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Original Article

Sensitivity and specificity of the Neuropad for distal sensory peripheral neuropathy (DSPN) in subjects with HIV-Infection: A case controlled observational study

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ABSTRACT

Objective: The aim of the current study was to assess the diagnostic accuracy of the Neuropad for detecting distal sensory peripheral neuropathy (DSPN) related to human immunodeficiency virus (HIV)-infection.
Design: Single-gate observational case-control study.
Methods: A convenience sample of 30 HIV-infected subjects with a mean age of 48.9 years (95% CI 45.6–52.3) and no other potential causes of neuropathy were recruited from the Ian Charleson Day Centre (London, UK). Sudomotor function was assessed with the Neuropad, an adhesive patch designed to measure sweat production, using a 10-min cut-off. Vibration perception threshold (VPT) testing with a neurothesiometer and the painDETECT questionnaire (PD-Q) were used as reference standard measures of DSPN.
Results: 16 participants with sudomotor dysfunction and 14 with healthy sudomotor function were included in analysis. Compared to VPT (> 25 V) the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Neuropad yielded 66.7%, 48.1%, 12.5% and 92.9% respectively. For the PD-Q (> 12) sensitivity of the Neuropad was 100% and specificity was 58.3%, with PPV and NPV of 37.5% and 100% respectively.
Conclusions: The Neuropad may be a suitable screening tool for HIV-related DSPN, and can reliably exclude healthy individuals in the clinical setting. Abnormal Neuropad results require referral to confirm diagnosis. Limitations exist in the sampling method and study size. The use of reference standard measures of small nerve fibre function and continuous Neuropad quantification would strengthen future research.

Introduction

The accuracy of a diagnostic test describes its ability to differentiate between individuals who have a target condition and those who do not. Using a single group of participants, a diagnostic test's accuracy can be validated by comparing the outcomes of the test with the results of a reference standard measure. A two-by-two contingency table (Table 1) can be used to summarize these findings and generate values of sensitivity (sensitivity = TP/(TP + FN)) and specificity (specificity = TN/(TN + FP)) for the test being evaluated. While sensitivity refers to the test's ability to correctly identify individuals with the condition of interest, specificity provides a measure of the test's ability to correctly identify individuals without the condition [1].

The accuracy of diagnostic testing for neurological disorders is of particular interest to those who work in the clinical setting. Distal sensory peripheral neuropathy (DSPN) is the most common neurological complication of human immunodeficiency virus (HIV), with more than one-third of patients affected [2,3]. DSPN predominately affects the small sensory fibres, and is characterised by distal axonal degeneration [4]. Symptoms of burning or stabbing pain, paraesthesia, and numbness are reported in a symmetrical distribution affecting the feet more commonly than the hands [5–7]. Reduced vibration and pressure perception are found in a similar distribution, and ankle tendon reflexes are diminished or absent [7–12]. DSPN can lead to substantial disability and a significantly reduced quality of life, emphasising the importance of timely diagnosis [13,14].

Sudomotor dysfunction is another common feature of DSPN. Sudomotor dysfunction causes the skin of the feet to become dry and susceptible to ulceration as a result of damage to the small sympathetic C-fibres that innervate sweat glands [15,16]. Quantitative sudomotor

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Table 1
A 2 × 2 contingency table for evaluating a test's diagnostic accuracy.

	Patients with Condition	Patients without Condition	Totals
Positive Test Result	True Positives (TP)	False Positives (FP)	TP + FP
Negative Test Result	False Negatives (FN)	True Negatives (TN)	FN + TN
Totals	TP + FN	FP + TN	TP + FP + TN + FN

axon reflex testing (QSART) is useful for detecting sudomotor neuropathy [15,17]. Unfortunately this method is expensive and time-consuming, and does not lend itself well to triage in clinical practice [18]. The Neuropad is a non-invasive diagnostic test of sudomotor function that is simple and cost-effective [19,20]. The indicator test is an adhesive patch designed to measure sweat production on the plantar surface of the foot. Healthy sweat production causes a cobalt II compound to change colour from blue to pink.

