Peripheral nerve damage : diagnosis and prediction of recovery

Datema, M.

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The diagnostic value of water immersion skin wrinkling and Neuropads® in small fiber neuropathy

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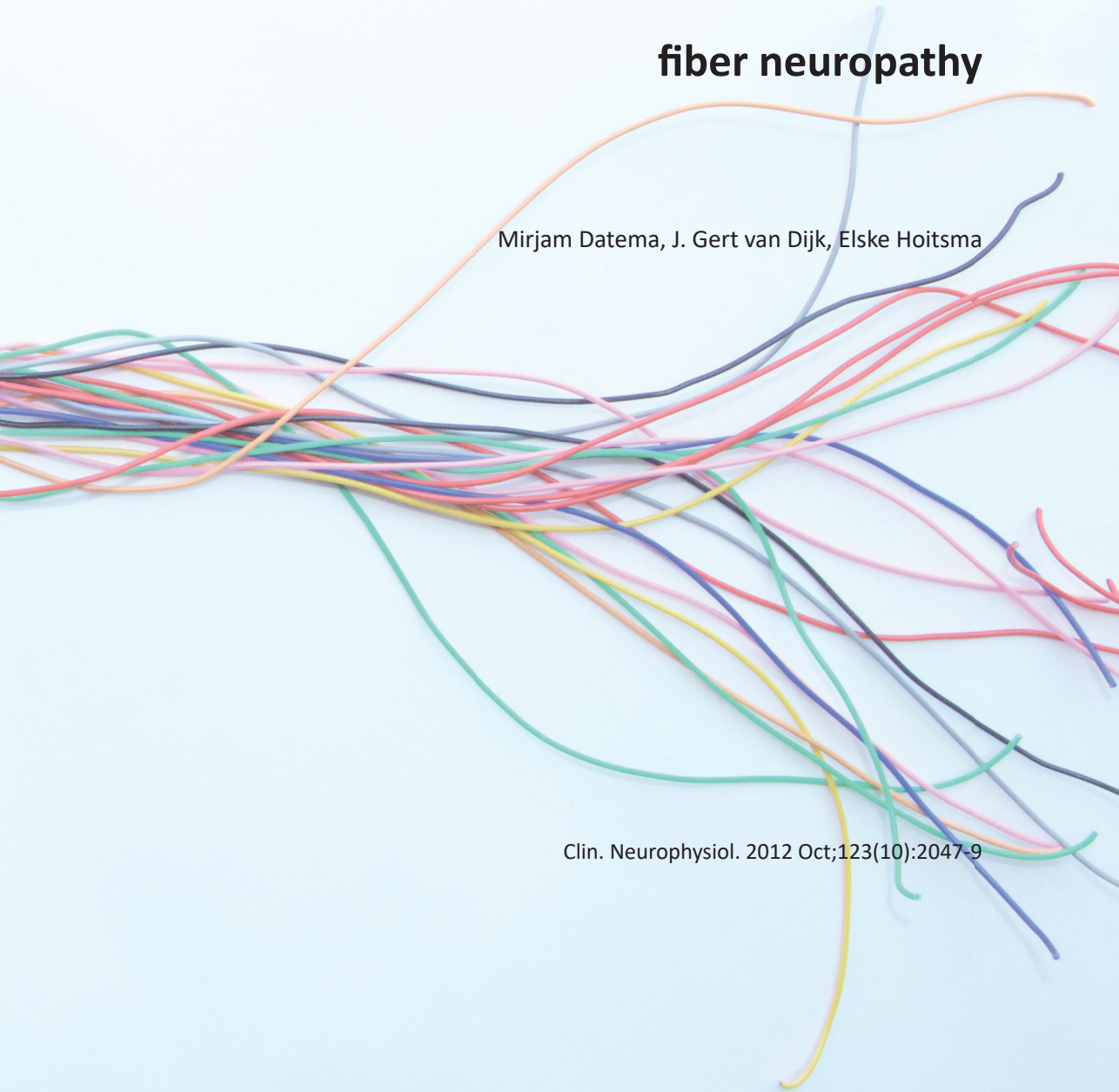


Clin. Neurophysiol. 2012 Oct;123(10):2047-9

ABSTRACT

Objective: To investigate the diagnostic yield of two cheap and practical tests for small fiber neuropathy: water immersion skin wrinkling (WISW) and Neuropads®.

