstrated a decrease in delta range activity and an increase in theta range activity during apomorphine induced penile erection, which was not present under the influence of apomorphine without penile erection.
Conclusion: These data demonstrate correlated activity (positive and negative) between PVN neurones and erectile activity and synchronous network activity during erection. These data support the involvement of the PVN of the hypothalamus in male sexual function and in dopamine receptor agonist induced sexual arousal.

P-02-018

Sexual quality of life of 10,161 subjects over 40 years old: Partial results

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Objective: Evaluate sexual quality of life of 10,161 subjects sample and identify elements suggesting Androgen Deficiency in the Aging Male (ADAM).
Methods: A self-administered and anonymous questionnaire with 71 questions on several aspects of sexual health was applied to 10,161 subjects (men and women), 40 years old and above, in 20 Brazilian states. Questions on general and sexual health, sexual relationships, life habits and the Aging Male's Symptoms (AMS) questionnaire were answered in this survey. The data was analysed with chi-square tests ($p \leq 0.05$ was considered statistically significant).
Results: 1853 subjects from Sao Paulo/Brazil (57.1% men and 42.9% women) were the first ones analyzed and here presented. 20.6% of men and 22.2% of women have sedentary life; 18.5% of the sample refer stress; 6.1% have smoking habits. Women have in average 6.0 intercourses/month, and men, 7.9. 95.4% of men and 94.6% of women considered sex important/very important to the couple harmony. Orgasm is not reached by 26.0% of women. 48.1% of men refer some level of erectile dysfunction (2.0% complete; 10.0% moderate; 36.1% mild). AMS men's scores point to: no symptoms (46.7%); mild (34.9%); moderate (15.7%) and severe (2.7%).
Conclusion: 18.5% of the male subjects refer moderate/severe score to AMS. Unhealthy life habits contribute to high levels of not satisfactory sexual health of 40 years old and above men and women from Sao Paulo city. Sex is significantly important to the couple harmony, and the frequency of sexual intercourses vary from 1.5 to 2 times by week.
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足自主神经功能检测对糖尿病周围神经病变的早期诊断初探

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【摘要】 **目的** 评估泌汗神经功能检测在诊断2型糖尿病患者早期周围神经病变中的价值。**方法** 分别采用DNS评分法和欧米诺汗印法（新型诊断膏贴，Neuropad）对218例住院2型糖尿病患者进行外周神经病变评估及泌汗神经功能检测，计算欧米诺汗印法及10g单纤丝、振动觉、温度觉及针刺痛觉的单项检查相对于DNS评分法对周围神经病变诊断的灵敏度和特异度，评估欧米诺汗印法及其它4种筛查方法。**结果** 糖尿病合并周围神经病变组的平均欧米诺汗印法变色时间为19.1±8.1分钟，显著高于糖尿病无神经病变组的8.3±1.8和对照组的3.9±0.8（P<0.01）；欧米诺汗印法测得的周围神经病变发病率为 61.9% 略高于DNS测得的57.8%。相对于DNS评分法，欧米诺汗印法诊断周围神经病变的敏感性为92.8%，特异性为82.2% 阳性预告值82.6%；单项的10g单纤丝、振动觉、足背部温度觉及痛觉测试的灵敏度分别为69.0%、33.3%、67.4%和57.1% ，特异度分别为81.5%、90.2%、80.4%和84.8%。欧米诺汗印法变色时间与DNS评分呈显著正相关（r=0.46），优于其他4种单项检查。**结论** 欧米诺汗印法是一种客观、简易、可靠的检测方法，对糖尿病患者足部泌汗功能的检测有助于糖尿病周围神经病变的早期诊断；欧米诺汗印膏贴的量化特点可用于评估周围神经病变严重程度；

【关键词】 糖尿病；周围神经病变；糖尿病足；欧米诺

Preliminary evaluation of test for the function of foot autonomic nerve in the early diagnosis of peripheral neuropathy in diabetic patients

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[Abstract] **Objective** To evaluate the test for the function of sudomotor nerve in the diagnosis of peripheral neuropathy among type 2 Diabetic patients. **Methods** To test the function of sudomotor nerve and evaluate peripheral neuropathy in 218 patients with type 2 Diabetes Mellitus by Neuropad (A new diagnosis tlaster) and Neuropathy Disability Score (DNS) respectively. Then the sensitivity and specificity of the Neuropad, 10g monofilament, vibration sensation, temperature sensation and stabbing pain sensation in the diagnosis peripheral neuropathy was attained to evaluate and compare with DNS respectively. **Results** Time until complete colour change of Neuropad in patients with peripheral neuropathy was 19.1±8.1 min, which was higher than those without peripheral neuropathy (8.3±1.8min,p<0.1) and the control group(3.9±0.8min, p<0.1) significantly; The morbidity of peripheral neuropathy using the Neuropad (61.9%) was a bit higher than that using the DNS (57.8%);Compared with the DNS, the sensitivity, specificity and predictive value of positive list of Neuropad in the diagnosis of peripheral neuropathy was 92.8%, 82.2% and 82.6% respectively. The sensitivity of 10g monofilament, vibration sensation, temperature sensation and stabbing pain sensation was 69.0%,33.3%,67.4% and 57.1% respectively; The specificity of 10g monofilament, vibration sensation, temperature sensation and stabbing pain sensation was 81.5%、90.2%、80.4% and 84.8% respectively. Time until complete colour change of Neuropad has significant positive correlation with the DNS value and is better than the other 4 tests. **Conclusion** The Neuropad is an objective, convenient and reliable test; The test for the sudomotor function of

foot is helpful for the early diagnosis of peripheral neuropathy; The quantitative characteristic of the Neuropad can be used in the evaluation of the severity degree of peripheral neuropathy.
Key words Diabetes mellitus; Peripheral neuropathy; Diabetic foot; Neuropad

周围神经病变是导致糖尿病足部溃疡的重要原因，早期检测出可逆性的神经病变对预防糖尿病足的发生具有重要意义。周围自主神经其病变可通过影响足部泌汗及血管舒缩功能而导致溃疡发生[1]。德国学者报道[2]，欧米诺汗印法可通过检测外周自主泌汗神经病变，较其他足筛查方法更早的发现糖尿病外周神经病变。国内尚未获得一致性结论，本研究以218例2型糖尿病为对象，应用并比较多种周围神经病变筛查方法，评价了欧米诺汗印法在糖尿病周围神经病变的早期诊断中的价值。

一 对象与方法

1 研究对象 病例组为2005年4月至2006年11月我院门诊及住院2型糖尿病患者共218例（男123例、女95例），平均年龄55.3±13.4岁，平均病程6.4±6.7年，均符合1999年WHO糖尿病诊断标准。排除周围动脉闭塞性疾病、慢性酗酒、甲状腺疾病、腰椎疾病、服用影响自主神经功能药物及其他引起周围神经病变的疾病。对照组为40名健康体检人员（男23例女17例），平均年龄53.4±12.8岁。

2 研究方法

2.1 糖尿病周围神经病变诊断：采用密歇根大学提出的DNS (Neuropathy Disability Score)评分法[3][4]，振动觉阈值(128Hz TF)、足背部温度觉(Warm/cold)、痛觉测试(Neuro-tips)及踝反射检查，前三项存在记0分，缺失计1分。第四项存在计0分，减弱计1分，消失计2分。总分0～10分,DNS≥3可诊断。

2.2 欧米诺汗印法 采用新诊断膏贴欧米诺（Neuropad，德国GmbH公司），利用汗液与膏贴内化学成分结合后发生变色反应的原理进行检测，所有受试者除去鞋袜平卧病床5分钟后贴于双侧跖骨头I/II部位进行检测。使用标准的颜色表，测定开始变色及完全由蓝色变为粉红所需的时间（正常人10分钟内完全由兰色变为粉红色），诊断标准为10分钟内颜色未发生完全变化为阳性。

2.3 清晨空腹静脉取血,离心分离血清测定糖化血红蛋白并记录年龄、性别及糖尿病起病时间；

2.4 统计学方法：采用SPSS10.0统计软件进行统计分析,非正态分布的变量经自然对数转换使之正态化后再分析，计量资料以x±s表示,两组间均数比较用t检验,各变量间相关检验用相关和偏相关分析。