The usefulness of the indicator test for diabetic DSPN has received considerable attention [21–26]. The Neuropad has consistently demonstrated high sensitivity and excellent reproducibility [21–23,27]. In a systematic review, the indicator test exhibited average sensitivity and specificity values of 86% and 65% respectively [20]. Compared to vibration perception threshold (VPT) testing, the Neuropad exhibits a sensitivity of 97.8% and a specificity of 67.2% [23]. These findings demonstrate the instrument's potential for DSPN screening. Given that sudomotor dysfunction precedes sensory loss, Neuropad testing may enable early detection and prevent disabling complications associated with disease progression [28].

In HIV-infection, DSPN is characterised by a clinical profile similar to that of diabetic DSPN [29,30]. However the diagnostic utility of the Neuropad has yet to be evaluated in this population. With the introduction of highly active antiretroviral therapy (HAART) during the last 20 years, the lifespan of HIV-infected individuals has dramatically increased [31]. Osteopaths are now more likely to work with individuals affected by what has become a chronic, progressive illness.

The objective of the current study was to evaluate the diagnostic accuracy of the Neuropad for assessing DSPN in an HIV-infected population. It was hypothesised that the Neuropad would achieve diagnostic sensitivity of at least 95% for DSPN in HIV-infected individuals.

Materials & methods

Study design

A single-gate observational case-control design was used to evaluate the diagnostic accuracy of the Neuropad index test (Fig. 1). This design is beneficial for assessing diagnostic utility in studies where material and human resources are limited [32]. VPT and the painDETECT questionnaire (PD-Q) were both used as reference standard measures of DSPN.

Participants

Convenience sampling was used to recruit outpatients prospectively from the Ian Charleson Day Centre (ICDC) at Royal Free Hospital in London, UK. The study was performed at the ICDC between 4 and 17 August 2015. Clinical staff identified potentially eligible participants aged 18–65 years with known HIV-infection for screening by members of the research team. Individuals with type I or type II diabetes, lower extremity amputation, and central or peripheral neurological disease unrelated to HIV-infection were excluded.

Ethical approval for this study was granted by the British School of Osteopathy's Research Ethics Committee and the Royal Free Ethics Committee. All participants took part in the study voluntarily and gave informed consent.

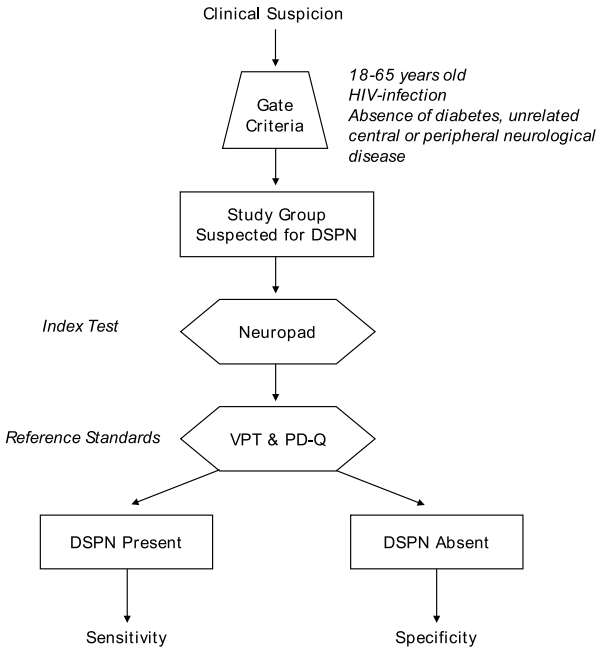

Fig. 1. Single-gate case-control design for assessing the diagnostic accuracy of the Neuropad.

Test methods

Prior to testing, all subjects were seated in an air condition controlled room held at a constant temperature of 20–25 °C with shoes and socks removed for an acclimatisation period of 10 min. This procedure has been consistently implemented in previous Neuropad investigations [18,23,24,26,33–36].

