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Evaluation of clinical tools and their diagnostic use in distal symmetric polyneuropathy

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Abstract

Aims: To compare the diagnostic usefulness of tuning fork, monofilament, biothesiometer and skin biopsies in peripheral neuropathy in individuals with varying glucose metabolism.

Methods: Normoglycaemic, impaired glucose tolerance (IGT) and type 2 diabetes (T2DM) individuals were recruited. Nerve conduction studies (NCS) and thermal threshold tests were performed. Vibrotactile sense was tested with a biothesiometer and a 128-Hz tuning fork.

Touch/pressure perception was examined with a 10-gram monofilament. Skin biopsies were performed and intraepidermal nerve fibres were quantified. Distal symmetric polyneuropathy (DSPN) was defined as neuropathy disability score ≥ 2 and abnormal NCS. Thermal threshold tests were used to define small nerve fibre neuropathy (sDSPN) in cases where NCS (large nerve fibres) were normal. *Results:* The prevalence of DSPN and sDSPN in the whole group (n=119) was 18% and 23%, respectively. For the biothesiometer, a cut-off of ≥ 24.5 volts had a sensitivity of 82% and specificity of 70% (AUC=0.81, 95% CI 0.71-0.91) when evaluating DSPN. An intraepidermal nerve fibre density cut-off of ≤ 3.39 fibres/mm showed a sensitivity of 74% and specificity of 70% in the detection of sDSPN, whereas the sensitivity of the tuning fork and the biothesiometer were relatively low, 46% and 67%, respectively. When combining skin biopsies with the tuning fork, 10 more sDSPN cases were identified. Adding skin biopsy to the combination of the tuning fork and biothesiometer increased the sensitivity of finding sDSPN cases, but not DSPN, from 81% to 93%. *Conclusion:* Using a biothesiometer in clinical routine might be a sensitive method to detect large nerve fibre dysfunction in the lower extremity, whereas skin biopsies in combination with methods measuring vibrotactile sense could increase the diagnostic sensitivity of detecting peripheral neuropathy at an early stage.

Keywords

biothesiometer; diabetes mellitus; nerve fibres; neuropathy; skin biopsy

Introduction

Distal symmetric polyneuropathy (DSPN) is one of the most frequent complications of diabetes with a reported prevalence ranging from 30% to 50% [1,2]. DSPN can occur early in diabetes [3,4] with damage to peripheral nerve due to prolonged hyperglycaemia [5]. However, the condition may be unrelated to diabetes and result from other causes as well [6,7].

Neuropathy, being a clinical diagnosis, has been proposed to be accurately assessed when the combination of symptoms and signs together with nerve conduction studies are considered [8,9]. It has been proposed that DSPN could be confirmed in the presence of abnormal nerve conduction and symptom(s) *or* sign(s) of neuropathy [5, 9].

Assessing DSPN in routine clinical practice, methods such as the tuning fork and the 10-gram monofilament are frequently used to measure large nerve fibre function, i.e. vibrotactile and pressure sensation, respectively [10-12]. Biothesiometry for measuring vibration perception thresholds (VPT) is most often used for risk assessment of foot ulceration as a consequence of neuropathy [13,14]. In addition, skin biopsy with quantification of intraepidermal nerve fibre density (IENFD) has been suggested to be a useful method for assessing small nerve fibre neuropathy [15]. Still, it is uncertain whether these methods are sensitive and specific enough for detecting DSPN at an early stage, and whether the combination these methods provides any further diagnostic improvement and if the methods overlap.

Thus, our aim was to compare the diagnostic usefulness of the 128 Hz tuning fork, the 10-gram monofilament, the biothesiometer and the skin biopsy in a well-defined population consisting of normoglycemic (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2D) individuals.

Methods

Study population

Participants were recruited from November 2004 to April 2007 from the population-based Västerbotten Intervention Programme (VIP) [16]. The study population has been described elsewhere [17]. The glycaemic status of the NGT and IGT individuals was verified by two standardized oral glucose tolerance tests (OGTT). Participants with type 2 diabetes also performed an OGTT except those with fasting plasma glucose of > 15 mmol/L. Of the 129 recruited participants, four withdrew, three were excluded due to vitamin B12 and folate deficiency and three were excluded due to stroke or sciatica. All participants gave informed consent and the regional ethical review board of Umeå University, Umeå, Sweden, approved the study.

Measurements

Height and weight were measured and BMI was calculated (kg m^{-2}). Blood pressure was measured with standard sphygmomanometer in supine position after 10 minutes' rest. Total cholesterol and triglycerides (Vitros 5.1 FS analyser, Johnson&Johnson, Raritan, NJ), HbA1c (HPLC, TOSOH, Tokyo, Japan), and fasting and 2-hour plasma glucose (HemoCue, HemoCue AB, Ängelholm, Sweden) were measured in blood samples. HbA1c values were converted to the Diabetes Control and Complications Trial (DCCT) standard values using the formula: $\text{HbA1c (DCCT)} = 0.923 \times \text{HbA1c (Mono S)} + 1.345$ and are presented in both the

DCCT (%) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (mmol/mol) units. Conversion between DCCT and IFCC was done using the following equation: $\text{HbA1c (mmol/mol)} = [\text{HbA1c (\%)} - 2.15] \times 10.929$. Chronic alcoholism (evaluated by carbohydrate deficient transferrin and gamma glutamyl transferase), thyroid disease (thyroid stimulation hormone, thyroid hormones T₄ and T₃), and vitamin B12 and folate deficiencies (homocystein, methylmalonic acid) were considered as other possible causes of polyneuropathy, and if present those participants were excluded.

Neurophysiological assessment

Standardized nerve conduction studies were performed on the tibial, peroneal and sural nerves at the clinical neurophysiology laboratory in Umeå University, Sweden. All nerve attributes measured were performed in one lower extremity [5]. The motor conduction velocity, amplitude and latency of the tibial and peroneal nerves were measured. The sensory conduction velocity, amplitude and latency of the sural nerve were measured. F-wave studies of the tibial and peroneal nerves were performed.