二 结果

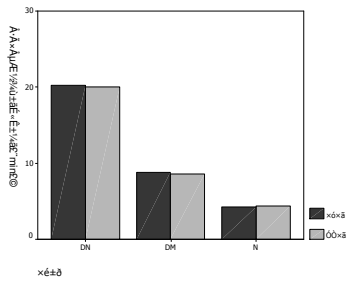
1、 欧米诺膏贴对218例糖尿病患者周围神经病变的诊断结果（表1）：使用欧米诺汗印法测得的2型糖尿病周围神经病变发病率为61.9% 高于DNS测得的57.8%。16例DNS评分诊断阴性的糖尿病患者存在泌汗功能障碍。与DNS相比，欧米诺汗印法测试周围神经病变的灵敏度为92.9%，特异度为82.2%，阳性预告值88.1%，阴性预告值82.6%，总符合率达89.4%。

2、 各组临床资料及不同检测方法与欧米诺汗印法变色时间的比较（表1）：DN组平均年龄、病程、HBA1c均显著高于DC组（P<0.05），DN组的欧米诺汗印法完全变色时间明显长于DC组（P<0.05），在所有测试对象中，左右足颜色完全变化的时间无明显差异（图1）（p > 0.05），40名健康志愿者欧米诺汗印法颜色均在10分钟内完全变化。单项的10g单纤丝、振动觉、足背部温度觉及痛觉测试的灵敏度分别为69.0%、33.3%、67.4%和57.1% ，特异度分别为81.5%、90.2%、80.4%和84.8%。

表1 各组临床资料及不同检测方法与汗印法变色时间的比较

组别	例数 n	年龄（岁）	病程（年）	HBA1c（%）	10g单纤丝（n）	振动觉异（n）	温度觉（n）	痛觉（n）	欧米诺（n）	右足 min	左足 min
DN组(DNS≥3)	126	57.9±13.8	6.35±5.92	11.1±2.4	87	42	85	72	119	18.4±7.0	19.1±8.1
DC组(DNS<3)	92	50.7±12.6	4.45±3.4	9.2±2.7	17	9	18	14	16	8.3±1.8	8.3±1.8
N组	40	53.4±12.8	-	-	0	0	0	0	0	4.0±0.6	3.9±0.8

3、 相关分析：欧米诺 汗印法完全变色时间与年龄、病程、 HBA1c及DNS评分均呈显著正相关（r=0.31、0.33、0.24及0.46，p<0.01, n=218），扣除年龄、病程因素后，变色时间仍与DNS评分呈显著正相关，与性别无相关关系。



3 讨论

周围神经病变和缺血是目前公认的糖尿病患者并发足部溃疡的主要原因，单纯的缺血性溃疡约占糖尿病足部溃疡的10%～15%，而由神经病变所致的足部溃疡占60%～70%[5][6]。目前对周围神经病变的定义局限在对大神经病变的检测，如神经电生理检测，而忽略了占到周围90%的小纤维神经[7]。受目前检测工具的限制，当感觉神经和运动神经病变的症状在临床上得到确认时，通常已是糖尿病足神经病变的晚期，治疗起来相当棘手，且难以取得良好效果。早期检测出仍具有可逆性的神经病变对预防糖尿病足具有非常重要的意义。

研究发现，无论尸检或活检，在光学显微镜或电镜下糖尿病人的小纤维神经受累均较大纤维神经为著，后者在其传导速度中度减慢时才出现震动觉的丧失小纤维神经病变可能出现在大神经病变之前[8]。目前常用的单纤丝、振动仪等检测只能对患者的大纤维神经病变作出诊断，针对小纤维神经病变的温度觉及痛觉检查均存在易受主观因素影响及可重复性差等缺点。自主神经的节后传出纤维直径<1um，是典型的小纤维，分布与其它周围神经一致，其病变可以导致支配区域出

现皮肤干燥、皲裂、营养障碍，并通过影响血管舒缩功能，使足部微循环调节异常，皮肤缺血、水肿，以致溃疡发生。长期以来，由于方法学的原因，对周围自主神经病变的诊断一直未得到足够的重视。现有的检查如：量化排汗神经轴突反射试验（QSART）、温度调节性发汗试验（TST）、交感皮肤电位反应（SSR）等都因为检查程序复杂耗时，花费昂贵及需要高技术医务人员等缘故不能得到推广。欧米诺汗印法诊断膏贴具有快速简便的特点，Zick[9]等发现欧米诺泌汗功能检测与上述精密、昂贵和耗时的排汗神经功能异常测试具有高度的相关性。

我们采用国际公认的糖尿病周围神经病变评分法（DNS评分）与欧米诺汗印法检测结果做了对比，观察到欧米诺汗印法变色时间随DNS评分的升高而延长，其与DNS评分结果的诊断符合度最高，灵敏度优于单项的10g单纤丝、振动觉、足背部温度觉及痛觉测试，特异度高于10g单纤丝及温度觉检查。作为一种简易的筛查方法，这一结果令人满意。本研究中,DNS评分法测得的阳性率为57.2%，欧米诺汗印法测得的泌汗神经功能异常率为62.2 % 有17%DNS评分正常的糖尿病患者经欧米诺汗印法检测已经存在泌汗功能异常，提示糖尿病发展过程中小纤维神经病变的发生可能先于大纤维神经病变，而欧米诺汗印法检测有助于早期糖尿病周围神经病变的筛查。有报道[10]DNS评分为6分时其预测糖尿病足溃疡发生的RR值是6.3，此分值时欧米诺汗印法的变色时间是25分钟，该变色时间可否高度预测糖尿病足溃疡的发生还需进一步的研究。欧米诺汗印法变色时间与HBA1c的正相关关系提示血糖控制可能改善泌汗神经功能，这一推测仍需进一步验证。

综上，欧米诺汗印法诊断膏贴作为一种客观、简易、可靠的糖尿病足部自主神经病变（泌汗功能异常）的诊断方法，其结果与糖尿病足部周围神经病变有密切关系。相对于标准音叉、10克尼龙丝、Tip-Therm等检查方法,欧米诺汗印法可以更早期的对糖尿病足部周围神经病变做出诊断，有利于促进早期治疗并采取相应的保护措施。欧米诺汗印法的时间量化特点可用于评估周围神经病变严重程度并可能预测糖尿病足溃疡的发生风险。

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Erectile dysfunction in diabetic men is linked more to microangiopathic complications and neuropathy than to macroangiopathic disturbances

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Summary

The aim of this study was to evaluate the importance of macro- and microangiopathic diabetic complications and especially diabetic neuropathy (DN) as risk factors for diabetic erectile dysfunction (DED). **Patients and methods:** In this cross-sectional study, the clinical records of 150 consecutive patients with mean age±SD 53.0±12.5 years, DM 1/2 = 39/111 and diabetes duration of 8.6±6.7 years were analyzed. Data about macroangiopathy (arterial hypertension, coronary artery- and cerebral vascular disease (CAD/CVD), dyslipidemia) and microangiopathy (nephropathy, retinopathy, symptoms and predetermined diagnosis of DN) was collected. Modified Neuropathy Disability Score (NDS): vibration (VP), thermal and 10g monofilament perceptions and Achilles reflexes for somatic DN and a new test for sudomotor autonomic DN – Neuropad were used as diagnostic tools. DED was diagnosed with a questionnaire, based on the answer to the question about having erectile problems. **Results:** DED was present in 44.7% of men with significant dependence on age and diabetes duration. The prevalence of arterial hypertension ($p<0.05$) and CAD/CVD ($p<0.05$) was higher in the DED group, but the differences in nephropathy ($p<0.01$), retinopathy ($p<0.001$) and neuropathy ($p<0.001$) were more significant. After adjustment for age, the duration of diabetes OR(CI;p) - 1.054(1.010-1.099;$p<0.05$), retinopathy - 5.512(2.469-12.305;$p<0.001$), symptoms of DN - 2.428(1.138-5.179;$p<0.05$) and diagnosis of DN - 2.805(1.406-5.597;$p<0.01$) remained as risk factors for DED. All neuropathic tests were significantly more unfavorable in the DED group. After adjustment for age and diabetes duration, best predictors for DED were NDS and VP. Only 4.5% of men have been treated for DED. **Conclusions:** Erectile dysfunction in diabetic patients is a specific entity. Microvascular diabetic complications are more important risk factors for erectile dysfunction than macrovascular ones. Our data support the hypothesis that in the complex pathogenesis of diabetic ED, diabetic neuropathy is more important pathogenic factor than macroangiopathy.