Following the acclimatisation period, subjects were asked to complete the PD-Q in pencil-and-paper form. The PD-Q is a patient-reported questionnaire designed to screen for neuropathic pain [37–39]. It is comprised of 7 questions that address the quality of neuropathic pain symptoms, 1 temporal question regarding pain patterns, and 1 spatial item on pain radiation. Symptoms are measured by self-rating on a scale from 0 (never) to 6 (very strongly), while temporal patterns are graded from −1 to +1. Spatial patterns are graded 0 if radiations are absent or +2 if radiating pain is present. A final score between −1 and 38 is given. The authors of the PD-Q recommend a test positivity cut-off score of 19. The questionnaire has been validated as a reliable screening tool using this protocol, demonstrating 85% sensitivity and 85% specificity [37]. It has good internal consistency for HIV-related DSPN, and has higher sensitivity and specificity than other screening questionnaires for DSPN, including the Leeds Assessment of Neuropathic Symptoms and Signs, the Douleur Neuropathique 4, and the Neuropathic Pain Questionnaire [38,39].

After the PD-Q was completed, VPT was used to assess vibration sense at the feet. Impaired vibration sense as a result of large fibre dysfunction has been well documented in those with DSPN related to both HIV-infection and diabetes [4,5,29,30,40]. VPT is an established measure of diabetic DSPN and has been used as a reference standard for evaluating the performance of the Neuropad in previous investigations [17–19,28,46]. It has high sensitivity (77.3–100%) and specificity

(72.8–81.0%) and demonstrates excellent intra-rater reliability [41,42]. VPT testing is less invasive, less expensive, and less time consuming than other reference standard measures of HIV-related DSPN, making it a practical choice for the research setting.

VPT was measured with a neurothesiometer (Bailey Instruments, Manchester, UK) from the pulp of the dorsum of the hallux of both feet. This testing site is reliable for neuropathy screening [14–17,45,46]. The voltage was gradually increased until the participant reported first feeling the vibration. The test was repeated three times at each site and the mean voltage (V) was recorded [10]. In order to familiarise subjects with the procedure, testing was first performed on each subject's forearm. DSPN was defined in participants exhibiting a high VPT value (> 25 V) in at least one foot. VPT is an effective clinical predictor of neuropathic foot ulceration for this test positivity cut-off in prospective studies [9,10].

The Neuropad (miro Verbandstoffe, Wiehl, Germany) was subsequently applied to a callus-free area on the plantar surface of both feet between the first and second metatarsal heads. The plasters were assessed for colour change 10 min after initial application. Complete colour change from blue to pink in both feet within this 10-min period was graded as a normal sudomotor response. Subjects were considered to have sudomotor impairment if the time until complete colour change exceeded 10 min in at least one foot [21,23]. Although there has been some disagreement in the literature regarding a suitable application time for differentiating normal and abnormal sudomotor responses, Ziegler et al. (2011) demonstrated that extending the screening time beyond 10 min does not improve the test's diagnostic performance [36].

Neuropad and VPT testing were performed by separate investigators. To prevent researcher bias, the individuals responsible for VPT and Neuropad assessment were blinded to the results of the other tests. As the current investigation formed part of a larger study, biomechanical features of the feet of these participants were also measured but not assessed for the purposes of the current investigation. All measures were performed in a single session.

Analysis

Statistical analysis was performed using SPSS 22.0. (Statistical Package for Social Sciences). Receiver operating characteristic analysis generated sensitivity and 1-specificity values to assess the diagnostic accuracy of the Neuropad for VPT and the PD-Q. Data derived from crosstabulation was used to calculate positive predictive values (PPV) and negative predictive values (NPV) [44]. Fisher's exact test was used to test for associations between qualitative variables. Indeterminate or missing Neuropad, PD-Q and VPT results were excluded.

Assuming a sensitivity of at least 95%, a confidence interval of 0.05, and DSPN prevalence of 0.7, a minimum sample size of 27 was required to ensure adequate statistical power [45].

Methods and procedures have been reported according to the most recently published guidelines for studies of diagnostic accuracy [46].

Results

31 outpatients were assessed for eligibility and invited to participate in the study. Fig. 2 shows the flow of participants and outlines the primary outcome measures. Patients who were excluded from the study are noted (Fig. 2). In total, 30 HIV-infected participants (4 women), mean age of 48.9 years (95% CI 45.6–52.3), completed the study. No adverse events occurred using any of the testing modalities. The demographic profile and clinical characteristics of the study population are reported in Table 2.

High VPT (> 25 V) was detected in 3 participants (10%). Sudomotor impairment was identified in 16 subjects (53.3%), 2 with abnormal VPT (66.7%) and 14 without (51.9%) (Table 3). Sensitivity of the Neuropad was 66.7% and specificity was 48.1%, while positive and negative predictive values were 12.5% and 92.9% respectively (Table 4).