Methods: We studied 35 patients with SFN and 61 age- and sex-matched healthy controls. Wrinkling was judged as absent (abnormal), or present (normal) after immersion of the hands for 30 minutes. Neuropads® were stuck to the soles of the feet; without moisture pads remained blue (abnormal) or turned pink (normal).

Results: The sensitivity of the Neuropad® was 29% and its specificity 93%. The sensitivity of WISW was 66% and its specificity 70%. Regarding abnormality of at least one test to define the combination as abnormal yielded a sensitivity of 71% and specificity 67%. When both tests had to be abnormal for the combination to be abnormal, sensitivity of the combination was 23% and specificity 97%.

Conclusions: The Neuropad® has a high specificity, so an abnormal result can be used to confirm SFN confidently. WISW has a moderate sensitivity and specificity. Combining these two tests can be helpful: when both tests are abnormal the diagnosis SFN is highly likely.

Significance: The Neuropad® and WISW can be helpful in diagnosing small fiber neuropathy in daily practice.

INTRODUCTION

Small fiber neuropathy (SFN) is a disorder of the peripheral nerves predominantly affecting C and Aδ- fibers. SFN is clinically characterized by neuropathic pain and paraesthesias in a stocking-glove pattern along with autonomic symptoms including impaired sudomotor function. Clinical examination and nerve conduction studies can be wholly normal, creating a need for practical diagnostic tests. Currently used tests mainly involve quantitative sensory testing (QST)[1-4] cardiovascular and autonomic tests[5] and intraepidermal nerve fiber density measurement in skin biopsy (IENFD).[6] QST, while useful, is not ideal, as it is most effective *in concert* with other diagnostic modalities.[2] It requires cooperation and judgment from patients, and QST devices are expensive. Skin biopsy is expensive and only available in a few centres.

The Neuropad® is marketed as a cheap and easy test of sudomotor function to screen for early peripheral neuropathy in patients with diabetes mellitus. Several studies reported sensitivity ranging from 94-98% and specificity 70-96%, comparing diabetic patients with and without clinical neuropathy.[7-9] To our knowledge, the Neuropad has not been evaluated in disorders other than diabetes mellitus. Skin-wrinkling after prolonged immersion in water is a normal phenomenon dependant on the sympathetic nervous system.[10] Impairment of water-induced wrinkling was found to correlate with low IENFD in 21 patients with foot and hand dysaesthesia by Teoh et al.[11] The objective of this prospective study was to investigate the diagnostic yield of two cheap, non-invasive, practical bed-side tests for SFN: Neuropads® and water-immersion skin wrinkling (WISW).

MATERIAL AND METHODS

The study was performed with approval of the local ethics committee. From April 1 until September 15, 2010, all consecutive patients visiting the neurology department of the Diaconessenhuis in Leiden with a clinical diagnosis of SFN were included.

Subjects

The following definition of clinical SFN was adopted as gold standard for this study: *Symptoms of SFN*; *at least two of these*: 1. distal symmetrical dys-/paraesthesias 2. burning/painful feet worsening at night 3. intolerance of sheets touching the legs or feet. The *neurological examination should be consistent with isolated SFN,* meaning that tendon reflexes should be normal, muscle weakness should be absent, while sensory testing may be either normal or abnormal.[12,13] Complaints of autonomic failure such as sicca syndrome, hyper/anhydrosis, constipation/diarrhea, blurry vision, and orthostatic intolerance could be present but were not part of the definition.

Sixty-one healthy age-matched controls were recruited from partners of SFN patients, partners of other patients visiting the hospital, friends and hospital staff. Exclusion criteria for healthy controls were diabetes mellitus, alcohol abuse defined as drinking more than two units of alcohol per day, auto-immune disease, complaints of burning/tingling feet or hands and actual or previous neurological condition.