Key words: diabetes, erectile dysfunction, macrovascular and microvascular complications, Neuropad, NDS

Introduction

Cardiovascular diseases (CVD) have been suggested to be one of the main causes of erectile dysfunction (ED) [1,2]. CVD and ED are late manifestations of a process that begins many years earlier with endothelial dysfunction. ED is usually diagnosed later than CVD because it is a difficult topic for many men. However, if actively sought, ED can be found as an early and sensitive marker for insidious CVD. ED preceded ischemic symptoms by more than 3 years in 67% of a cohort patients [3].

ED is 3 to 4 times more common in men who have diabetes mellitus than is those who do not have diabetes [3]. Symptoms of ED can be recognized in more than half of patients who have had diabetes for 10 years [4]. The prevalence of ED increases from 9% of men aged 20-29 years to more than 95% of men aged over 70 years. This increase correlates with diabetes duration, poor metabolic control and complications [5]. Although traditionally affiliated with other microangiopathic complications, diabetic neuropathy (DN) is a multifactorial process, in which microvascular damage is only one aspect. Advanced glucation end-products (AGEs), oxidative stress, polyol pathway, nerve growth factor deficiency, dysfunction of protein kinase C, tissue remodelling etc. are other important factors for neuronal damage. The pathogenesis of diabetic ED is complex and includes atherosclerosis, neuropathy, hypogonadism and corporeal erectile tissue alterations like endothelial dysfunction, abnormal collagen deposition and smooth muscle degeneration [6]. AGEs, which increase in human diabetic penile tissue, are considered important contributors to ED [7]. AGEs quench nitric oxide (NO) and through up-regulation of inducible NO-synthase induce oxidative-nitrosative stress and down-regulation of endothelial NO-synthase, which may mediate ED.
Diabetes is a major risk factor for CVD and over the last decades more attention has been focused on this relation than on that of microangiopathic diabetic complications as risk factors for ED. This study assesses the relative importance of macro- and microangiopathic diabetic complications, especially DN, as risk factors for ED.

Patients and methods:

In this cross-sectional study, the clinical records of 150 consecutive patients, visiting a general endocrinology clinic, were evaluated.

Inclusion criteria were:

- Diagnosis of diabetes mellitus type 1 or 2 according to contemporary criteria for the disease, independent of its duration.
- Fasting plasma glucose stable over the previous week and within the range ≥3.5 to ≤ 12 mmol/L.

Exclusion criteria were

- Unstable glucaemic control. Although very often used, this term does not have any universally accepted definition. Neural function is vulnerable to variations in blood sugar levels ("insulin neuritis", hypo- or hyperglycaemia-induced changes in electrophysiologic tests etc.). We considered a lack of large blood sugar level excursions (>5 mmol/L for corresponding from day to day points in the profile) more important than the particular value.
- Acute diabetic complications like ketoacidosis or severe hypoglycaemia in the past month.
- Consumption of drugs that can interfere with neurological examination, i.e. analgesics, tricyclic antidepressants, anticonvulsants etc.

Patient database

Most of the patients were with poor glucaemic control, a state which hindered accurate measurement of proteinuria and lipids during their short stay in hospital. For this reason, the main criterion for diagnosis and treatment of a micro- or macroangiopathic diabetic complication was whether the complication had been noted in the patient's medical records. A database was created for every patient comprising

- Demographic, anthropometric and diabetic data (Table 1).
- The presence of ED determined by the patient's answer to the question "Do you have problems achieving and/or sustaining erections sufficient for satisfactory intercourse, but not characterized as premature or late ejaculation?" If the answer to this question was "Yes", the questions, "How long have you had this problem?" and "Have you sought medical advice?" were asked.
- Predetermined diagnosis and/or treatment and duration of arterial hypertension, dyslipidaemia, coronary artery disease and/or cerebral vascular disease

(CAD/CVD), nephropathy, retinopathy. We accepted these earlier diagnoses according to the criteria: "diabetic nephropathy", an earlier recognized non-inflammatory macroalbuminuria in conditions of a good glucaemic control, in the absence of any other known glomerulopathy; "diabetic retinopathy", background or more severe signs observed during ophthalmologic examination; actual hypertension or its treatment; documented and/or treated dyslipidaemia; history of a coronary and/or cerebral event or appropriate treatment for CAD/CVD.

- Special attention was paid to DN. We distinguished three diagnostic levels: (1) presence of neuropathic symptoms like numbness, nocturnal pain, paresthesias etc.; (2) an earlier diagnosis of "diabetic neuropathy" made by a neurologist; (3) we applied objective neurologic tests, routinely used in the clinic - the modified Neuropathy Disability Score (NDS) and Neuropad.

NDS

The NDS test is widely used for diagnosing somatic DN. A detailed description of modified NDS is given elsewhere [8]. Instead of a pin-prick test, we used a 5.07 Semmes-Weinstein monofilament (MF). Briefly the diagnostic complex included 4 items tested on both feet:

1. Vibration perception (VP) with the threshold estimated with a 128-Hz Rydel-Seiffer tuning fork (Thio-Vib, Germany), graduated in 8 stages on both vibrating branches. The fork was applied to the apex of the big toe and the following assessments were made: normal (can distinguish $\geq$ 6/8) = 0 points, abnormal (cannot distinguish $\leq$ 5/8) = 1 point.
2. Temperature perception (TP) using a simple instrument (Thio-Term, Germany) based on differences in thermal conductivity which result in different subjective feelings, e.g. a metal is colder than a plastic surface. The instrument was applied to the skin of the sole 1-2 cm distally from the mid-point between metatarsal heads I-II, but not on callus. The following assessments were made: normal (can distinguish) = 0 points, abnormal (cannot distinguish) = 1point.
3. MF (10g, Thio-Feel, Germany) placed in the same way as TP and applied with smooth pressure until the filament bent. The following assessments were made: normal (can feel) = 0 points, abnormal (cannot feel) = 1point.

4. Achilles reflex (AR) as routinely examined. The following assessments were made: when present = 0 points, present with reinforcement = 1 point and when absent = 2 points.

The maximum NDS was 10 points (5 points for each foot). All procedures were performed in at least 3 "active" and some "placebo" trials, in a quiet room with a temperature of 18-22 C° and with the instruments hidden from the patient.

Neuropad

The diagnostic test Neuropad (miro Verbandstoffe GmbH, Wiehl-Drabenderhöhe, Germany) was developed for early detection of sudomotor disturbances as a marker of diabetic autonomic neuropathy of the feet and for early recognition of the diabetic foot syndrome. This adhesive indicator test changes colour when applied to the skin of the foot. The indicator material is a cobalt-containing compound which changes from blue to pink. After completion of all DN procedures, the plaster was stacked on the soles of both feet in the same position as the MF and TP tests had been applied. The colour of the indicator was noted after 10 minutes and scored as normal if there was a complete change from blue to pink = 0 points, borderline if a mottled blue/pink colour = 1point and abnormal if the colour remained blue = 2 points.

Statistical methods

The data was processed with the statistical package SPSS 13.0.1. The level of significance for rejecting the null hypothesis was $p<0.05$. We applied the following statistical methods: descriptive analysis, variation analysis, Kolmogorov-Smirnov's one sample nonparametric test, Student's t-test for two independent samples, Kruskal-Wallis' nonparametric test for several independent samples, Mann-Whitney's nonparametric test for two independent samples, binary logistic regression, multinomial logistic regression, and the chi-squared test.

Results

Demographic and diabetic characteristics are shown in Table 1. The distribution of the variables was checked with the Kolmogorov-Smirnov test. For variables with normal and abnormal distribution Student's t-test and Mann-Whitney's nonparametric test were used.

respectively. For categorical variables the chi-squared test was applied. The mean age ± SD was 53.0 ± 12.5 (18 – 86 years). Three men over 80 years of age were included in the group of 9 men aged over 75 years. The rate of ED in this group was 66.7%. All patients were divided into two groups: 67 (44.7%) with ED (ED+) with a mean duration of 4.9 ± 3.8 years and the remaining 83 (55.3%) without ED (ED-). Only 3 men (4.5%) had been treated for ED. Additionally, the prevalence of diabetes complications was determined in both groups (Table 1). A significant association of ED was observed with age, diabetes type 2 and its duration. The prevalence of arterial hypertension and CAD/CVD was higher in the ED+ group, but the differences in microangiopathic complications – nephropathy, retinopathy and neuropathy – were more significant. The rate of diagnosis of neuropathy was lower than that of neuropathic symptoms.