High PD-Q scores (≥ 19) were not observed in any of the study participants. There is strong evidence for the use of alternate PD-Q cut-off scores for detecting DSPN in HIV-infection [39]. The original validation study reported the absence of neuropathic pain for scores of 12 and less, and the PD-Q has high sensitivity for this cut-off (> 12) [37,47]. Using a moderate cut-off (> 12), DSPN was identified in 6 subjects (20.0%), and all 6 subjects demonstrated sudomotor dysfunction (Table 5). The sensitivity of the Neuropad was 100% and the specificity was 58.3%, with positive and negative predictive values of 37.5% and 100% respectively (Table 4). Fisher's exact test revealed an association between PD-Q scoring and sudomotor function.

Discussion

This study evaluated the diagnostic accuracy of the Neuropad indicator test for DSPN in HIV-infected individuals using two reference standard measures.

The performance of the Neuropad for the PD-Q could not be evaluated using the recommended test positivity cut-off. When the moderate cut-off (> 12) was used the Neuropad performed better than in previous investigations, yielding 100% sensitivity and 58.3% specificity [20–23]. Low PPV suggests that HIV-infected individuals with a positive Neuropad result should be referred on for specialist assessment to confirm the presence of DSPN. Individuals with a negative Neuropad result can be safely excluded from DSPN diagnosis based on the high NPV exhibited for both reference standards.

The Neuropad did not perform as well as hypothesised when referenced to VPT and demonstrated sensitivity and specificity values that were short of those previously reported for diabetic DSPN [20–23]. This discrepancy may be accounted for by advances in HAART. While microvascular damage driven by poor glycaemic control is believed to be the cause of large nerve fibre damage in diabetic neuropathy [48], the virus itself is thought to play an important role in the aetiology of large fibre dysfunction in HIV-infection [49]. The effectiveness of current HAART therapy for minimizing viral load may reduce complications that are typically characteristic of established DSPN, such as impaired vibration sense. While high VPT (> 25 V) is a significant predictor of diabetic foot ulceration, these findings suggest that it may not be appropriate for evaluating the diagnostic performance of the Neuropad in HIV-infected outpatients [9,10].

The modest specificity of the Neuropad can be explained by the high proportion of participants diagnosed with sudomotor dysfunction despite normal VPT and PD-Q results. As small fibre injury occurs early in the development of DSPN, the Neuropad may enable detection before signs of large fibre dysfunction or pain are reported [15]. Reference standard tests for small fibre function, such as QSART, would strengthen future investigations but were not feasible for the present study [12,50].

Methodological constraints and small sample size are limiting factors and may account for the absence of high PD-Q scores (≥ 19) and the low prevalence of high VPT (> 25 V) observed in the current investigation. The method of assessing Neuropad results could also be improved upon in future study. Categorical estimation of the indicator test is a highly subjective method that does not permit discrimination of the severity of sudomotor dysfunction [51]. Ponirakis et al. (2015) developed image analysis software that generates a measure of the percentage colour change for scanned Neuropad plasters. Compared to categorical estimations, automated quantification was shown to improve the diagnostic efficacy of the Neuropad for detecting small fibre diabetic neuropathy [51]. These findings emphasize the inherent limitations of the Neuropad protocol utilized here, and provide encouraging results for the use of continuous output measures in subsequent study design.

