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Diagnosis and prevalence of Diabetic Polyneuropathy: A Cross-Sectional Study of Danish Patients with Type 2 Diabetes

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Data available on request from the authors

Background
Diabetic polyneuropathy (DPN) is a common complication of diabetes. Using the Toronto criteria for diabetic polyneuropathy and the grading system for neuropathic pain, we assessed the performance of neuropathy scales and questionnaires by comparing them to a clinical gold standard diagnosis of DPN and painful DPN in a cohort of patients with recently diagnosed type 2 diabetes.
Methods

A questionnaire on neuropathy and pain was sent to a cohort of 5,514 Danish Type 2 diabetes patients. A sample of 389 patients underwent a detailed clinical examination and completed neuropathy questionnaires and scales.

Results

Of the 389 patients with a median diabetes duration of 5.9 years, 126 had definite DPN (incl. 53 with painful DPN), 88 had probable DPN, and 53 had possible DPN. There were 49 patients with other causes of polyneuropathy, neuropathy symptoms, or pain, 10 with subclinical DPN and 63 without DPN. The sensitivity of the Michigan Neuropathy Screening Instrument questionnaire to detect DPN was 25.7% and the specificity 84.6. The sensitivity of the Toronto Clinical Neuropathy Scoring System, including questionnaire and clinical examination was 62.9% and the specificity was 74.6%.

Conclusions

DPN affects approximately one in five Danish patients with recently diagnosed type 2 diabetes but neuropathic pain is not as common as previously reported. Neuropathy scales with clinical examination perform better compared with questionnaires alone, but better scales are needed for future epidemiological studies.

Diabetic polyneuropathy (DPN) is a length-dependent symmetrical sensorimotor polyneuropathy and is one of the most common and troublesome complications of diabetes [1]. The reported prevalence of DPN ranges from 13% to 55% [2-4], and 25% to 50% of these patients have neuropathic pain [3, 5]. The large variation in the prevalence may be explained by differences in study populations (e.g., diabetes duration and type) and different criteria for polyneuropathy and neuropathic pain.

Correct and early identification of patients with DPN is essential for preventive purposes. Finding a screening instrument that is simple and easy to administer with a high positive and negative predictive value is of importance. However, the identification of DPN and painful DPN is not straightforward [6]. The current gold standard for DPN is the hierarchical grading system by the Toronto Diabetic Neuropathy Expert Group and for neuropathic pain the NeuPSIG (The Neuropathic Pain Special Interest Group of the International Association for the Study of Pain) grading system. These require a medical history, neurologic examination, and neurophysiological or neuropathological testing [7, 8].

Clinical scores based on recorded symptoms and signs are expected to have higher diagnostic accuracy than symptoms alone [9], but questionnaires are useful in large epidemiological studies [10]. The Michigan Neuropathy Screening Instrument questionnaire

part (MNSIq) has been used to identify DPN and is validated for DPN in patients with type 1 diabetes [2, 11, 12]. Similarly, the Douleur Neuropathique 4 questionaire (DN4q) is a screening tool used to identify painful DPN [10, 13].

Only a few large studies, which included a detailed examination done by experienced neurologists and employed clinical, neurophysiological, and neuropathologic consensus criteria, have assessed the prevalence of DPN and often in unclear study populations [3, 14]. In this study, we performed a detailed clinical examination in a sample of a large well-characterized nationwide cohort of patients with recently diagnosed type 2 diabetes using the Toronto classification system and NeuPSIG grading system as gold standards for DPN and neuropathic pain, respectively. The aim was to determine the prevalence of DPN in newly diagnosed type 2 diabetes patients and to evaluate critically the diagnostic performance of questionnaires and neuropathy scales.

RESEARCH DESIGN AND METHODS

This was a cross-sectional population-based study of patients with recently diagnosed type 2 diabetes in Denmark, conducted at two study-sites, Aarhus and Odense. Patients were recruited from a questionnaire survey study conducted in 2016 among 5,514 patients from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. In the questionnaire survey study, all patients were screened for symptoms of DPN and painful DPN using the MNSIq and DN4q and the prevalence of possible DPN and painful DPN was calculated [15].

In order to limit patient transportation, we started by inviting all patients living close to the two study sites, where the only requirement for invitation was a valid answer on the questionnaire survey (random sample). To ensure a larger number of patients with DPN, we invited patients with symptoms of neuropathy based on the questionnaire survey study (MNSIq ≥ 4 *and/or* DN4q ≥ 3) from the rest of Denmark, except the Capital region and Eastern part of Zealand (selected sample). The Danish healthcare system is tax paid and offers free and equal access to all citizens, therefore we did not expect regional differences in health care access and treatment. The patient inclusion is illustrated in Figure 1. One reminder was sent to those who did not reply.