Study protocol

For both patients and controls tests were performed according to the same protocol. Age, weight, length, current medication and medical history were recorded. The severity of disease was established using the Small Fiber Neuropathy Screening List (SFNSL).[14] Subjects were asked to lie down with bare feet. Room temperature was between 18 and 24°C. Testing of all subjects was performed prospectively by the same examiner (MD).

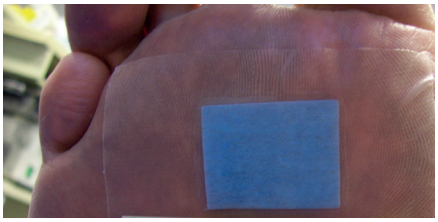
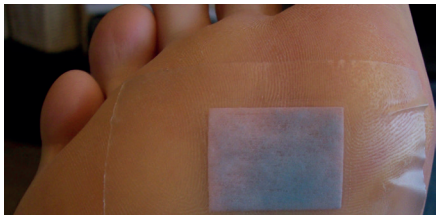
The Neuropad

The Neuropad® (Miro Verbandstoffe GmbH, D-51674 Wiehl/Germany, www.neuropad.com) is an adhesive pad impregnated with a blue cobalt II chloride solution. When cobalt gets in contact with water (sweat) its color changes from blue to pink. According to manufacturer's instructions, the Neuropad test is considered normal when the pad turns completely pink after 10 minutes. When the pad is only partially pink or remains completely blue, the test is considered abnormal. The Neuropad was applied on both feet on the metatarsal head of the first toe of both feet, or, in case of extensively callused feet, on the metatarsal head of the small toe. Subjects took off shoes and socks at least 10 minutes before the Neuropad was applied. (2007) Skin temperature of the feet was recorded (FirstTemp Genius Sherwood medical UK.) Photographs of the Neuropad on the feet were taken at 10 and 15 minutes after applying. (Kodak EasyShare C613). One observer blinded to the health status of the subjects graded the photographs of the Neuropad. Results were classified into three categories: a score of 0.0 = blue, 0.5 = patchy, 1.0 = pink for the Neuropad on each foot at 10 and at 15 minutes (figure 1a, 1b and 1c). We combined the results of both feet: the test was considered abnormal when the Neuropad remained blue in one or both feet.

WISW

Photographs of the palmer side of the hands were taken before and after testing. Subjects placed both hands in a bowl of warm tap water for 30 minutes. For practical purposes this was done while Neuropads were applied to the feet. At the start of the test, the temperature of the water was 40^{o}C.

Results were scored by the same observer who scored the Neuropads. Score 0.0 = not wrinkled 0.5 = minimally wrinkled and 1.0 = obviously wrinkled, compared with the pictures of the hand before water immersion. The pulp of the fingertips of digit II-V was judged. Wrinkling had to be present in at least one digit to merit a positive score (figure 2a, 2b and 2c). In case wrinkling had been already present before water immersion, which was very rare, the observer needed to be convinced wrinkling had increased to be able to assign a positive score. We analyzed results of both hands: when one or both hands showed absence of wrinkling the test was considered abnormal.

a)  b) 

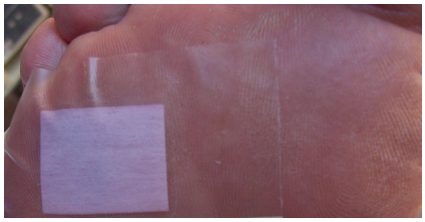
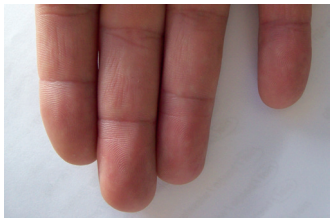
c) 

Figure 1a-c. The results of the Neuropad after 15 minutes on the foot soles of three different subjects.