Type 1 and type 2 diabetes were analysed separately. 39 men aged 36.0 ± 11.4 years with a mean duration of the disease of 9.1 ± 8.9 years had type 1 and the rate of ED was 23.1%. 111 men aged 59.0 ± 8.6 years with a mean duration of the disease of 8.4 ± 5.9 years had type 2 and the rate of ED was 52.3 %.

Further, the role of all individual factors studied (unadjusted, adjusted for age, and for age and diabetes duration) as risk factors for ED, was determined with binary logistic regression. Only age, type 2 diabetes, diabetes duration, retinopathy and DN (symptoms and diagnosis) proved to be independent risk factors for ED (Table 2). The OR did not reach significant values for height, weight, BMI, waist and hip circumferences and weight:height ratio, HbA1c, arterial hypertension presence, duration and number of drugs, dyslipidaemia presence and its duration and treatment, presence of CAD/CVD and their duration, nephropathy and its duration.

The predictive value of diabetic ED was also estimated. If a man with diabetes has ED, the adjusted risk (OR;95% CI; p) for having retinopathy was 5.576 (2.479-12.541; $p<0.001$), for presence of DN symptoms was 2.386 (1.119-5.087; $p<0.05$), and for diagnosed DN was 2.780 (1.395-5.540; $p<0.001$). There was a clear tendency for nephropathy too with 2.925 (0.962-8.895).

ED and neuropathy tests

The next part of the study focused on the predictive value of different neurological tests for ED. Positive (>0) results for DN by different tests were 117 of all men (78.0%) for

Neuropad, 115 (76.7%) for VP, 76 (50.7%) for TP, 62 (41.3%) for MF, 131 (87.3%) for AR and 139 (92.7%) for NDS. Significant differences in the prevalence of neuropathic disturbances, registered by different tests, were established between the ED+ and ED- groups (Table 3; the same statistical methods were used as for Table 1). The mean NDS score for all patients was 6.0±2.3 points. ED+ men had a significant higher NDS score (6.9±1.6 points), than ED- men (5.3±2.5 points; $p<0.001$). We also calculated the percentage of patients with an NDS score ≥6. This cut-off was originally used to identify patients at a high risk for diabetic foot problems and such data can be analyzed retrospectively from different medical sources. The difference between ED+ and ED- men with the cut-off of ≤5/≥6 was even more significant than with cut-off of 0/≥0.

Most patients had similar results from both feet by separate tests. For this reason and to simplify the statistics, we used the mean value from the readings of both feet (0&0=0; 1&1=1; 2&2=2). In a small number of men, the differences between both feet resulted in intermediate groups after calculating the mean value for both feet (0&1=0.5; 1&2=1.5). Dependent on these data, there were 3 different results (0, 0.5 and 1) for VP, TP and MF. For the 3-grade tests, Neuropad and AR, more combinations appeared from the basic readings (0; 0.5; 1; 1.5 and 2). Because of the small number of intermediate groups, 6 men with 0.5 score and 3 men with 1.5 score, they were included in the groups with a Neuropad score of 0 and 2, respectively. For AR the intermediate sub-groups consisted of 4 men with a 0.5 and 3 men with a 1.5 score, and they were included in the corresponding larger groups as well (Table 4). Multinomial logistic regression was applied in these calculations.

Except for MF, all tests showed statistically significant increases in the calculated risk for ED on the higher tested neuropathy level, determining a more severe DN. Neuropad was sensitive and demonstrated a statistically significant increase in risk at both levels (1 and 2). Because of the small number of patients after adjustment for age and diabetes duration, only NDS ≥ 6 and VP (level 2) still determined a significantly higher risk. A special calculation showed that every 1 point of NDS increases the unadjusted risk for ED to OR = 1.242 (1.094-1.409; $p<0.01$) and adjusted for age risk to 1.186 (1.035-1.359; $p<0.5$).

Discussion

In this study to evaluate the relative importance of macro- and microangiopathic diabetic complications, especially that of DN, as risk factors for ED, data was collected and analysed

- To estimate the prevalence of ED, and of type 1 diabetes and of type 2 diabetes in the cohort
- To evaluate the differences in demographic indices and diabetes complications between men with and without ED.
- To estimate the contribution of different diabetic micro- and macroangiopathic complications as risk factors for ED.
- To determine the reliability of some common neurological tests including NDS and Neuropad, a new test for autonomic functions, as predictors for ED.

According to the literature the prevalence of diabetic ED varies from 20 to >70% depending on differences in age, diabetes type, duration and severity, and diagnostic methods for ED (summarized data in [5]). Our cohort was 53.0 ± 12.5 years old with a mean duration of diabetes of 8.6 ± 6.7 years. The presence of diabetic ED in our cohort was determined as described in large epidemiological studies [5]. Among our patients 44.7% had ED. These men were older (57.4 ± 10.2 vs. 49.4 ± 13.1 years; $p<0.001$), more often had type 2 diabetes (86.6% vs. 63.9%, $p<0.001$) and had had the disease for longer (10.9 ± 6.6 vs. 6.7 ± 6.1 years; $p<0.01$), than men without ED. Age is a well-proven risk factor for ED, even in non-diabetic men. In our study, the risk of ED increased by 3.7% per year ($p<0.01$). Unlike others [9], we found a higher prevalence of ED in patients with type 2 (52.3%) than in patients with type 1 (23.1%) diabetes, even though the patients in both groups had had diabetes for a similar length of time. Men with type 2 had a 3.6 times ($p<0.01$) higher risk of having ED than those with type 1 diabetes. After adjustment for age, the risk decreased to 2.2 times and the significance was lost. Likewise in a study of two cohorts of men with type 1 diabetes of equivalent age (20-70 years) no difference was found in diabetic ED prevalence (36 and 35%, respectively) [10]. Diabetes duration is a strong predictor of diabetic ED and in our study every year increased the adjusted risk by 5.4% ($p<0.05$).

The presence of ED has been reported to be a predictor for dyslipidaemia, arterial hypertension and CAD/CVD [3,17]. Surprisingly, all investigated macroangiopathic

diabetic complications, or their duration, failed to reach significant values as risk factors for ED in any of our analyses. One explanation could be the small number of patients and the research approach we used, which was based on hospital records and predetermined diagnoses. Another reason could be the specific type of complex diabetic ED we studied. In our opinion, microangiopathic diabetic disturbances and DN are at least equal, if not more important, pathogenic causal factors in diabetic ED than macrovascular ones. Because of this specific microvascular and neuronal deterioration, in interventional studies with inhibitors of phosphodiesterase-5, diabetic patients have a less satisfactory response, than those without diabetes.

No studies analogous to ours have been conducted in Bulgaria. A study of 310 patients aged 20-78 years with a 63.2% ED rate in a close geographic region reported ED not to be correlated with the type of diabetes, hypertension, ischaemic heart disease, nephropathy or dyslipidaemia [11]. Data from Hong Kong did also show retinopathy, nephropathy and sensory neuropathy to be more important risk factors for ED than macrovascular disease [12].

Other studies have found a significant correlation between ED and microvascular disease [5,11,13]. The prevalence of microangiopathic complications differed significantly between men with and without ED in our cohort. The presence of retinopathy, symptoms of neuropathy and a diagnosis of DN increased the age-adjusted risk for diabetic ED by 5.5 ($p<0.001$), 2.4 ($p<0.05$) and 2.8 ($p<0.01$) times, respectively. More patients ($p<0.05$) had symptoms, rather than a predetermined diagnosis of DN. The neurological examination gave other figures for the prevalence of DN. We used three methods (symptoms, predetermined diagnosis and objective tests) to establish the presence of DN for the following reasons.

The diagnosis "DN" has not been standardized and is a matter of debate. One of the most widely accepted definitions of DN is "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" [14]. This definition emphasizes two important messages:

- A diabetic patient with neuropathic symptoms does not necessarily have DN. In the Rochester Diabetic Neuropathy Study, up to 10% of peripheral neuropathy in patients with diabetes was of non-diabetic origin [15].

- A patient who has diabetes without neuropathic symptoms can have DN. The natural course of DN begins insidiously. The first changes can be detected only by specific electrodiagnostic investigations, later changes can be detected by quantitative and semi-quantitative tests (some of them used in this study), and finally, neuropathic symptoms appear.