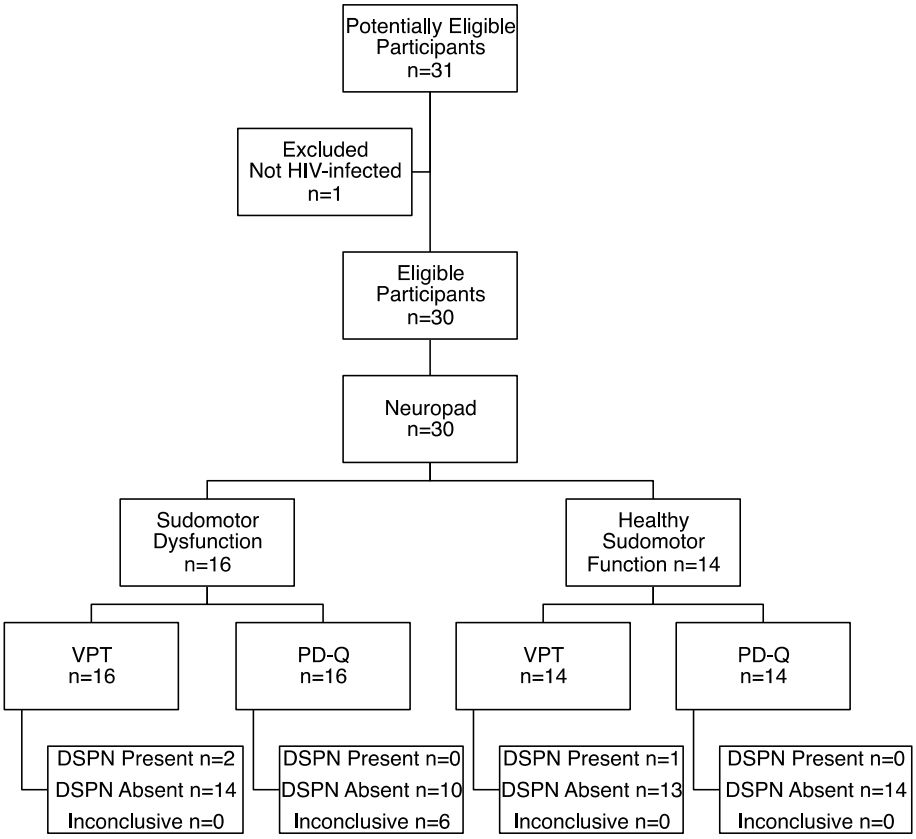

Fig. 2. Flow of participants to evaluate the diagnostic accuracy of the Neuropad for DSPN.

Table 2
Demographics and clinical characteristics of the HIV-infected population.

	Age (years)[a]	Females, *n* (%)	Males, *n* (%)
with sudomotor dysfunction	52.7 ± 10.3	2 (12.5%)	14 (87.5%)
without sudomotor dysfunction	51.1 ± 9.1	2 (14.3%)	12 (85.7%)
VPT > 25V	56.0 ± 15.1	0 (0.0%)	3 (100.0%)
VPT ≤ 25V	51.0 ± 8.3	4 (14.8%)	23 (85.2%)
PD-Q ≥19	0.0 ± 0.0	0 (0.0%)	0 (0.0%)
PD-Q > 12	56.8 ± 6.6	2 (33.3%)	4 (66.7%)
PD-Q ≤ 12	50.1 ± 9.0	2 (8.3%)	22 (91.7%)

[a] Mean ± standard deviation.

Table 3
Sudomotor function compared with VPT, where results > 25 V indicate DSPN.

Patients	VPT > 25 V	VPT ≤25 V	total
with sudomotor dysfunction	2	14	*16*
without sudomotor dysfunction	1	13	*14*
total	*3*	*27*	*30*

Table 4
Diagnostic performance of the Neuropad for the PD-Q and VPT.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PD-Q (> 12)	100	58.3	37.5	100
VPT (> 25V)	66.7	48.1	12.5	92.9

Conclusions

The current study investigated the diagnostic accuracy of the Neuropad for DSPN related to HIV-infection. Sensitivity of the Neuropad for VPT was modest and lower than previous reports. When referenced to a moderate PD-Q cut-off (> 12), the Neuropad yielded 100% sensitivity. For both reference standards the indicator test exhibited moderate specificity, low PPV, and excellent NPV. These findings suggest that the Neuropad may not be robust enough to be used as a standalone diagnostic tool for DSPN in the HIV-infected population. The high NPV of the test suggests that its utility may lie in its ability to reliably rule out healthy individuals. Interpretation of these findings is limited by sampling methods and study size. However, as the first to investigate the accuracy of the indicator test in this population, these observations are promising and merit future exploration. The simplicity of the Neuropad lends itself well to the clinical setting and makes it a suitable and inexpensive instrument for DSPN screening by non-specialist practitioners. Neuropad screening by osteopaths may facilitate early intervention and prevent disabling complications associated with advanced DSPN related to HIV-infection.