An experienced neurophysiologist, blinded to the group identity of all participants, performed all nerve conduction studies. The neurophysiologist considered the nerve attributes representative of neurophysiological abnormality in DSPN and determined whether the participants had evidence of abnormal nerve conduction or not. The limb temperature was monitored prior and during recordings using a skin surface probe; hot-water bath and hot-water blankets were used to keep the limb temperature above 31 °C when needed.

Thermal threshold testing

Thermal threshold tests were performed with the method of limits, using ThermoTest® equipment (Somedic AB, Hörby, Sweden). Thermal stimulations were applied bilaterally to the dorsum of both feet, one at a time. The starting temperature was 32°C for both the heat and cold sensation measurements. The rate of temperature change was 1°C/s and 10 stimulations were applied for each thermal sensation. By using the method of limits (17), the individuals were told to give a response when perceiving a noticeable thermal sensation. The results are mean values and the standard laboratory cut-off values (adjusted for age and sex) were used for delimiting pathological findings: 40.6°C for heat and 26.7°C for cold perceptions (mean \pm 2 SD). Only individuals with bilaterally abnormal thresholds were considered to have an abnormal outcome. The limb temperature was kept above 31 °C when needed.

Neuropathy disability score (NDS)

A modified version [18,19] of Dyck's original Neuropathy Disability Score [20] was used to evaluate clinical signs of neuropathy in the leg. The modified version of NDS included examination of sensory perception, muscle strength and reflexes in the lower extremities. Sensory perception (NDS-A) was tested by considering modalities of light touch (cotton wool), vibration (128 Hz tuning fork), pin prick (needle) and cold (cold metal item). The assessments were on the greater toe, medial malleolus and medial side of the knee. Patellar and ankle reflexes (NDS-B) were evaluated together with muscle strength at toe, foot and knee level (NDS-C). For NDS-A and NDS-B, normal findings were scored as 0, reduced reflexes or sensation as 1, and absent reflexes or sensation as 2; for NDS-C, normal as 0, reduced muscle strength as 1, considerable reduction as 2, and paralysis as 3. The same physician performed all the NDS examinations.

Definition of DSPN

The definition presented by the Toronto Diabetic Neuropathy Expert Group, states that an abnormal nerve conduction study and symptom(s) *or* sign(s) of neuropathy confirms clinical DSPN [5, 9] In order to assess DSPN objectively, we did not include subjective symptoms in the definition of DSPN. The guidelines also recommend considering a validated measure of small nerve fibre neuropathy if NCS are normal [9]. Thus, our minimal criterion for DSPN was defined as having abnormal nerve conduction according to the neurophysiologist and presence of concurrent objective clinical neuropathic signs ($NDS \geq 2$); i.e. a large nerve fibre weighted definition. Hence, we also defined a group with neuropathy weighted more on small nerve fibres, called sDSPN and consisting of individuals with normal NCS, but abnormal thermal thresholds (abnormal heat and/or cold thresholds) in presences of clinical signs of neuropathy ($NDS \geq 2$).

Biothesiometer, monofilament and tuning fork

Vibration thresholds were tested with a hand-held biothesiometer vibrating at 100 Hz (Bio-Medical Instrument Co, ROVA Company Inc, Newbury, OH). The rubber tractor of the biothesiometer was bilaterally balanced perpendicular to the bony part the medial malleolus. The vibration perception was also tested with a 128 Hz tuning fork applied bilaterally with the on-off method to the medial malleolus; absent or reduced perception on either malleolus was noted.

The pressure perception was examined with a Semmes-Weinstein 5.07/10-g monofilament (Gertab AB, Stockholm, Sweden) on three standard points (plantar surface of distal hallux, 1st and 5th metatarsal heads) bilaterally on the sole of the foot; absence of sensation at one or more sites on either foot was noted [21]. The 128 Hz tuning fork and the Semmes-Weinstein 5.07/10-g monofilament are clinical tools used in Sweden in the assessment of peripheral

nerve dysfunction. The same physician, who also performed the NDS examinations, performed all of the physical examinations.

Skin biopsy

Skin biopsies were performed and published guidelines were considered [15]. The site of the punch skin biopsy was approximately 10 cm proximal to the right lateral malleolus and performed under local anaesthesia using a three mm disposable circular needle (Dermal Biopsy Punch; Miltex, Inc, PA, USA). The procedure was well tolerated by the participants and without any complications. The histotechnical preparation and the subsequent microscopical quantification of the intraepidermal nerve fibre density followed a routine developed during 2004 [22] and was slightly modified from a previous study on similar thin 5 μm sections [23]. Two observers, both blinded to the identities of the participants, assessed all the sections once. One of the observers re-counted a random selection of 100 samples and the intra-observer reliability ($r_s=0.98$) and the inter-observer reliability was obtained ($r_s=0.84$). The intraepidermal nerve fibre density was expressed as the number of fibres/mm of epidermal length.

Statistical analyses

Data are presented as numbers (n), proportions (%) and distribution as mean and standard deviation or median and interquartile range (IQR). Differences between groups were tested by *ANOVA* and subsequent Student's t-test for normally distributed variables. For not normally distributed variables the Kruskal-Wallis test was applied with subsequent Mann-Whitney U-testing. Differences in proportions were tested using the Chi-square test and trends were tested with linear-by-linear associations. The dependent variable was the occurrence of DSPN/sDSPN. Receiver-operating characteristic (ROC) curves were produced where

appropriate and the area under the curve (AUC) and optimal sensitivity and specificity were determined. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS 19.0 (SPSS Inc, Chicago, IL).