Specific objective methods used in our study showed the different prevalences of DN: MF (41.3%), TP (50.7%), VP (76.7%), Neuropad (78.0%), AR (87.3%) and NDS (92.7%). The wide range depended therefore on the diagnostic method used, which sheds light on the differences in prevalence of DN found in the literature and the previously mentioned discrepancy between neuropathic symptoms and diagnoses in this study. The composite somatic score, NDS with a cut-off 0/>0, was the most sensitive of the methods used in our study. We introduced this reading of NDS for a more earlier diagnosis of DN. The classical and commonly used cut-off (≤5/≥6 points) has been shown to be a good predictor for a diabetic foot at risk, what represents a later stage in DN.

Neuropad alone as an indicator for autonomic dysfunction showed good sensitivity. The unadjusted risk of diabetic ED increased 2.4 or 4.6 times if a mottled colour pattern or no change in the plaster colour occurred. After adjustment, this tendency remained but because of the small number of patients it did not reach significance. We believe this is the first study demonstrating the diagnostic value of Neuropad in patients with diabetic ED. Adding such a simple test to the somatic neuropathy diagnostic armamentarium is not unreasonable because autonomic neuropathy is one of the cornerstones in the pathogenesis of diabetic ED, as shown by analysis of nearly 10,000 men with diabetes and ED [5]. This analysis ranked the risk factors for diabetic ED as follows (OR in parenthesis): autonomic neuropathy (5.0), diabetic foot (4.0), peripheral neuropathy (3.3), peripheral arterial disease (2.8), nephropathy (2.3), poor glucaemic control (2.3), retinopathy (2.2), hypertension (2.1), diabetes duration (2.0).

Data concentrating on a detailed description of DN as a factor for diabetic ED are scarce. Men with diabetic ED were compared with men with other types of neurogenic ED in an instrumental study [16]. Only 1 out of 13 men with diabetic ED and 3 out of 21 with neurogenic ED were found to have vascular dysfunction. The results of other tests (diabetic ED vs. neurogenic ED) were as follows: frequencies of autonomic symptoms other than ED (64% vs 64%), abnormal electromyogram (33% vs 40%), abnormal quantitative sensory testing (vibratory perception 83% vs 84%, cold perception 9% vs

19%, warm perception 42% vs 43%), abnormal clinical findings (50% vs 33%), nerve conduction studies (75% vs 50%). Heart rate variability testing (39% vs 25%) were slightly, but not substantially, more frequent in men with diabetic ED than those with neurogenic ED. The investigators concluded that their findings supported the hypothesis that neuropathy contributes significantly to the pathophysiology of ED in diabetes. Our corresponding data are close to these results.

Our results indicate that microangiopathic complications, which are underestimated in the everyday practice, have a higher predictive value for diabetic ED. Physicians rarely ask patients about ED and patients rarely complain spontaneously about it. Even after diagnosis, ED remains untreated in most cases [18]. In our study only 4.5% of men with ED had been treated. Likewise in another study only 7% had been treated [13].

Conclusions: Erectile dysfunction in diabetic patients is a specific entity. Microvascular diabetic complications are more important risk factors for erectile dysfunction than macrovascular ones. Our data support the hypothesis that in the complex pathogenesis of diabetic ED, diabetic neuropathy is more important pathogenic factor than macroangiopathy.

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Table 1. Demographic characteristics of the patients and characteristics of their diabetes and its complications

Variable	All patients	ED+	P value	ED-
N	150	67		83
Age (years#)	53.0±12.5	57.4±10.2	***	49.4±13.1
Height (cm#)	173.1±5.6	172.4±5.7		173.7±5.3
Weight (kg#)	87.0±14.8	87.0±14.5		87.1±15.0
BMI (kg/m^2#)	29.0±4.3	29.2±3.8		28.8±4.6
Waist (cm#)	101.6±12.1	101.9±10.6		101.3±12.7
Hip (cm#)	103.6±7.9	103.6±7.6		103.6±8.0
W/H#	0.98±0.06	0.98±0.05		0.97±0.07
Diabetes type 2	111 (74.0)	58 (86.6)	***	53 (63.9)
Duration of diabetes (years#)	8.6±6.7	10.9±6.6	**	6.7±6.1
FPG (mmol/L#)	8.9±1.7	7.1±1.6		7.1±1.5
HbA1c (%#)	7.1±1.6	8.7±1.6		9.1±1.7
Hypertension	111 (74.0)	52 (77.6)	*	59 (71.1)
Duration of hypertension (years#)	9.7±6.8	9.3±6.9		10.1±6.7
Antihypertensive drugs (N#)	2.1±0.9	2.2±1.0		2.0±0.8
Dyslipidemia	70 (46.7)	31 (46.3)		39 (47.0)
Duration of dyslipidemia (years#)	2.5±2.3	3.3±3.3		1.8±1.3
CAD/CVD	42 (28.0)	21 (31.3)	*	21 (25.3)
Nephropathy	17 (11.3)	11 (16.4)	**	6 (7.2)
Duration of nephropathy (years#)	2.6±2.3	2.7±2.2		2.5±2.7
Retinopathy	54 (36.0)	41 (61.2)	***	13 (15.7)
Duration of retinopathy (years#)	3.7±2.6	4.0±3.1		3.0±1.6
Neuropathy symptoms	101 (67.3)	54 (80.6)	***	47 (56.6)
Neuropathy diagnosis	77 (51.3)	44 (65.7) †	***	33 (39.8) ††

values are given as mean±SD. Other data presented as number of patients (%).

*p<0.05; **p<0.01; ***p<0.001 – differences between ED+ and ED- groups.

†p<0.05; ††p<0.01 – between neuropathy symptoms and diagnosis – groups.

Table 2. Risk factors for ED

Studied parameter		Risk for ED (Odds ratio(95%CI))
Age (increase per 1 year)	NA	1.037** (1.013-1.061)
Diabetes type 2	NA	3.648** (1.586-8.389)
	ADJ[1]	2.223 (0.791-6.245)
	ADJ[2]	3,597* (1,125-11,505)
Diabetes duration (increase per 1year)	NA	1.063** (1.019-1.108)
	ADJ[1]	1.054* (1.010-1.099)
Retinopathy	NA	4.923*** (2.298-10.545)
	ADJ[1]	5.512*** (2.469-12.305)
	ADJ[2]	5,135*** (2,045-12,898)
DN symptoms	NA	2.900** (1.395-6.027)
	ADJ[1]	2.428* (1.138-5.179)
	ADJ[2]	1,967 (0,880-4,401)
DN diagnosis	NA	2.899** (1.485-5.659)
	ADJ[1]	2.805** (1.406-5.597)
	ADJ[2]	2,251* (1,043-4,858)

NA – non-adjusted; ADJ[1] – adjusted for age;

ADJ[2] - adjusted for age and duration of diabetes

*p<0.05; **p<0.01; ***p<0.001

Table 3. Prevalence of neuropathical disturbances, registered by different tests in men with and without diabetic ED.

Variable	All patients	ED+	P value	ED-
N	150	67		83
Neuropad	117 (78.0)	58 (86.6)	**	59 (71.1)
VP	115 (76.7)	60 (89.6)	***	55 (66.3)
TP	76 (50.7)	40 (59.7)	**	36 (43.4)
MF	62 (41.3)	33 (49.3)	**	29 (34.9)
AR	131 (87.3)	63 (94.0)	**	68 (81.9)
NDS (≥ 0)	139 (92.7)	66 (98.5)	**	73 (88.0)
NDS (≥ 6) #	96 (64.0)	53 (79.1)	***	43 (51.8)

Normal/abnormal cut-off in all tests is 0/>0, except for NDS # where cut-off is ≤5/≥6.

All values are given as N (%).