(a) Blue Neuropad = score 0,0 ; (b) Patchy Neuropad = score 0,5; (c) Pink Neuropad = score 1,0

Interobserver variability

Two additional examiners observed pictures of both Neuropad and WISW in order to assess interobserver variability. The examiners were blinded for the health status of the subjects and for each other's scores. All examiners were given the same set of written instructions including pictures to illustrate what should be seen as absence of wrinkling, minimally wrinkled and obviously wrinkled. After reading these carefully, all three observers judged the pictures of the hands and feet. Note that data on diagnostic yield in this study derived from one examiner to mimic daily practice.

a)  b) 

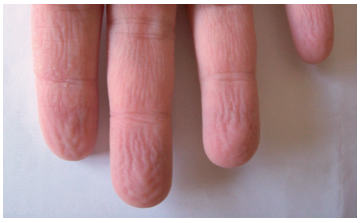
c) 

Figure 2a-c. The results of WISW of the fingertips of three different subjects.

(a) Not wrinkled = score 0,0; (b) Minimally wrinkled = score 0,5; (c) Obviously wrinkled = score 1,0

Statistical analysis.

Statistical analyses were performed using the Statistical Package for Social Sciences version 17.0. Age and BMI and sex of patients and controls were compared using the two-sample t-test and chi-square test respectively. Bivariate analysis was performed on results of the Neuropad and water-immersion skin wrinkling using chi-square test. Interobserver variability was assessed using kappa and improvement of observer variability by paired-samples t-test. Significance was defined as $p < 0.05$.

RESULTS

Table 1 shows demographic and clinical data of 35 patients and 61 healthy controls. Age, sex and BMI did not differ significantly between groups. Patients scored significantly higher on the SFNSL than controls, with a mean difference of 28.6 points. Eight patients had an established diagnosis of sarcoidosis. Five patients had diabetes mellitus, two of them using insulin. Another five patients had disturbances of glucose metabolism but did not use medication yet. Three patients had hypothyroidism; two indulged in alcohol use; one had Sjögren's syndrome, and one had psoriasis. One patient had a history of breast cancer and chemotherapy. Ten patients were diagnosed idiopathic small fiber neuropathy after testing for diabetes mellitus, vitamin B deficiency and thyroid dysfunction. Routine nerve conduction studies were performed on 23 patients with normal results. The other twelve patients either refused nerve conduction studies or were not tested because the a priori chance on finding abnormalities was deemed too low. No adverse events, specifically no allergic reactions, were reported by any of the subjects.

Table 1. Demographic and clinical characteristics of studied control subjects and patients with SFN.

Characteristic	Patients with SFN n = 35	Healthy controls n = 61	P-value
Age (year)	56.4 ± 14.3	52.4 ± 15.8	0.216
Female (%)	69	53	0.123
BMI	25.3 ± 4.9	24.4 ± 2.9	0.267
SFNSL	33.7 ± 16.3	5.1 ± 5.4	<0.001

Data are expressed as mean ± SD. n = number of cases; SFN = small fiber neuropathy; BMI = body-mass index; SFNSL = score on small fiber neuropathy screening list

Interobserver variability

The Neuropad

Interobserver agreement was fair for both feet after 10 and 15 minutes with kappa ranging from 0.64 to 0.79 ($p < 0.001$). Variability was due to disagreement about category 0 or 0.5 and 0.5 or 1. Disagreement on categories 0 versus 1 did not occur.

WISW

Interobserver agreement was moderate, with kappa ranging from 0.48 to 0.65. As with the Neuropad variability was largely due to disagreement about category 0.5.

Diagnostic accuracy

The Neuropad

In contrast with the manufacturer's instructions, we considered the test abnormal only when the Neuropad remained blue entirely (the manufacturer's instructions suggest that a partial change of colour should also be considered abnormal). The agreement between results of both feet was fair after 10 minutes (kappa 0.75) but less good after 15 minutes (kappa 0.59). Further analysis was performed with the results of both feet combined: when the Neuropad remained blue in one or both feet the test was considered abnormal. When both feet showed some degree of color change the result was considered normal. After 10 minutes 17 of 35 patients had one or two blue Neuropads and 47 of 61 healthy subjects showed color change on both Neuropads. Sensitivity of the Neuropad after 10 minutes was 49% and specificity 77% (table 2). After 15 minutes sensitivity lowered to 29% with an increase of specificity to 93% (table 2).