Results

Population characteristics

In our study 22 individuals (18%) were considered to have DSPN in the whole group, e.i. those with abnormal NCS and signs of neuropathy. We also observed 27 individuals with sDSPN, (23%), i.e. those with normal NCS, but abnormal thermal thresholds and signs. In the NGT group, the prevalence of DSPN and sDSPN was 16% and 13%, respectively. In the IGT group we observed a relatively higher prevalence of sDSPN (32%) than DSPN (12%), whereas in the T2DM group the prevalence of DSPN (28%) and sDSPN (30%) were quite similar (Table 1). The prevalences of DSPN and sDSPN were not significantly different between NGT and IGT individuals. DSPN and sDSPN individuals had higher concentration of HbA1c, but lower cholesterol than individuals without neuropathy (Table 2). Both height and weight were higher in the DSPN group than in the non-neuropathy group, whereas only height was higher for the sDSPN as compared to the non-neuropathy group (Table 2).

Routine clinical tools

The tuning fork was a relatively good method to identify DSPN cases in terms of sensitivity (17/22, 77%). For sDSPN, the tuning fork identified 12 out of 27 sDSPN cases, resulting in a lower sensitivity (44%) when compared to identification of DSPN cases. In the whole study group (n=119), 52 individuals had absent or clearly reduced vibration perception when using the tuning fork. However, within the non-DSPN/sDSPN group, the tuning fork recognized 41 out of 61 cases with no neuropathy, giving a specificity of 67% for both DSPN and sDSPN.

The 5.07/10-g monofilament had a low sensitivity (6%), but a high specificity (97%); three individuals with DSPN/sDSPN were identified, and two non-neuropathic individuals had abnormal monofilament outcome.

Biothesiometry

Analysing areas under the ROC curve (AUC) for the biothesiometer, an optimal sensitivity of 82% and a specificity of 70% (AUC=0.81, 95% CI 0.71-0.91) were found at the cut-off value of ≥ 24.5 V at the medial malleolus for the DSPN group (Fig. 1). Accordingly, the biothesiometer identified 18 out of 22 DSPN cases. The ROC curve (AUC=0.55, 95% CI 0.43-0.65) for the sDSPN group (not illustrated), with a cut-off value of ≥ 20.5 V resulted in a sensitivity and specificity of only 67% and 46%, respectively.

Skin biopsy

Considering the DSPN group, the ROC curve for the skin biopsies showed an almost equally high AUC as the biothesiometer (AUC=0.73, 95% CI 0.61-0.86, $p=n.s.$) with a sensitivity of 73% and specificity of 70% at an IENFD cut-off ≤ 2.96 fibres/mm (Fig. 1). Thus, the skin biopsy method identified 16 out of 22 individuals with DSPN. When considering the ROC curve (AUC=0.63, 95% CI 0.52-0.75) for the sDSPN group (not illustrated), an IENFD cut-off of ≤ 3.39 fibres/mm gave a sensitivity of 74% and a specificity of 61%. Thus, the skin biopsy method identified 20 out of 27 individuals with sDSPN.

Concordance of methods for DSPN

We observed an overlap in diagnostic sensitivity; 15 individuals out of 22 that had DSPN were identified by both the skin biopsy (≤ 2.96 fibres/mm) and the biothesiometer (≥ 24.5 V). Similarly, 13 individuals out of 22 that had DSPN were identified by both the skin biopsy and

the tuning fork. The identification overlap for the tuning fork and the biothesiometer was 14 individuals out of 22 DSPN cases. Taken together, all three methods mentioned above overlapped in 12 out of 22 DSPN cases (Fig. 2). The 10-g monofilament, which identified only two DSPN cases, overlapped with all other methods in those two cases.

Combining of methods for DSPN

When combining skin biopsies with the biothesiometry, three more DSPN cases were found by the biothesiometer where the skin biopsies did not, and one case was found by the skin biopsy where the biothesiometer did not, identifying a total of 19 out of 22 DSPN cases.

Similarly, skin biopsies in combination with the tuning fork, resulted in 20 out of 22 DSPN cases to be identified; four DSPN cases were found by the tuning fork when the skin biopsy method had failed to do so, likewise three DSPN cases were identified by the skin biopsies where the tuning fork did not; those three DSPN cases that were not identified by the tuning fork but with the skin biopsy were also identified with the biothesiometer.

The combination of the tuning fork with the biothesiometer identified all (n=21) but one of the DSPN cases, and 16 out of those cases had IGT or diabetes. Adding skin biopsy to this combination did not change the sensitivity of detecting DSPN further. The monofilament in combination with the other methods respectively did not provide additional diagnostic sensitivity.

Concordance of methods for sDSPN

We observed an overlap for the sDSPN group as well; 14 individuals out of 27 that had been identified as sDSPN by the biothesiometer (≥ 20.5 V) were also identified by the skin biopsy (≤ 3.39 fibres/mm). Similarly, 10 individuals out of 27 that had sDSPN were identified by both the skin biopsy and the tuning fork. The identification overlap for the tuning fork and the

biothesiometer was 8 individuals out of 27 sDSPN cases. Taken together, all three methods mentioned above overlapped only in 7 out of 27 sDSPN cases (Fig. 3). The 10-g monofilament, which identified only one sDSPN case, overlapped with all other methods in that case.

Combining of methods for sDSPN

When combining skin biopsies (≤ 3.39 fibres/mm) with the biothesiometer (≥ 20.5 V), 6 more sDSPN cases were found by the skin biopsy, where the biothesiometer did not, and 4 cases were found by the biothesiometer, where the skin biopsy did not, identifying a total of 24 out of possible 27 sDSPN cases.

Similarly, skin biopsies in combination with the tuning fork resulted in 22 out of 27 sDSPN cases to be identified; two sDSPN cases were found by the tuning fork when the skin biopsy method had failed to do so. We observed that 10 additional sDSPN cases were identified by the skin biopsies, where the tuning fork did not; 7 out of those 10 sDSPN cases were also identified with the biothesiometer (Fig. 3). The combination of the tuning fork with the biothesiometer identified 22 out of 27 possible sDSPN cases; four sDSPN cases were found by the tuning fork when the biothesiometer did not, and 10 sDSPN cases were identified by the biothesiometer, where the tuning fork did not (Fig. 3). Consequently, three out of those four sDSPN cases that were not identified by the biothesiometer, but with the tuning fork, were also identified by skin biopsies (Fig. 3).