Table 5
Sudomotor function compared with PD-Q status where scores > 12 indicate DSPN. Fisher's exact test showed a significant association between PD-Q and Neuropad results (p = 0.0135).

Patients	PD-Q > 12	PD-Q ≤ 12	total
with sudomotor dysfunction	6	10	*16*
without sudomotor dysfunction	0	14	*14*
total	*6*	*24*	*30*

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication. There has been no financial support for this work that could have influenced its outcome.

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Ethical approval

Ethical approval for this study was granted by the University College of Osteopathy Research Ethics Committee and the Royal Free Ethics Committee (#15/NS/0066).

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References

[1] Lalkhen AG, McCluskey A. Clinical tests: sensitivity and specificity. Cont Educ Anaesth Crit Care Pain 2008;8:221–3. https://doi.org/10.1093/bjaceaccp/mkn041.
[2] Gonzalez-Duarte A, Robinson-Papp J, Simpson DM. Diagnosis and management of HIV-associated neuropathy. Neurol Clin 2008;26:821–32. https://doi.org/10.1016/j.ncl.2008.04.001. x.
[3] Ghosh S, Chandran A, Jansen JP. Epidemiology of HIV-related neuropathy: a systematic literature review. AIDS Res Hum Retrovir 2011;28:36–48. https://doi.org/10.1089/aid.2011.0116.
[4] Nicholas PK, Corless IB, Evans LA. Peripheral neuropathy in HIV: an analysis of evidence-based approaches. J Assoc Nurses AIDS Care 2014;25:318–29. https://doi.org/10.1016/j.jana.2014.01.002.
[5] Centner CM, Bateman KJ, Heckmann JM. Manifestations of HIV infection in the peripheral nervous system. Lancet Neurol 2013;12:295–309. https://doi.org/10.1016/S1474-4422(13)70002-4.
[6] Fuller GN, Jacobs JM, Guiloff RJ. Nature and incidence of peripheral nerve syndromes in HIV infection. J Neurol Neurosurg Psychiatry 1993;56:372–81.
[7] Tagliati M, Grinnell J, Godbold J, Simpson DM. Peripheral nerve function in HIV infection: clinical, electrophysiologic, and laboratory findings. Arch Neurol 1999;56:84–9.
[8] Kumar S, Fernando DJS, Veves A, Knowles EA, Young MJ, Boulton AJM. Semmes-Weinstein monofilaments: a simple, effective and inexpensive screening device for identifying diabetic patients at risk of foot ulceration. Diabetes Res Clin Pract 1991;13:63–7. https://doi.org/10.1016/0168-8227(91)90034-B.
[9] Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJ. Multicenter study of the incidence of and predictive risk factors for diabetic neuropathic foot ulceration. Diabetes Care 1998;21:1071–5.
[10] Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. Diabetes Care 1994;17:557–60.
[11] Armstrong DG, Lavery LA, Vela SA, Quebedeaux TL, Fleischli JG. Choosing a practical screening instrument to identify patients at risk for diabetic foot ulceration. Arch Intern Med 1998;158:289–92.
[12] Cherry CL, Wesselingh SL, Lal L, McArthur JC. Evaluation of a clinical screening tool for HIV-associated sensory neuropathies. Neurology 2005;65:1778–81. https://doi.org/10.1212/01.wnl.0000187119.33075.41.
[13] Wulff EA, Wang AK, Simpson DDM. HIV-associated peripheral neuropathy. Drugs 2012;59:1251–60. https://doi.org/10.2165/00003495-200059060-00005.
[14] Rith-Najarian S, Branchaud C, Beaulieu O, Gohdes D, Simonson G, Mazze R. Reducing lower-extremity amputations due to diabetes. Application of the staged diabetes management approach in a primary care setting. J Fam Pract 1998;47:127–32.
[15] Tesfaye S, Boulton AJM, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285–93. https://doi.org/10.2337/dc10-1303.
[16] Tentolouris N, Marinou K, Kokotis P, Karanti A, Diakoumopoulou E, Katsilambros N. Sudomotor dysfunction is associated with foot ulceration in diabetes. Diabet Med J Br Diabet Assoc 2009;26:302–5. https://doi.org/10.1111/j.1464-5491.2009.02677.x.
[17] 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. https://doi.org/10.1002/mus.880150605.
[18] Ponirakis G, Petropoulos IN, Fadavi H, Alam U, Asghar O, Marshall A, et al. The diagnostic accuracy of Neuropad for assessing large and small fibre diabetic neuropathy. Diabet Med J Br Diabet Assoc 2014;31:1673–80. https://doi.org/10.1111/dme.12536.
[19] Papanas N, Boulton AJM, Malik RA, Manes C, Schnell O, Spallone V, et al. A simple new non-invasive sweat indicator test for the diagnosis of diabetic neuropathy. Diabet Med J Br Diabet Assoc 2013;30:525–34. https://doi.org/10.1111/dme.12000.
[20] Tsapas A, Liakos A, Paschos P, Karagiannis T, Bekiari E, Tentolouris N, et al. A simple plaster for screening for diabetic neuropathy: a diagnostic test accuracy systematic review and meta-analysis. Metabolism 2014;63:584–92. https://doi.org/10.1016/j.metabol.2013.11.019.
[21] Papanas N, Papatheodorou K, Christakidis D, Papazoglou D, Giassakis G, Piperidou H, et al. Evaluation of a new indicator test for sudomotor function (Neuropad) in the diagnosis of peripheral neuropathy in type 2 diabetic patients. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc 2005;113:195–8. https://doi.org/10.1055/s-2005-837735.