WISW

Obvious and minimal wrinkling were considered normal; only complete absence of wrinkling was considered abnormal. The agreement of test results between left and right hands was moderate (kappa 0.69). Results of both hands were used in the analysis: when one or both hands showed absence of wrinkling the test was considered abnormal. Of 35 patients, 23 showed absence of wrinkling on at least one hand; 43 of 61 healthy subjects showed some degree of wrinkling on both hands. Sensitivity of WISW was 66% and specificity 70% (table 2).

Combination of WISW and the Neuropad

Counting abnormality of one or two tests as an abnormal combination yielded a sensitivity of 71% and specificity of 67% (combination 1, table 2). When both tests had to be abnormal for the combination to be abnormal, sensitivity of the combination was 23% and specificity 97% (combination 2, table 2)

Table 2. Results of the Neuropad, WISW and combinations of these, n = 96.

Test	Patients with SFN n = 35	Healthy controls n = 61	P-value
Neuropad 10 min.	17 (49)	14 (23)	0.030
Neuropad 15 min.	10 (29)	4 (7)	0.003
WISW	23 (66)	18 (30)	0.001
Combination 1	25 (71)	20 (33)	<0.001
Combination 2	8 (23)	2 (3)	0.003

Data are numbers of subjects with abnormal result on the tests with percentage in parentheses.
Neuropad: abnormal = the Neuropad remained blue in one or both feet; normal = colour change in both feet;
WISW: abnormal = absence of wrinkling in one or both hands; normal = some degree of wrinkling in both hands;
Combination 1: abnormal = either result of WISW or result of the Neuropad abnormal, or both abnormal; normal = results of WISW ánd the Neuropad normal;
Combination 2: abnormal = result of WISW ánd result of Neuropad abnormal; normal = normal result on WISW or Neuropad or normal result on both tests; SFN = small fiber neuropathy; min. = minutes; WISW = water immersion skin wrinkling

Foot temperature

Measurements of foot temperature were missing for three subjects. Mean temperature of left and right feet did not differ significantly (p = 0.8). The mean temperature of the left foot of patients (28.3 $\pm$ 3.2 °C) did not differ significantly from that of controls (29.7 $\pm$ 3.5 °C; p = 0.061). There was a significant difference in foot temperature between subjects with abnormal and normal Neuropad results. Temperature of the left foot of 11 subjects with an abnormal Neuropad result was 26.6 $\pm$ 1.5 °C, while it was 29.5 $\pm$ 3.5 °C in 81 subjects with a patchy or pink Neuropad (p <0.001).

Age

Subjects with abnormal result of the Neuropad were 15.3 years older than subjects with normal result of the Neuropad (p = <0.001). The mean age of subjects with normal or abnormal results of WISW did not differ significantly (table 3).

Score on SFNSL

Subjects with abnormal test results of the Neuropad scored 15.0 points more on the SFNSL than subjects with a normal result of the Neuropad (p = 0.003). For WISW this difference was 8.9 points (p = 0.006; table 3). These data are graphically displayed in figure 3.

Table 3. Mean age and mean score on SFNSL of subjects with abnormal and normal results of WISW and the Neuropad.

Test result (n)	Age (y)	p-value	SFNSL	p-value
Neuropad abnormal (14)	66.9 ± 11.0	<0.001	28.3 ± 18.7	0.003
Neuropad normal (82)	51.6 ± 14.9	<0.001	13.3 ± 16.4	0.003
WISW normal (55)	51.8 ± 15.3	0.127	11.2 ± 18.8	0.006
WISW abnormal (41)	56.6 ± 15.2	0.127	20.1 ± 15.2	0.006

Data are expressed as mean ± SD. n = number of cases; WISW = water immersion skin wrinkling; y = year; SFNSL = score on small-fiber neuropathy screening list