Thus, by adding skin biopsy to this combination (skin biopsy + tuning fork + biothesiometer) the sensitivity of finding sDSPN increased from 81% to 93% by identifying three more sDSPN cases (total of 25 sDSPN cases, from which 21 had IGT or T2DM). Choosing a higher cut-off for the skin biopsy, e.g. ≤ 4.0 fibres/mm or even up to 4.4 fibres/mm, did not further

increase the sensitivity. The monofilament, in combination with the other methods, respectively, did not provide additional diagnostic sensitivity.

Discussion

In our cross-sectional study, the biothesiometer, a method for assessing the vibrotactile perception threshold at 100 Hz, and thus mainly large nerve fibre dysfunction with their nerve receptors, proved to be a sensitive and specific method in evaluating the occurrence of mainly large nerve fibre dysfunction (DSPN) in the lower extremity in normoglycaemic, IGT and type 2 diabetes individuals. There was a significant overlap between the biothesiometer, skin biopsy the tuning fork in the identification of individuals with DSPN. Such an overlap was less prominent in the sDSPN group. However, we observed that skin biopsy with the quantification of IENFD served as a sensitive and specific method to detect small, but also large, nerve fibre dysfunction. We also observed that additional sDSPN individuals were identified by the skin biopsies when used in combination with the 128 Hz tuning fork and the biothesiometer.

Skin biopsies and neuropathy

We found a gain of combining skin biopsies with measures of large nerve fibres, such as the tuning fork and the biothesiometer in a group of individuals with sDSPN. Thus, combining a simple technique for immunohistochemical staining small nerve fibres in punch biopsies with methods assessing vibrotactile sense could be a complementary manner of assessing neuropathy in the lower extremity at an early stage where small nerve fibre dysfunction might develop prior to advanced large nerve fibre abnormalities that are detectable by NCS. Thus, as proposed by Lauria *et al.* [15] we have shown that quantification of IENFD is a sensitive measure in the detection of peripheral neuropathy, especially since small nerve fibre

dysfunction can also occur before development of symptoms and signs [24]. The use of skin biopsy, a minimal invasive method, gives a minor wound and may theoretically lead to pain, but none of the participants in our study reported any complications. In addition, the biopsies were taken above the foot, thus with less risk of foot ulcers or delayed wound healing. However, whether skin biopsies will become a widespread measure or a useful alternative clinical tool in clinical practice is uncertain.

Tuning fork and neuropathy

We observed that when combining the tuning fork with a measure of small nerve fibre function, such as skin biopsies, additional neuropathy cases were found, especially in the case of normal NCS (large nerve fibres). The use of the tuning fork in detection of early neuropathy in the lower extremity has been the subject of many studies. When comparing the tuning fork with a biothesiometer, the tuning fork alone was reported to be a reliable method of detecting neuropathy [11]. Similarly, using the tuning fork for screening purposes proved to be a reliable method, and better than the 10-g monofilament [25], perhaps due to the fact that different anatomical sites are tested. Conventional tuning forks found at primary health care clinics only examine vibrotactile sense at 128 Hz. When a multi-frequency method was used, there were higher vibration thresholds in finger pulps in the upper extremities in individuals with type 1 and 2 diabetes than NGT at frequencies higher as well as lower than 128 Hz [26,27]. In addition, such method may also be used to detect neuropathy in the sole of the foot, particularly at low frequencies (i.e. 8-32 Hz) [28]. Thus, assessing vibration perception at 128 Hz by conventional tuning fork might overlook early neuropathology.

Biothesiometry and neuropathy

Biothesiometry has not been taken into routine clinical use, at least not in Sweden. The reason could be its possible limitations, e.g. the confounding effect of limb temperature, the amount of pressure applied by the vibratory probe, psychological factors, and choice of limb site and tactile surface of the skin. Despite potential limitations, the biothesiometer was the best method of those used in our study at detecting advanced neuropathy in the lower extremity in a population with abnormal NCS. Hence, this might suggest that the criteria applied ($NDS \geq 2$ and abnormal NCS) may be too strict so as to detect less advanced or early neuropathy (small nerve fibres). Moreover, it has suggested that assessing vibrotactile perception by a biothesiometer is a sensitive method for screening neuropathy individuals at risk of developing foot complications, even better than the 10-g monofilament [14], and thus useful in detecting advanced neuropathy. We further observed that biothesiometry was not a sensitive enough method for detection of small nerve fibre weighted neuropathy (i.e. those with normal NCS, but abnormal thermal thresholds). However, the use of a biothesiometer might be of value for detection and diagnosis of early neuropathy in the lower extremity when used in combination with measures of small nerve fibre function, such as skin biopsies. The method utilizes 100 Hz, a frequency in between the function of Paccini corpuscles (around 250 Hz) and Meissner's corpuscles (around 30 Hz). In addition, variability in vibration thresholds has been reported [29]. Variability in vibration thresholds have also been reported between different anatomical sites [30]. Moreover, the use of biothesiometry to detect neuropathy in the lower extremity in young individuals with diabetes [31] has been shown to have a sensitivity of 82% and a specificity of 75%, similar to our findings in an older population.