[22] 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. Diabetes Educat 2007;33:257–60. https://doi.org/10.1177/0145721707299661.
[23] Papanas N, Papatheodorou K, Papazoglou D, Monastiriotis C, Christakidis D, Maltezos E. A comparison of the new indicator test for sudomotor function (Neuropad) with the vibration perception threshold and the clinical examination in the diagnosis of peripheral neuropathy in subjects with type 2 diabetes. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc 2008;116:135–8. https://doi.org/10.1055/s-2007-984455.
[24] Tentolouris N, Voulgari C, Liatis S, Kokkinos A, Eleftheriadou I, Makrilakis K, et al. Moisture status of the skin of the feet assessed by the visual test neuropad correlates with foot ulceration in diabetes. Diabetes Care 2010;33:1112–4. https://doi.org/10.2337/dc09-2027.
[25] Spallone V, Morganti R, Siampli M, Fedele T, D'Amato C, Cacciotti L, et al. Neuropad as a diagnostic tool for diabetic autonomic and sensorimotor neuropathy. Diabet Med J Br Diabet Assoc 2009;26:686–92. https://doi.org/10.1111/j.1464-5491.2009.02760.x.
[26] Quattrini C, Jeziorska M, Tavakoli M, Begum P, Boulton AJM, Malik RA. The Neuropad test: a visual indicator test for human diabetic neuropathy. Diabetologia 2008;51:1046–50. https://doi.org/10.1007/s00125-008-0987-y.
[27] 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. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc 2005;113:577–81. https://doi.org/10.1055/s-2005-872912.
[28] Levy DM, Reid G, Rowley DA, Abraham RR. Quantitative measures of sympathetic skin response in diabetes: relation to sudomotor and neurological function. J Neurol Neurosurg Psychiatry 1992;55:902–8.
[29] Jay GW, Barkin RL. Neuropathic pain: etiology, pathophysiology, mechanisms, and evaluations. Dis Mon 2014;60:6–47. https://doi.org/10.1016/j.disamonth.2013.12.001.
[30] Freeman R, Baron R, Bouhassira D, Cabrera J, Emir B. Sensory profiles of patients with neuropathic pain based on the neuropathic pain symptoms and signs. Pain 2014;155:367–76. https://doi.org/10.1016/j.pain.2013.10.023.
[31] Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA study group. Lancet Lond Engl 1998;352:1725–30.
[32] Rutjes AWS, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PMM. Case-control and two-gate designs in diagnostic accuracy studies. Clin Chem 2005;51:1335–41. https://doi.org/10.1373/clinchem.2005.048595.
[33] Kamenov ZA, Petrova JJ, Christov VG. Diagnosis of diabetic neuropathy using simple somatic and a new autonomic (Neuropad) tests in the clinical practice. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc 2010;118:226–33. https://doi.org/10.1055/s-0030-1247565.
[34] Liatis S, Marinou K, Tentolouris N, Pagoni S, Katsilambros N. Usefulness of a new indicator test for the diagnosis of peripheral and autonomic neuropathy in patients with diabetes mellitus. Diabet Med J Br Diabet Assoc 2007;24:1375–80. https://doi.org/10.1111/j.1464-5491.2007.02280.x.
[35] Manes C, Papanas N, Exiara T, Katsiki N, Papantoniou S, Kirlaki E, et al. The indicator test Neuropad in the assessment of small and overall nerve fibre dysfunction in patients with type 2 diabetes: a large multicentre study. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc 2014;122:195–9. https://doi.org/10.1055/s-0034-1367061.
[36] Ziegler D, Papanas N, Roden M. GDC Study Group. Neuropad: evaluation of three cut-off points of sudomotor dysfunction for early detection of polyneuropathy in recently diagnosed diabetes. Diabet Med J Br Diabet Assoc 2011;28:1412–5. https://doi.org/10.1111/j.1464-5491.2011.03345.x.
[37] Freynhagen R, Baron R, Gockel U, Tölle TR. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. Curr Med Res Opin 2006;22:1911–20. https://doi.org/10.1185/030079906X132488.
[38] Mathieson S, Lin C. painDETECT questionnaire. J Physiother 2013;59:211. https://doi.org/10.1016/S1836-9553(13)70189-9.
[39] Cappelleri JC, Koduru V, Bienen EJ, Sadosky A. A cross-sectional study examining the psychometric properties of the painDETECT measure in neuropathic pain. J Pain Res 2015;8:159–67. https://doi.org/10.2147/JPR.S80046.
[40] Boulton AJM, Malik RA, Arezzo JC, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care 2004;27:1458–86. https://doi.org/10.2337/diacare.27.6.1458.
[41] Garrow AP, Boulton AJM. Vibration perception threshold–a valuable assessment of neural dysfunction in people with diabetes. Diabetes Metab Res Rev 2006;22:411–9. https://doi.org/10.1002/dmrr.657.
[42] Lanting S, Craike P, Spink M, Casey S, Chuter V. The reliability of non-invasive neurological examinations in people with diabetes. J Foot Ankle Res 2013;6:O6. https://doi.org/10.1186/1757-1146-6-S1-O6.
[44] Petrie A, Sabin C. Medical statistics at a glance. Oxford: Blackwell Science Ltd; 2000.
[45] Jones SR, Carley S, Harrison M. An introduction to power and sample size estimation. Emerg Med J EMJ 2003;20:453–8.