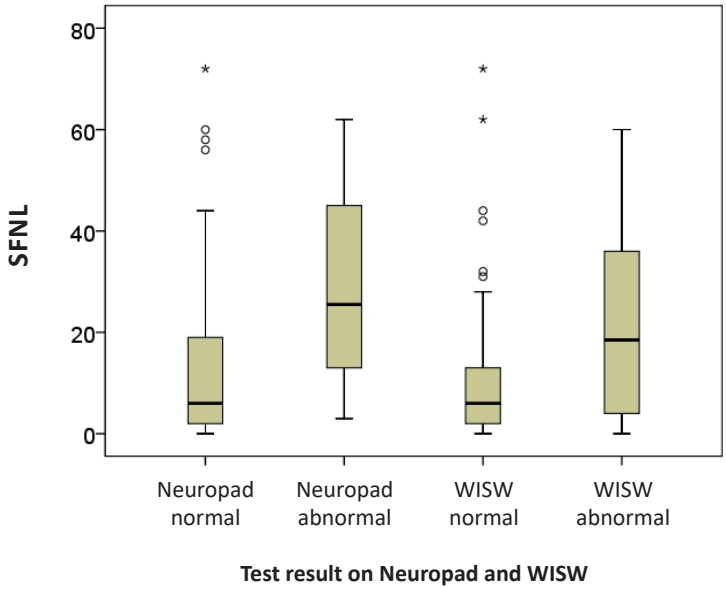


Figure 3 Scores on the SFNSL in boxplots of the patients with normal and abnormal test results on the Neuropad and WISW.

SFNSL = score on small-fiber neuropathy screening list. WISW = water immersion skin wrinkling. The circles and asterisks represent outliers. Significance: Neuropad abnormal versus normal p = 0.003, WISW abnormal versus normal p = 0.006.

DISCUSSION

The present study shows the diagnostic value of WISW and the Neuropad. Both WISW and the Neuropad revealed significantly more abnormal outcomes in a population of SFN patients compared to a population of healthy subjects. Neuropad® had a high specificity when applied for 15 minutes and can thus be used in daily practice to confirm SFN. Water immersion skin wrinkling had a moderate sensitivity and specificity. Combining these two tests did not raise diagnostic accuracy much, but the diagnosis SFN can be considered quite certain when both tests are abnormal.

Test results were scored blindly. Interobserver variability was studied by comparing independent judgments of three observers. Reproducibility was not investigated in this study, but the Neuropad showed good reproducibility in an earlier study with a coefficient of variability of 4.4-4.9%.[15] Wilder-Smith et al repeated WISW and EMLA-induced skin wrinkling in eight subjects on several occasions and reported good reproducibility, although without a quantitative measurement. [16]

The Neuropad

The Neuropad was found to have a high specificity but only when a partial discoloration of the patch was considered normal, in contrast to both the manufacturer's instructions and to the practice of other authors. [8,9,17-20] If we had defined partial discoloration abnormal, sensitivity after 10 minutes application would have been 83% with a very low specificity of 36%. This might be explained by different selection of patients and by different choice of reference standards. Moreover, the Neuropad has never before been tested in patients with SFN or on a large group of healthy controls in a wide age range (20-84 year) as in the present study. The increase of specificity due to the longer interval, i.e., judging Neuropad after 15 minutes instead of 10 minutes, is in accordance with findings by Spallone et al.[20]

To our knowledge no other study on the Neuropad took temperature into consideration. Our results show a relation between temperature and the abnormality rate, to the effect with warmer feet show normal results more often. There are two possible explanations for this result: the first is that abnormal autonomic control is likely to cause low temperature as well as impaired sweating, so both phenomena correctly identify the underlying abnormality. The second explanation is that colder feet produce less sweat than warm ones, in which case the abnormal result could be a measurement artifact. This will have to be assessed in future studies.

Subjects with an abnormal Neuropad were almost 15 years older than subjects with a normal Neuropad. The four healthy controls with abnormal Neuropads were 68-84 years old opposed to the patients with abnormal Neuropads who were 42-73 years old. These four people had no complaints of small fiber neuropathy. Assuming the Neuropad is an adequate measurement of autonomic neuropathy, this suggests that peripheral autonomic sudomotor function deteriorates with age.