**p<0.01; ***p<0.001 – differences between ED+ and ED- groups.

Table 4. Association between neuropathy tests and diabetic ED

Test			Risk for ED (Odds ratio(95%CI))
Neuropad	**1**	NA	2.393* (1.072-5.342)
		ADJ[1]	1.781 (0.764-4.147)
		ADJ[2]	1,441 (0,601-3,455)
	2	NA	4.582* (1.253-16.757)
		ADJ[1]	3.353 (0.572-12.894)
		ADJ[2]	2,504 (0,625-10,028)
VP	**0.5**	NA	1.714 (0.184-6.075)
		ADJ	0.998 (0.246-4.054)
		ADJ[2]	0,952 (0,229-3,960)
	1	NA	5.268*** (2.094-13.251)
		ADJ[1]	3.350* (1.193-9.410)
		ADJ[2]	2,918* (1,024-8,320)
TP	**0.5**	NA	1.160 (0.373-3.615)
		ADJ[1]	1.022 (0.316-3.299)
		ADJ[2]	0,934 (0,272-3,205)
	1	NA	2.192* (1.097-4.38)
		ADJ[1]	2.005 (0.981-4.096)
		ADJ[2]	1,841 (0,890-3,809)
MF	**0.5**	NA	1.324 (0.375-4.675)
		ADJ[1]	1.068 (0.285-3.977)
		ADJ[2]	0,816 (0,215-3,102)
	1	NA	1.934 (0.961-3.889)
		ADJ[1]	1.677 (0.814-3.454)
		ADJ[2]	1,398 (0,659-2,966)
AR	**1**	NA	3.553 (0.990-12.746)
		ADJ[1]	2.607 (0.694-9.793)
		ADJ[2]	2,311 (0,605-8,833)
	2	NA	3.424* (1.053-11.138)
		ADJ[1]	2.078 (0.591-7.306)
		ADJ[2]	1,557 (0,426-5,690)
NDS	**≥ 6**	NA	3.864*** (1.838-8.123)
		ADJ[1]	2.957** (1.348-6.485)
		ADJ[2]	2,419* (1,068-5,479)

*p<0.05; **p<0.01; ***p<0.001
NA – non-adjusted; ADJ[1] – adjusted for age;
ADJ[2] - adjusted for age and duration of diabetes

The performance of Neuropad in diabetic neuropathy

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Backgroud

1. Classification of diabetic neuropathy

Most common:

- Chronic sensorimotor distal symmetric polyneuropathy
- The autonomic neuropathies

Generalized symmetric polyneuropathies
- Acute sensory
- Chronic sensorimotor
- Autonomic

Focal and multifocal neuropathies
- Cranial
- Truncal
- Focal limb
- Proximal motor (amyotrophy)
- Coexisting CIDP

Adapted from Thomas (4). Note: Clinicians should be alert for treatable neuropathies (CIDP, monoclonal gammopathy, vitamin B_{12} deficiency, etc.) occurring in patients with diabetes.

2. The prevalence of diabetic neuropathy

- The prevalence: 47%-90%;
- Morbidity: non-traumatic amputation (50—70%)
- Mortality: 25—50%
- Burden: very heavy
- major risk factor for the development of diabetic foot syndrome
- predicted a poor prognosis

3. Diagnosis of Diabetic peripheral neuropathy (DPN)

- Nerve conduction velocity:

  most objective, accurate measures only large fibre function

  but not early stage

- Quantitative sensory tests

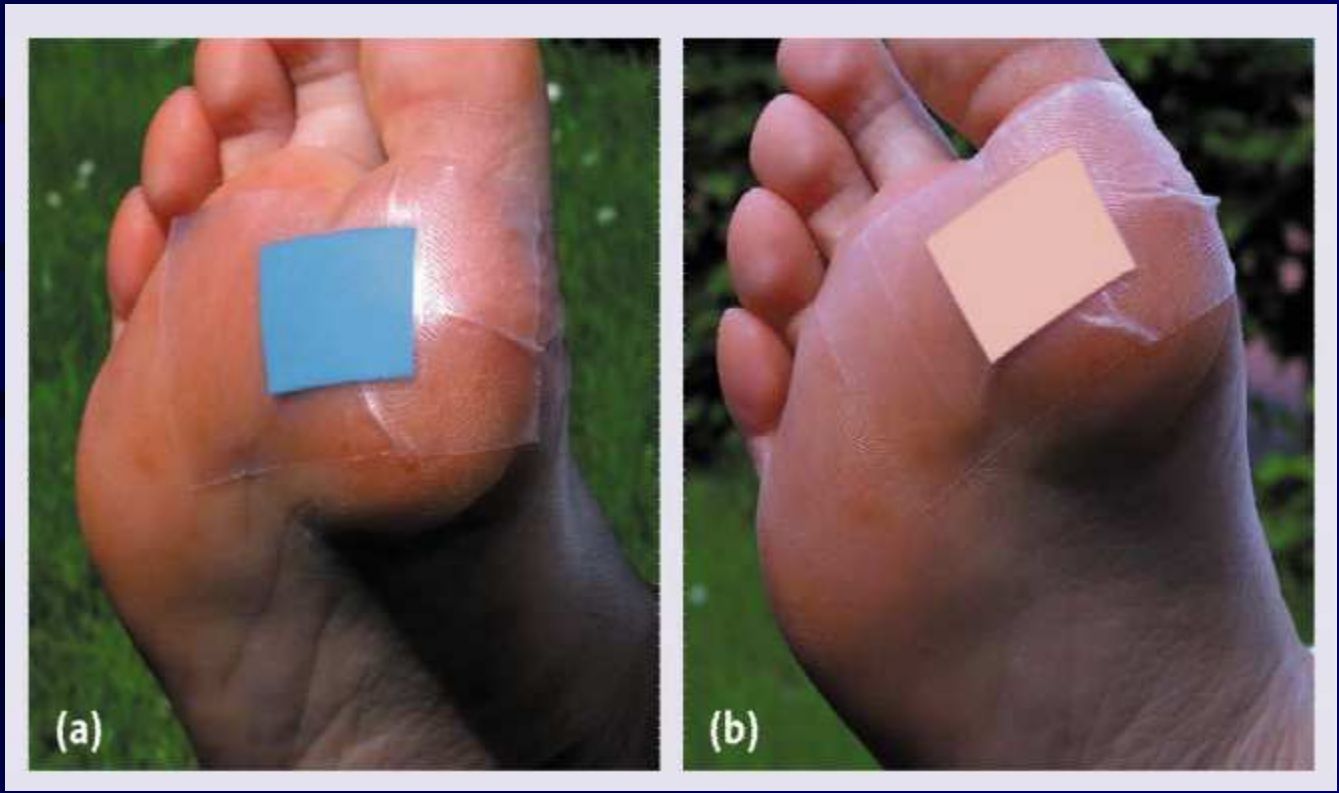
  measure both small/large fibre deficit
  less objective

- Sensory tests (pain, cool, vibration, 10g monofilament)

  less objective

Earliest damage is to Small Fibres!