[46] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. Clin Chem 2003;49:1–6.

[47] Moreton BJ, Tew V, das Nair R, Wheeler M, Walsh DA, Lincoln NB. Pain phenotype in patients with knee osteoarthritis: classification and measurement properties of painDETECT and self-report Leeds assessment of neuropathic symptoms and signs scale in a cross-sectional study. Arthritis Care Res 2015;67:519–28. https://doi.org/10.1002/acr.22431.

[48] Nukada H. Ischemia and diabetic neuropathy. Handb Clin Neurol 2014;126:469–87. https://doi.org/10.1016/B978-0-444-53480-4.00023-0.

[49] Kokotis P, Schmelz M, Papadimas GK, Skopelitis EE, Aroni K, Kordossis T, et al. Polyneuropathy induced by HIV disease and antiretroviral therapy. Clin Neurophysiol Off J Int Fed Clin Neurophysiol 2013;124:176–82. https://doi.org/10.1016/j.clinph.2012.05.022.

[50] Simpson DM, Kitch D, Evans SR, McArthur JC, Asmuth DM, Cohen B, et al. HIV neuropathy natural history cohort study: assessment measures and risk factors. Neurology 2006;66:1679–87. https://doi.org/10.1212/01.wnl.0000218303.48113.5d.

[51] Ponirakis G, Fadavi H, Petropoulos IN, Azmi S, Ferdousi M, Dabbah MA, et al. Automated quantification of neuropad improves its diagnostic ability in patients with diabetic neuropathy, automated quantification of neuropad improves its diagnostic ability in patients with diabetic neuropathy. J Diabetes Res 2015;2015:1–7. https://doi.org/10.1155/2015/847854, 10.1155/2015/847854.