Monofilament and neuropathy

The use of monofilament in the assessment of the diabetic foot is well established [32]. However, the role of monofilament in detecting early neuropathy has also been studied [10,12,33,34]. In our study, the use of a monofilament, although using only the 5.06/10-g monofilament, was not a suitable method in the detection of neuropathy in the lower extremity. This false negative outcome of the monofilament has been recognized in a study where it also was concluded that the use of monofilament is valuable in the identification of very high-risk individuals [14]. In the present study, a single monofilament (i.e. 5.06/10 g) was used to examine perception of touch. However, a more sensitive technique may be to use a set of 20 different monofilaments, where results of such an approach correlated with vibrotactile thresholds evaluated at several frequencies in the sole of the foot [28]. Hence, one could argue that the 5.06/10-g monofilament probably only detects advanced neuropathy and therefore did not add to the sensitivity of the methods in our study. However, the outcome of that study [14] was risk of foot complications and not neuropathy per se, which is an important distinction. We had neuropathy as primary outcome, and tested only one type of monofilament (5.07/10-g); using monofilaments with different calibres might be of value in the detection of early neuropathy [10]. Moreover, there is no standardized method for monofilament assessment and there are many important factors that vary between studies, e.g. differences in the site on the foot that is used, the number of sites, using the plantar or dorsal side of the foot and the definition of pathological outcome [10,33-35]. Thus, having different location of testing makes comparison to prior studies difficult. In addition, the number of different monofilaments used and technical issues, such as environmental effects of humidity, temperature and filament aging and durability are other factors that might influence the outcome [36].

Limitations and strengths

Our study included a low number of individuals with DSPN, especially women, which hampered the possibility of studying differences between sexes. In our study we observed that a number of NGT persons had neuropathy. This observation might call into question the definition of neuropathy; however idiopathic peripheral neuropathy is a common problem encountered in clinical practice. In addition, symptoms were not included in the definition of neuropathy. Also, picking the cut-off for the different tests may overstate their actual sensitivity and specificity in clinical practice.

The strength of our study was that our IGT and NGT individuals were well defined since their glucose tolerance status was based on the results of two OGTTs. We also tried by using electrophysiological measures to define both large and small nerve fibre dysfunction. In addition, all participants were recruited consecutively from a population-based sample and were of the same age.

Conclusion

The biothesiometer is a sensitive and specific method to be used in a clinical setting when evaluating large nerve fibre dysfunction in the lower extremity. Combining skin biopsies with routine clinical tools, such as the 128 Hz tuning fork is of greater use when considering less advanced neuropathy than more advanced large nerve fibre neuropathy. In conclusion, the diagnostic usefulness of detecting early peripheral neuropathy, especially when NCS are normal, can be increased by combining measures of intraepidermal nerve fibres and vibrotactile sense.

Conflict of interest statement

The authors state that they have no conflict of interest.

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References

- [1] P.J. Dyck, K.M. Kratz, J.L. Karnes, et al., The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study, *Neurology* 43 (1993) 817-824.
- [2] M. Young, A. Boulton, A. MacLeod, et al., A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population, *Diabetologia* 36 (1993) 150-154.
- [3] S. Novella, S. Inzucchi, J. Goldstein, The frequency of undiagnosed diabetes and impaired glucose tolerance in patients with idiopathic sensory neuropathy, *Muscle Nerve* 24 (2001) 1229-1231.
- [4] N. Papanas, A.I. Vinik, D. Ziegler, Neuropathy in prediabetes: does the clock start ticking early?, *Nat. Rev. Endocrinol.* 7 (2011) 682-690.
- [5] P.J. Dyck, J.W. Albers, H. Andersen, et al., Toronto Expert Panel on Diabetic Neuropathy, Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity, *Diabetes Metab. Res. Rev.* 27 (2011) 620-628.

- [6] K.C. Gorson, A.H. Ropper, Additional causes for distal sensory polyneuropathy in diabetic patients, *J. Neurol. Neurosurg. Psychiatry* 77 (2006) 354-358.
- [7] P. Lozeron, L. Nahum, C. Lacroix, et al., Symptomatic diabetic and non-diabetic neuropathies in a series of 100 diabetic patients, *J. Neurol.* 249 (2002) 569-575.
- [8] J. England, G. Gronseth, G. Franklin, et al., Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation, *Neurology* 64 (2005) 199-207.
- [9] S. Tesfaye, A.J. Boulton, P.J. Dyck, et al., Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments, *Diabetes Care* 33 (2010) 2285-2293.
- [10] N. Kamei, K. Yamane, S. Nakanishi, et al., Effectiveness of Semmes-Weinstein monofilament examination for diabetic peripheral neuropathy screening, *J. Diabetes Complications* 19 (2005) 47-53.
- [11] T. Kästenbauer, S. Sauseng, H. Brath, et al., The value of the Rydel-Seiffer tuning fork as a predictor of diabetic polyneuropathy compared with a neurothesiometer, *Diabet. Med.* 21(2004) 563-567.
- [12] M. Rahman, S.J. Griffin, W. Rathmann, N.J. Wareham, How should peripheral neuropathy be assessed in people with diabetes in primary care? A population-based comparison of four measures, *Diabet. Med.* 20 (2004) 368-374.
- [13] C.A. Abbott, A.L. Carrington, H. Ashe, et al., The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort, *Diabet. Med.* 19 (2002) 377-384.
- [14] H. Gin, V. Rigalleau, L. Baillet, C. Rabemanantsoa, Comparison between monofilament, tuning fork and vibration perception tests for screening patients at risk of foot complication, *Diabetes Metab.* 28 (2002) 457-461.
- [15] G. Lauria, S.T. Hsieh, O. Johansson, et al., European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fibre neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society, *Eur. J. Neurol.* 17 (2010) 903-912, e944-909.
- [16] M. Norberg, S. Wall, K. Boman, L. Weinehall, The Västerbotten Intervention Programme: background, design and implications, *Glob. Health Action* 3 (2010).
- [17] K. Pourhamidi, L.B. Dahlin, K. Boman, O. Rolandsson, Heat shock protein 27 is associated with better nerve function and fewer signs of neuropathy, *Diabetologia* 54 (2011) 3143-3149.