Neuropad: an new indicator plaster predicted the damage of small fibres



Blue

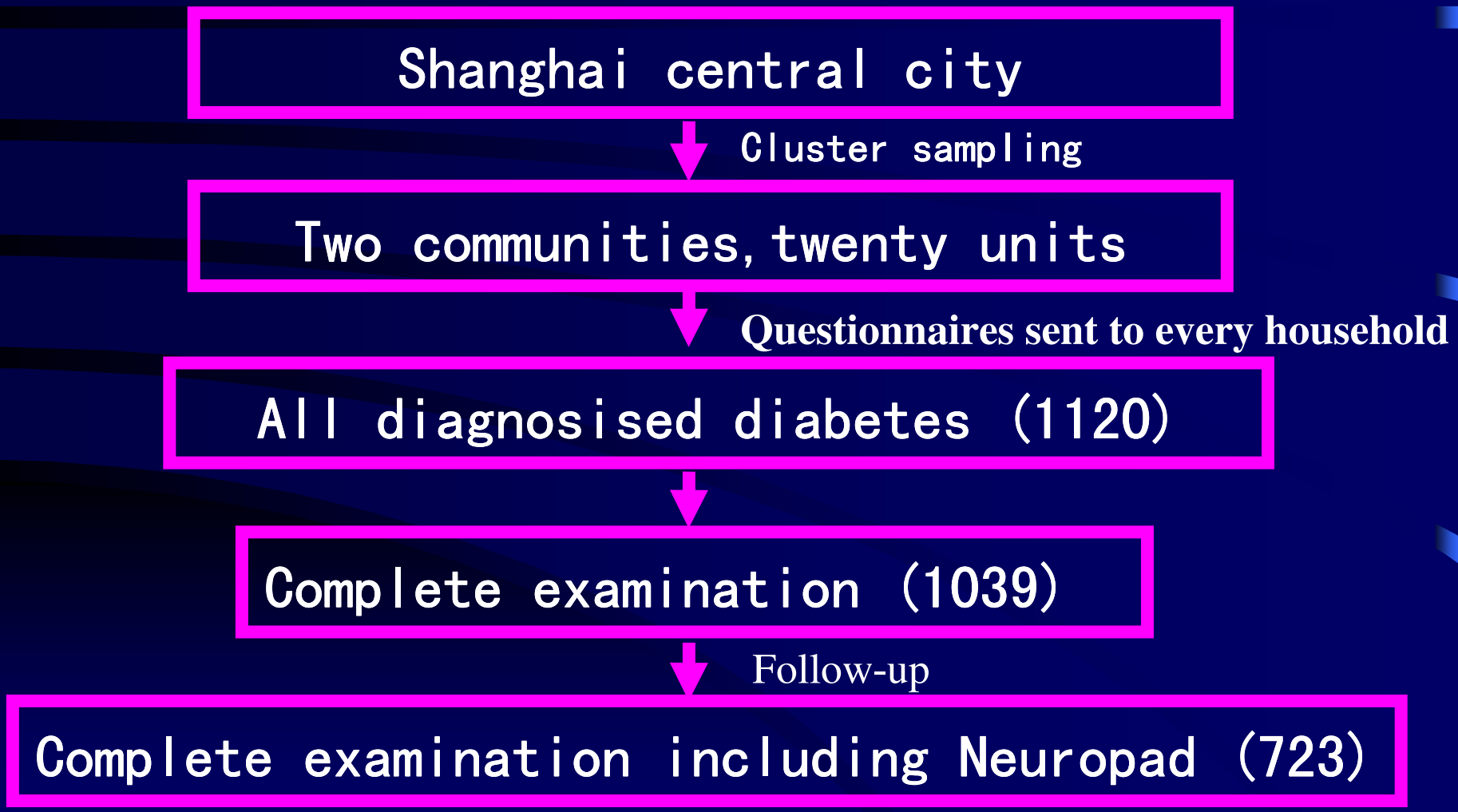
Pink

Aim

- To evaluate whether the new indicator plaster (Neuropad) was a suitable screening test for diabetic neuropathy
- To evaluate the agreement of the average colour change time of Neuropad between the right and left foot

Patients and Methods

1. Patients


Shanghai central city
Cluster sampling
Two communities, twenty units
Questionnaires sent to every household
All diagnosised diabetes (1120)
Complete examination (1039)
Follow-up
Complete examination including Neuropad (723)

2. Screening marker of chronic diabetic complications

(1) Neuropad:

Neuropad reaction times (seconds)

pink start and pink completely

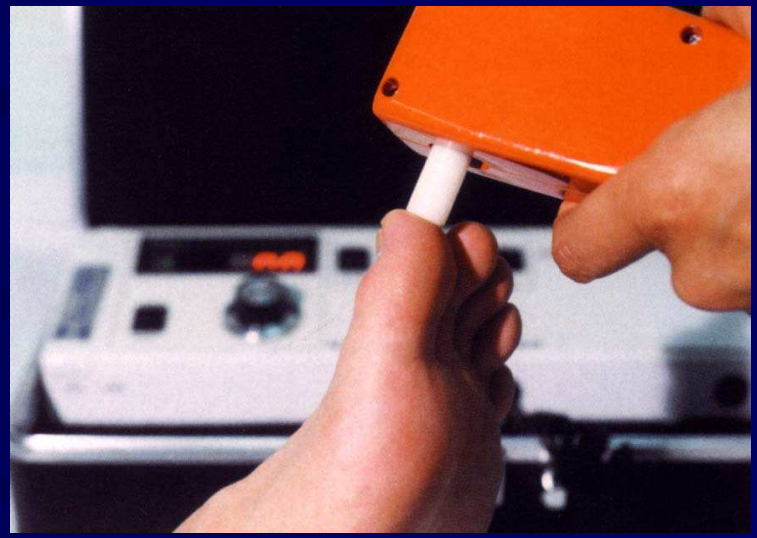
left and right foot

Recommended cutpoint

600 seconds (previous studies)

- Neuropad: the metatarsal bone I / II level on the right and left foot.
- During the examination itself, the patient sat on a chair situated in front of him or her. We allowed a minimum period of 5 minutes between removing the shoes and socks and taking the measurement.

(2) Vibration Perception Threshold (Biomedical Instrument Company, Newbury, OH)



Predicts foot ulceration

0-15V- low risk

16-25V- intermediate

>25V- high risk

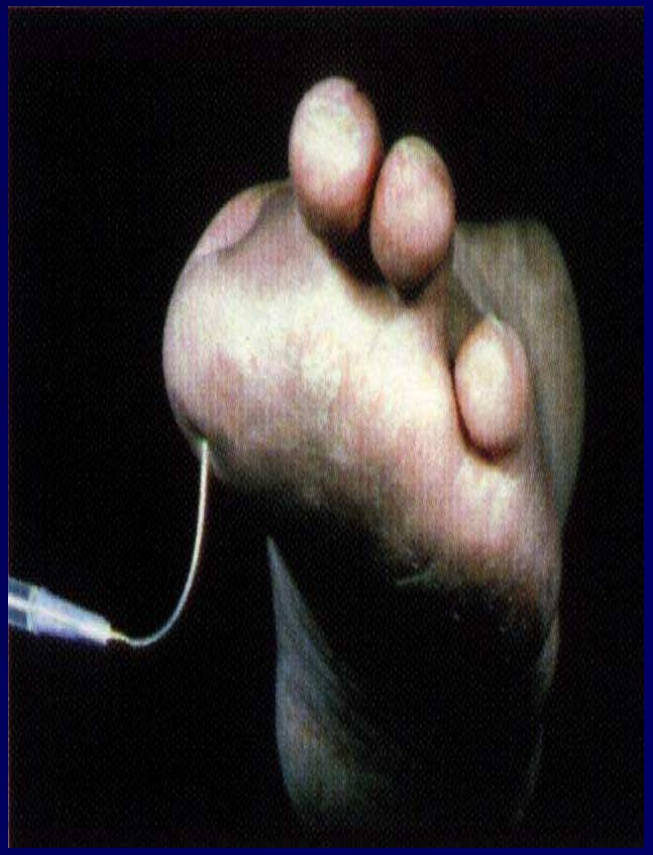
Abnormal: >25V （average of three measurements ）

(3) vibration perception (using a 128-Hz tuningfork)

Abnormal: no sense of vibration

(4) 10g monofilament

Abnormal: no sense <=7 point among the 10 points or no sense in the 1 or 5 MPJ



(5) Diagnosis of DPN

- Three tests： 128M tuning fork, the vibration perception threshold and 10 g monofilament
- at least two of the three tests were abnormal

(6) Diabetic retinopathy

- digital non-mydriatic fundus photography (Canon CR6-45NM camera)
- International clinical diabetic retinopathy and diabetic macular edema disease severity scales (Next page)

(1) No apparent Retinopathy: no abnormalities;

(2) Non-Proliferative Diabetic Retinopathy (NPDR):

1) Mild: Microaneurysms only;

2) Moderate: More than just microaneurysms but less than Severe NPDR;

3) Severe: Any of the following: Extensive (>20) intraretinal hemorrhages in each of 4 quadrants; Definite venous beading in 2+ quadrants; Prominent IRMA in 1+ quadrant and no signs of proliferative retinopathy;

(3) Proliferative Diabetic Retinopathy (PDR): One or more of the following: Neovascularization, Vitreous/preretinal hemorrhage.