WISW

Wrinkling after water immersion results from local vasoconstriction, influenced by vasomotor function.[11] Theoretically, assessing wrinkling of the toes might be more useful than of the fingers, in line with axonal length dependent character of SFN. From that perspective the lower sensitivity of the WISW compared to the Neuropad might simply reflect the measurement site: hands for WISW and feet for the Neuropad. For practical reasons, performing both tests in the same anatomical site was not possible, as the Neuropad is designed for the feet and skin wrinkling of the feet is hard to assess and reproduce. [21]

We took photographs of the hands after water immersion to facilitate blinded scoring of wrinkling, Taking pictures instead of observing the hands in reality has the advantage of the ability to document the result. Unfortunately, the quality of the pictures did not allow scoring in more than three categories as has been done in previous studies when observed in real life. [11,22] The observer assigned a score of 0,5 (minimal wrinkling) as soon as he felt that any change had occurred in the pulpa of one or more digits. This is in line with the cut-off score of grade 2 used by Teoh et al. [11] The results of WISW as a diagnostic test might be improved in future studies using different techniques.

Definition of SFN

Although there is increasing support for accepting skin biopsy with IENF assessment as best diagnostic test for the diagnosis of SFN, no validated objective gold standard yet exists. [3,23,24] In addition, the diagnostic value of skin biopsy is not yet established. Lauria et al. found a sensitivity of 45-90% and a specificity of 95-97% in a review in 2005, Nebuchennykh reported a sensitivity of 78% and a specificity of 64% in 2009.[6,25] In the same year Bakkers et al. found a sensitivity of IENFD of only 33% and a specificity of 86% compared to clinical symptoms of SFN in patients with sarcoidosis.[24] These differences may be the result of differences in neuropathy severity. As a reference standard, the clinical definition of SFN was therefore adopted in this study. [12,13,24] The scores on the previously validated SFNSL[14] correlated well with the results of WISW and the Neuropad, which supports the validity of these tests. Future studies are needed to relate the present results to other diagnostic modalities of SFN.

CONCLUSION

The aim of this study was to investigate the value of water immersion skin wrinkling and Neuropads® in diagnosing SFN. The sensitivity and specificity of the Neuropad after 10 minutes of application are less useful than the results after 15 minutes of application.

Neuropad® has a high specificity when applied for 15 minutes and can thus be used in daily practice to confirm SFN. Water immersion skin wrinkling has a moderate sensitivity and specificity. Combining these two tests does not raise diagnostic accuracy much, but in case both tests are abnormal the diagnosis SFN is quite certain.

It is hard to say whether the diagnostic yield of Neuropad and WISW is better or worse than current tests for SFN because data on diagnostic value differ. Clearly there are many favorable features of the Neuropad and WISW. They are cheap, non-invasive, easy accessible, non-time consuming and do not require patient cooperation or specialized personnel. Interobserver variability of the Neuropad was reasonably good. Interobserver variability of WISW is acceptable, although clear instructions for the observer are essential.

ACKNOWLEDGEMENTS

Our gratitude towards M. van Graas and M.C. van Breukelen for judging all the pictures. Neuropads were free of charge supplied by Bap Medical BV, Apeldoorn, the Netherlands.

REFERENCES

1. Magda P, Latov N, Renard MV, Sander HW. Quantitative sensory testing: high sensitivity in small fiber neuropathy with normal NCS/EMG. Journal of the peripheral nervous system : JPNS 2002;7:225-8.

2. Shy ME, Frohman EM, So YT, et al. Quantitative sensory testing: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2003;60:898-904.

3. Tavee J, Zhou L. Small fiber neuropathy: A burning problem. Cleveland Clinic journal of medicine 2009;76:297-305.

4. Vinik AI, Suwanwalaikorn S, Stansberry KB, Holland MT, McNitt PM, Colen LE. Quantitative measurement of cutaneous perception in diabetic neuropathy. Muscle & nerve 1995;18:574-84.

5. Santiago S, Ferrer T, Espinosa ML. Neurophysiological studies of thin myelinated (A delta) and unmyelinated (C) fibers: application to peripheral neuropathies. Neurophysiologie clinique = Clinical neurophysiology 2000;30:27-42.

6. Lauria G, Cornblath DR, Johansson O, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. European journal of neurology 2005;12:747-58.