- [18] S. Thrainsdottir, R. Malik, L. Dahlin, et al., Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man, *Diabetes* 52 (2003) 2615-2622.
- [19] S. Thrainsdottir, R.A. Malik, I. Rosén, et al., Sural nerve biopsy may predict future nerve dysfunction, *Acta Neurol. Scand.* 120 (2009) 38-46.
- [20] P. Dyck, Detection, characterization, and staging of polyneuropathy: assessed in diabetics, *Muscle Nerve* 11 (1988) 21-32.
- [21] J.G. Katon, G.E. Reiber, K.M. Nelson, Peripheral Neuropathy Defined by Monofilament Insensitivity and Diabetes Status: NHANES 1999-2004, *Diabetes Care* (2012) Dec 28, Epub ahead of print.
- [22] S. Thrainsdottir, Peripheral polyneuropathy in type 2 diabetes mellitus and impaired glucose tolerance. Correlations between morphology, neurophysiology and clinical findings. Lund University, Faculty of Medicine, Doctoral Dissertations series, 67 (2009) ISBN 978-91-86253-55-4, (<http://lup.lub.lu.se/luur/download?func=downloadFile&recordOId=1466061&fileOId=1466067>).
- [23] N.O. Thomsen, E. Englund, S. Thrainsdottir, et al., Intraepidermal nerve fibre density at wrist level in diabetic and non-diabetic patients, *Diabet. Med.* 26 (2009) 1120-1126.
- [24] M. Polydefkis, P. Hauer, S. Sheth, et al., The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy, *Brain* 127 (2004) 1606-1615.
- [25] J.W. Meijer, A.J. Smit, J.D. Lefrandt, et al., Back to basics in diagnosing diabetic polyneuropathy with the tuning fork!, *Diabetes Care* 28 (2005) 2201-2205.
- [26] L. Dahlin, S. Thrainsdottir, R. Cederlund, et al., Vibrotactile sense in median and ulnar nerve innervated fingers of men with Type 2 diabetes, normal or impaired glucose tolerance, *Diabet. Med.* 25 (2008) 543-549.
- [27] L.B. Dahlin, V. Granberg, O. Rolandsson, et al., Disturbed vibrotactile sense in finger pulps in patients with Type 1 diabetes - correlations with glycaemic level, clinical examination and electrophysiology, *Diabet. Med.* 28 (2011) 1045-1052.
- [28] J. Nelander, T. Speidel, A. Björkman, L.B. Dahlin, Vibration thresholds are increased at low frequencies in the sole of the foot in diabetes-a novel multi-frequency approach, *Diabet. Med.* 29 (2012) e449-456.
- [29] J. Duke, M. McEvoy, D. Sibbritt, et al., Vibrotactile threshold measurement for detecting peripheral neuropathy: defining variability and a normal range for clinical and research use, *Diabetologia* 50 (2007) 2305-2312.

- [30] G. Williams, J.S. Gill, V. Aber, H.M. Mather, Variability in vibration perception threshold among sites: a potential source of error in biothesiometry, *Br. Med. J. (Clin. Res. Ed.)* 296 (1988) 233-235.
- [31] E.A. Davis, T.W. Jones, P. Walsh, G.C. Byrne, The use of biothesiometry to detect neuropathy in children and adolescents with IDDM, *Diabetes Care* 20 (1997) 1448-1453.
- [32] American Diabetes Association, Consensus Development Conference on Diabetic Foot Wound Care: 7-8 April 1999, Boston, Massachusetts. American Diabetes Association, *Diabetes Care* 22 (1999) 1354-1360.
- [33] Y. Feng, F. Schlösser, B. Sumpio, The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy, *J. Vasc. Surg.* 50 (2009) 675-682.
- [34] B.A. Perkins, A. Orszag, M. Ngo, et al., Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study, *Diabetes Care* 33 (2010) 1549-1554.
- [35] G. Valk, P. Grootenhuis, J. van Eijk, et al., Methods for assessing diabetic polyneuropathy: validity and reproducibility of the measurement of sensory symptom severity and nerve function tests, *Diabetes Res. Clin. Pract.* 47 (2000) 87-95.
- [36] M.H. Haloua, I. Sierevelt, W.J. Theuvenet, Semmes-weinstein monofilaments: influence of temperature, humidity, and age, *J. Hand Surg. Am.* 36 (2011) 1191-1196.

Tables

Table 1 - Prevalence of DSPN/sDSPN in NGT, IGT and T2DM

	NGT n=39 ^a	IGT n=29	T2DM n=51
No DSPN/sDSPN, n (%)	27 (71)	14 (56)	20 (42)*
sDSPN, n (%)	5 (13)	8 (32)	14 (30)
DSPN, n (%)	6 (16)	3 (12)	13 (28)

^aPrevalence are presented where 1 NGT, 4 IGT and 4 T2DM individuals had missing NDS or NCS/thermal thresholds. *p <0.05 compared with NGT

Table 2 - Baseline clinical characteristics of the study population given by the incident of peripheral neuropathy (sDSPN and DSPN) in the lower extremity.

	Without DSPN or sDSPN ^a	sDSPN	DSPN
<i>n</i> (m/f)	61 (23/38)	27 (20/7)	22 (18/4)
Age (years)	61.2 ± 1.0	61.3 ± 0.7	61.2 ± 1.1
Height (m)	1.68 ± 0.09	1.75 ± 0.09*	1.78 ± 0.09*
Weight (kg)	78 ± 17	86 ± 12	90 ± 18*
BMI (kg/m ²)	27.3 ± 4.7	27.7 ± 5.2	28.3 ± 5.0
HbA1c (%)	5.5 (5.3–6.2)	6.0 (5.5–7.4)*	6.6 (5.5–8.1)*
HbA1c (mmol/mol)	39 (34–51)	42 (37–57)*	49 (37–65)*
Fasting plasma glucose (mmol/l)	5.3 (4.9–6.9)	5.9 (5.1–8.4)	7.1 (5.1–9.9)
Systolic blood pressure (mmHg)	128 ± 15	128 ± 13	133 ± 18
Diastolic blood pressure (mmHg)	75 ± 8	75 ± 9	77 ± 8
Total cholesterol (mmol/l)	5.5 ± 1.0	4.9 ± 0.8*	4.8 ± 0.6*
Triglycerides (mmol/l)	1.4 (1.0–1.6)	1.4 (1.0–1.7)	1.2 (0.9–2.1)
Creatinine (µmol/L)	71 (61–85)	74 (66–82)	76 (63–81)

Data are given as mean ± SD or median (interquartile range Q1–Q3), and proportions (%).