3. Statistical analysis

(1) The agreement of Neuropad reaction times (Neuropad placed at the plantar surface of left feet and both feet)

One-way ANOVA, Mann-Whitney Test or Kruskal-Wallis H Test

Spearman correlation

difference: times (L) – times (R)

absolute difference

mean prediction error ＝Σ偏差(i)/N (i=1～N)

(2) The correlation between Neuropad reaction times and DPN

One way ANOVA or spearman correlation

(3) The risk factors associated with the Neuropad reaction times were determined

Spearman correlation

(4) The sensitivity, specificity, positive predictive value and negative predictive value of Neuropad in diagnosing DPN

Results

1. Neuropad reaction time (Mean) graded by gender

parameter	Male	Female	P value
Pink start (Left)	200	195	0.926
Pink start (Right)	201	180	0.473
Pink completely (Left)	960	1020	0.283
Pink completely (Right)	1135	1145	0.461

Mann-Whitney Test

2. Neuropad reaction time (Mean) graded by age

parameter	Age				
	<=60	61-70	71-80	>80	P value
Pink start (Left)	132	188	240	300	<0.001
Pink start (Right)	123	180	240	315	<0.001
Pink completely (Left)	830	1020	1158	1635	<0.001
Pink completely (Right)	800	1170	1320	1710	<0.001

3. The agreement of Neuropad reaction times (Left and Right)

Discription of difference (seconds)

parameter	absolute difference (Mean)	mean prediction error
Pink start	5	-12.5
Pink completely	50	-15.0

Discription of difference (seconds)

parameter	Left	Right	P value
Pink start	200	190	0.468
Pink completely	1020	1140	0.998

Correlation of Neuropad reaction time (Left and Right)

parameter	correlation coefficient	P value
Pink start	0.892	<0.001
Pink completely	0.841	<0.001

Spearman correlation

Correlation of Neuropad reaction time (Left and Right) graded by age

Age	Neuropad Reaction Time	correlation coefficient	P value
<=60	Pink start	0.884	<0.001
	Pink completely	0.847	<0.001
61-70	Pink start	0.897	<0.001
	Pink completely	0.869	<0.001
71-80	Pink start	0.849	<0.001
	Pink completely	0.756	<0.001
>80	Pink start	0.761	<0.001
	Pink completely	0.774	<0.001

Correlation of Neuropad reaction time (Left and Right) graded by diabetic peripheral neuropathy (DPN)

Age	Neuropad Reaction Time	correlation coefficient	P value
DPN = yes	Pink start	0.936	<0.001
	Pink completely	0.776	<0.001
DPN = no	Pink start	0.890	<0.001
	Pink completely	0.855	<0.001

4. Risk factors associated with Neuropad reaction time --- pink start (Right)

	correlation coefficient	P value
Age	0.273	<0.001
Duration of diabetes	0.300	<0.001
Waist circumference	0.130	0.003
BUN	0.100	0.025
Cr	0.121	0.007
LDL	0.103	0.022
Cholesterol	0.091	0.042

Spearman correlation

Risk factors associated with Neuropad reaction time --- pink completely (Right)

	correlation coefficient	P value
Age	0.355	<0.001
Duration of diabetes	0.349	<0.001
BUN	0.098	0.013
Cr	0.082	0.039
Uric acid	0.117	0.009

Spearman correlation

5. Neuropad reaction time (Mean) and Diabetic peripheral neuropathy

parameter (seconds)	DPN=yes	DPN=no	P value
Pink start (Left)	251	180	0.009
Pink start (Right)	245	180	0.011
Pink completely (Left)	1200	1050	0.047
Pink completely (Right)	1230	970	0.013

Mann-Whitney Test

Correlation between Neuropad reaction time and DPN

	10g monofilament	128-Hz tuning fork	Vibration Perception Threshold	DPN
Pink start (Left)	0.089 (0.049)	0.072 (0.114)	0.200(<0.001)	0.119 (0.009)
Pink start (Right)	0.094(0.039)	0.067(0.140)	0.215(<0.001)	0.116(0.010)
Pink completely (Left)	0.061(0.181)	0.058(0.207)	0.112(0.015)	0.083(0.047)
Pink completely (Right)	0.061(0.180)	0.051(0.263)	0.142(0.002)	0.096(0.035)

Data were presented as correlation coefficient (P value)

Spearman correlation

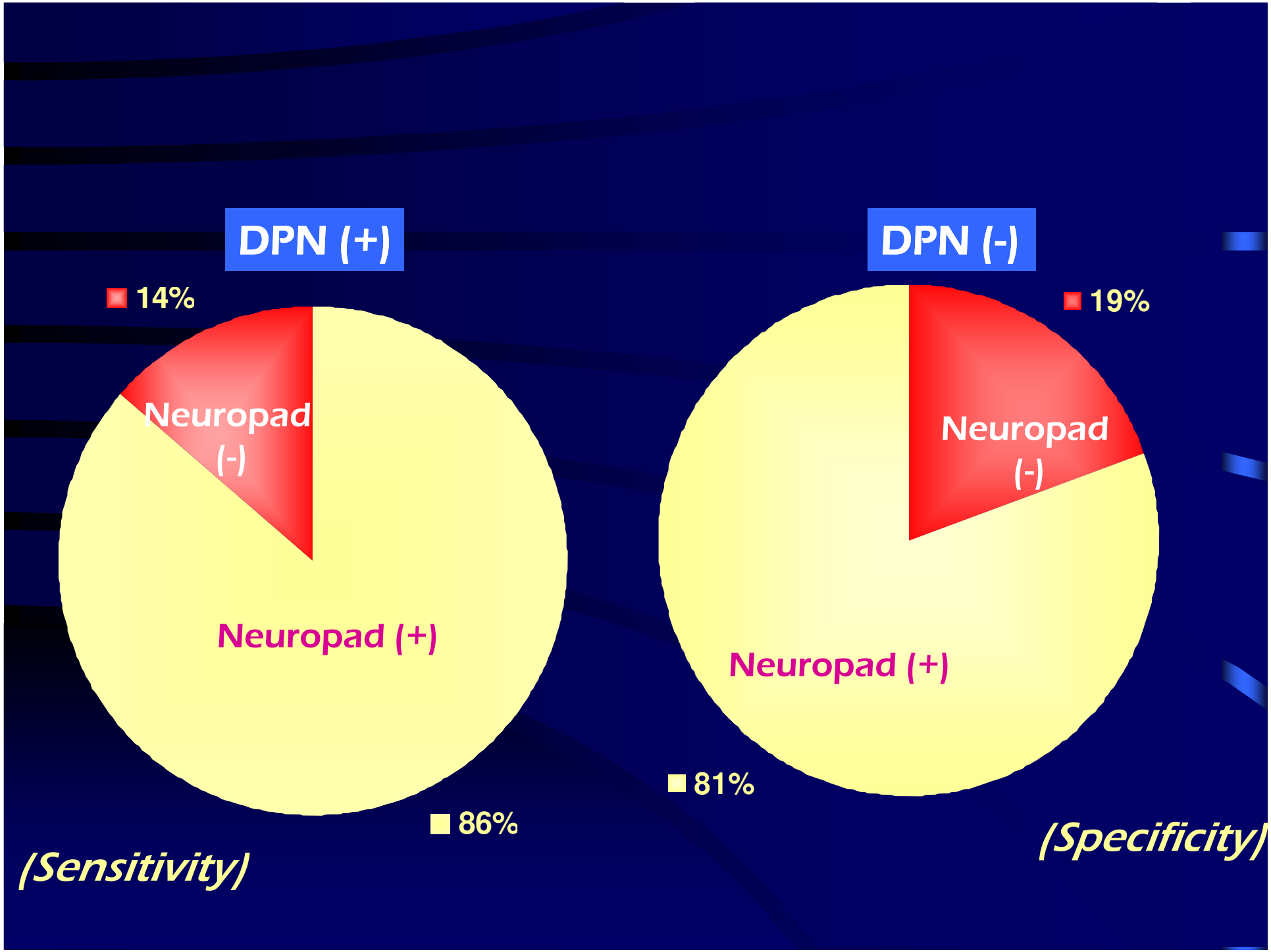
6. Neuropad reaction time (Mean) and Diabetic retinopathy (DR)

parameter (seconds)	DR=yes	DR=no	P value
Pink start (Left)	180	193	0.530
Pink start (Right)	180	190	0.475
Pink completely (Left)	1000	990	0.968
Pink completely (Right)	1140	1125	0.763

Mann-Whitney Test

7. The determination of the recommended cutpoint of Neuropad reaction time (pink completely)

	Left	Right
Sensitivity	86.3%	86.3%
Specificity	19.4%	19.4%
Positive predictive value	22.2%	22.2%
Negative predictive value	84.1%	84.1%


DPN (+)
14%
Neuropad (-)
Neuropad (+)
86%
(Sensitivity)
DPN (-)
19%
Neuropad (-)
Neuropad (+)
81%
(Specificity)

Conclusion

- Neuropad is an easy to handle, objective test
- The cutpoint of Neuropad reaction time should be graded by age
- The agreement of Neuropad reaction time (Left and Right) is satisfactory
- The risk factors associated with Neuropad reaction time are age, duration of diabetes, waist, BUN, Cr, LDL and so on

- Neuropad might be a good screening marker for diagnosing DPN
- The cutpoint of Neuropad reaction time (pink completely) recommended by previous studies might not be suitable for the Chinese population

Thank you