7. Papanas N, Giassakis G, Papatheodorou K, et al. Sensitivity and specificity of a new indicator test (Neuropad) for the diagnosis of peripheral neuropathy in type 2 diabetes patients: a comparison with clinical examination and nerve conduction study. Journal of diabetes and its complications 2007;21:353-8.

8. Papanas N, Papatheodorou K, Christakidis D, et al. Evaluation of a new indicator test for sudomotor function (Neuropad) in the diagnosis of peripheral neuropathy in type 2 diabetic patients. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2005;113:195-8.

9. Papanas N, Papatheodorou K, Papazoglou D, Christakidis D, Monastiriotis C, Maltezos E. The new indicator test (Neuropad): a valuable diagnostic tool for small-fiber impairment in patients with type 2 diabetes. The Diabetes educator 2007;33:257-8, 60, 62 passim.

10. Wilder-Smith EP. Water immersion wrinkling--physiology and use as an indicator of sympathetic function. Clinical autonomic research : official journal of the Clinical Autonomic Research Society 2004;14:125-31.

11. Teoh HL, Chow A, Wilder-Smith EP. Skin wrinkling for diagnosing small fibre neuropathy: comparison with epidermal nerve density and sympathetic skin response. Journal of neurology, neurosurgery, and psychiatry 2008;79:835-7.

12. Stewart JD, Low PA, Fealey RD. Distal small fiber neuropathy: results of tests of sweating and autonomic cardiovascular reflexes. Muscle & nerve 1992;15:661-5.

13. Tobin K, Giuliani MJ, Lacomis D. Comparison of different modalities for detection of small fiber neuropathy. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 1999;110:1909-12.

14. Hoitsma E, De Vries J, Drent M. The small fiber neuropathy screening list: Construction and cross-validation in sarcoidosis. Respiratory medicine 2011;105:95-100.

15. Papanas N, Papatheodorou K, Papazoglou D, Christakidis D, Monastiriotis C, Maltezos E. Reproducibility of the new indicator test for sudomotor function (Neuropad) in patients with type 2 diabetes mellitus: short communication. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2005;113:577-81.

16. Wilder-Smith E, Chow A. Water immersion and EMLA cause similar digit skin wrinkling and vasoconstriction. Microvascular research 2003;66:68-72.

17. Kamenov ZA, Petrova JJ, Christov VG. Diagnosis of diabetic neuropathy using simple somatic and a new autonomic (neuropad) tests in the clinical practice. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2010;118:226-33.

18. Papanas N, Papatheodorou K, Papazoglou D, Kotsiou S, Maltezos E. A prospective study on the use of the indicator test Neuropad(R) for the early diagnosis of peripheral neuropathy in type 2 diabetes. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2011;119:122-5.

19. Quattrini C, Jeziorska M, Tavakoli M, Begum P, Boulton AJ, Malik RA. The Neuropad test: a visual indicator test for human diabetic neuropathy. Diabetologia 2008;51:1046-50.

20. Spallone V, Morganti R, Siampli M, et al. Neuropad as a diagnostic tool for diabetic autonomic and sensorimotor neuropathy. Diabetic medicine : a journal of the British Diabetic Association 2009;26:686-92.

21. Clark CV, Pentland B, Ewing DJ, Clarke BF. Decreased skin wrinkling in diabetes mellitus. Diabetes care 1984;7:224-7.

22. Wilder-Smith EP, Guo Y, Chow A. Stimulated skin wrinkling for predicting intraepidermal nerve fibre density. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 2009;120:953-8.

23. England JD, Gronseth GS, Franklin G, et al. Evaluation of distal symmetric polyneuropathy: the role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). Muscle & nerve 2009;39:106-15.

24. Bakkers M, Merkies IS, Lauria G, et al. Intraepidermal nerve fiber density and its application in sarcoidosis. Neurology 2009;73:1142-8.

25. Nebuchennykh M, Loseth S, Lindal S, Mellgren SI. The value of skin biopsy with recording of intraepidermal nerve fiber density and quantitative sensory testing in the assessment of small fiber involvement in patients with different causes of polyneuropathy. Journal of neurology 2009;256:1067-75.