NGT, normal glucose tolerance; IGT, Impaired Glucose Tolerance; T2DM, type-2 diabetes

mellitus; **p* <0.05 compared with not having DSPN/sDSPN. ^aThose who were not considered

having DSPN nor sDSPN were individuals with no indication of electrophysiological abnormalities (NSC and/or thermal thresholds) and were otherwise not definable according to published guidelines to have confirmed neuropathy [5, 9].

Figures

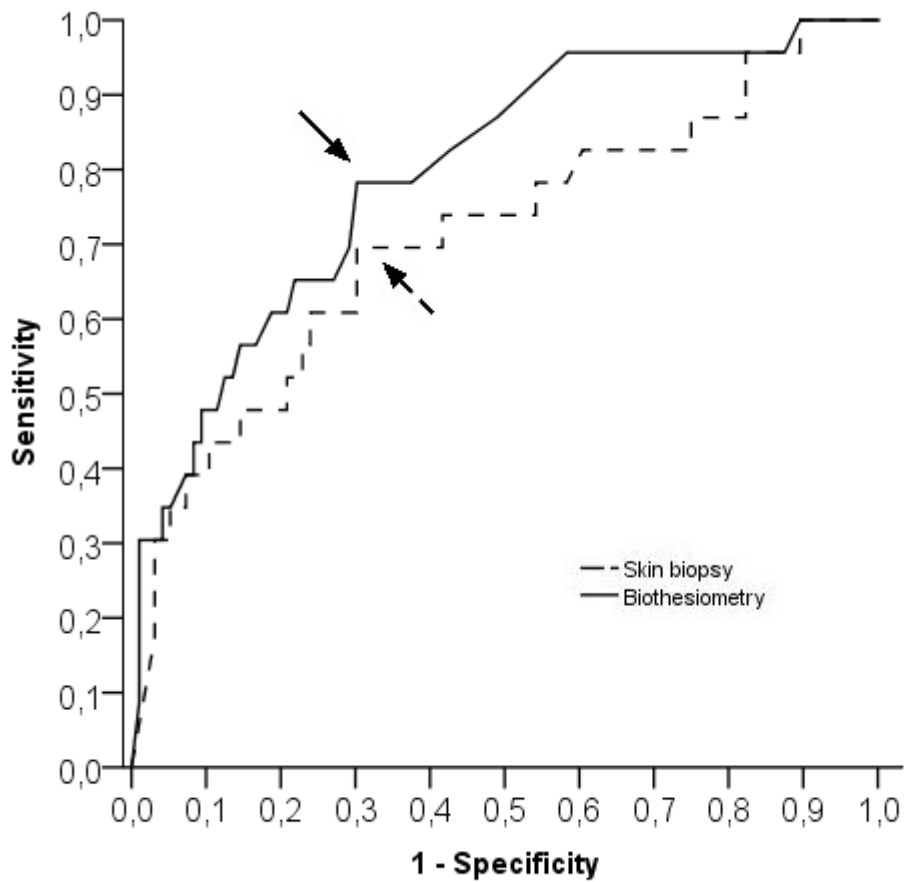


Figure 1 - ROC curves for biothesiometry and skin biopsy with the occurrence of DSPN (abnormal NCS and $NDS \geq 2$) as outcome. The arrow indicates the combination of optimal sensitivity and specificity.

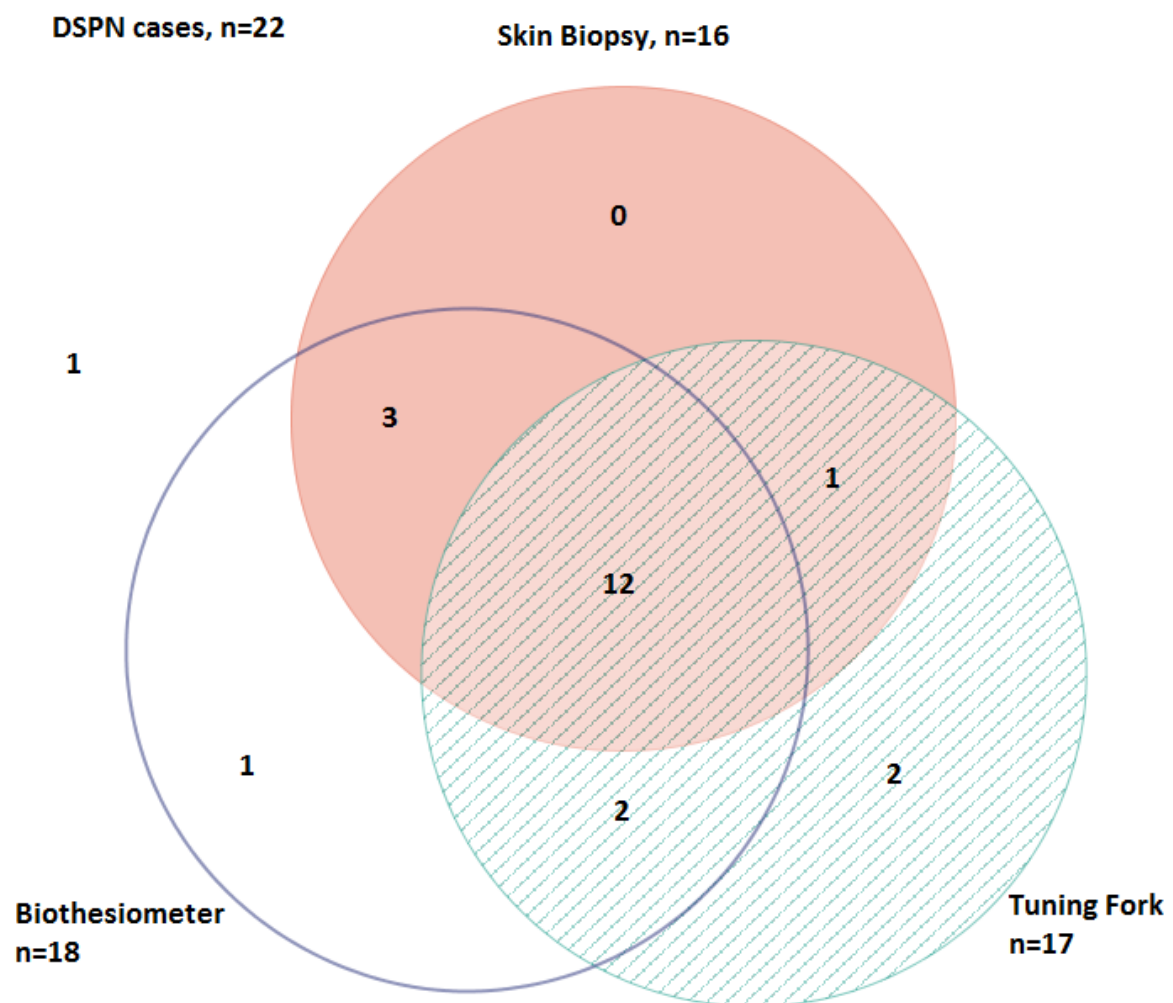


Figure 2 - Venn diagram showing the overlap of the various methods on identifying true DSPN cases. The numbers of DSPN cases are within each section. Skin biopsy overlapped with biothesiometer in 15 cases, with the tuning fork in 13 cases. The tuning fork and the biothesiometer overlapped in 14 cases. All three methods overlapped in 12 cases. The

combination of the tuning fork and the biothesiometer identified all but one of the DSPN individuals; adding skin biopsy to this combination did not identified any further DSPN cases.

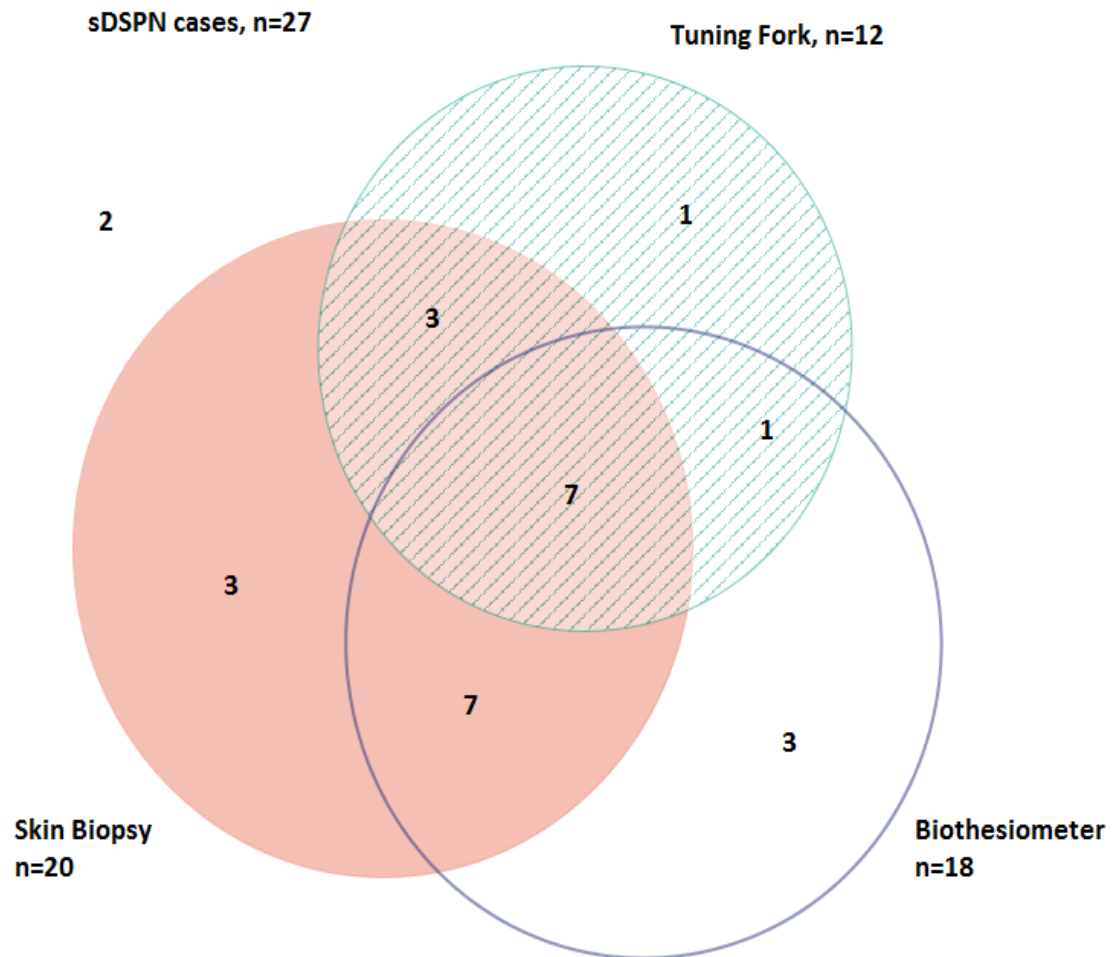


Figure 3 - Venn diagram showing the overlap of the various methods on identifying true sDSPN cases. The numbers of sDSPN cases are within each section. Skin biopsy overlapped with biothesiometer in 14 cases, with the tuning fork in 10 cases. The tuning fork and the biothesiometer overlapped in 8 cases. All three methods overlapped in 7 cases. The combination of the tuning fork and the biothesiometer identified 22 sDSPN individuals; adding skin biopsy to this combination resulted in identification of 3 further sDSPN cases.