Exclusion criteria were cognitive impairment, language difficulties, and pregnancy. Patients were included from October 2016 to the end of October 2018. In addition, we included 97 subjects without diabetes of similar age and sex during the same period, recruited from within the patients' social or work circle and by flyers. Baseline characteristics and in-/exclusion criteria for this group are provided in Supplementary Table 1.

The two clinical investigators at each center were trained neurologists (S.S.G. and M.I.). They interviewed all patients, focusing on the presence of symptoms of polyneuropathy including duration, localization, and type of symptoms, medication use and comorbidities. On the same day, all patients filled in a questionnaire, including questions on lifestyle habits (smoking, alcohol consumption, and physical activity), psychological functions, and the MNSIq and DN4q.

Height, weight were measured and waist circumference (average of two measurements) between the lower ribcage and iliac crest after expiration in standing position) were measured. Blood samples for glycated hemoglobin (HbA_{1c}), non-fasting blood sugar levels, cholesterol, and triglycerides were taken.

We conducted a detailed neurological examination of lower extremities, including a standard sensory examination and mapping for the following modalities: Light brush stroking (Somedic AB, Hörby, Sweden); pinprick (Owen Mumford Neuropen with sterile neurotips and Semmes-Weinstein monofilament no 5.88 (bending force 75.9 g/745 mN;, Stoelting, Wood Dale, IL) and cold (20°C) and warmth (40°C) thermal rolls (Somedic AB). We used the upper thigh or chest as the control area. We assessed knee and ankle reflexes with a Tromner reflex hammer (US Neurologicals LLC) and muscle strength in accordance with the Medical Research Council (MRC) scale. Vibration sense was determined with both a 128 Hz tuning fork and a biothesiometer (Biomedical Instruments, Ohio) on the dorsum of the great toe. Lastly, clinical examination parts of the MNSI [16], DN4 [17], the Toronto Clinical Neuropathy Scoring System (TCNS) [18, 19] and The Utah Early Neuropathy Scale (UENS) [20] were performed. Agreement between the primary investigators (S.S.G. and M.I.) was ensured by making detailed descriptions of all procedures, joint training, by regularly examining patients together during the inclusion period, and by comparing results and conclusions.

A single 3 mm punch biopsy was taken 10 cm above the right lateral malleolus for determination of Intra Epidermal Nerve Fiber Density (IENFD) with subcutaneous anesthesia using 10 mg/ml lidocaine if needed. The fixation, cryoprotection, staining, and analysis methods are described in detail elsewhere [21]. We considered IENFD abnormal if it was lower than the fifth percentile compared to age and sex matched healthy controls, as previously published [22]. Among the included controls without diabetes, 2 (2%) had abnormal IENFD (Supplementary Table 1).

Conventional Nerve Conduction Studies (NCS) of sural nerves were carried out bilaterally, the median, peroneal, and tibial nerves unilaterally [23]. The ulnar nerve was

examined on the same side as the median nerve if the median nerve was found to be abnormal. The results were compared to laboratory controls using z-scores. Polyneuropathy was defined as ≥ 2 nerves with ≥ 1 abnormal measure, including at least one abnormal sural nerve [23].

We performed quantitative sensory testing (QST) using a reduced version of the standardized protocol of the German Research Network for Neuropathic Pain (DFNS) [24]. We examined perception thresholds for warmth, cold, vibration, pinprick, and pain as well as dynamic mechanical allodynia, and paradoxical heat sensation. Patients were examined on the dorsum of the right foot, and the vibration detection threshold was measured on the right medial malleolus. We used Equista, a data analysis system that transfers data into standard normal distribution (z scores) adjusting for age, sex, and body localization [25].

The predefined case definition of DPN was as proposed by the Toronto Diabetic Neuropathy Expert Group [8]. In the definition, there are three levels of certainty: Possible DPN, which requires at least one of either sensory symptoms, signs, or reduced ankle reflexes, probable DPN, which requires two of the three and definite DPN, which requires one of the three, plus abnormal NCS or IENFD. Clinical diagnosis of painful DPN was made in accordance with the NeuPSIG grading system and adapted to painful DPN [7]. Possible painful DPN was defined as pain in both feet/legs, and a diagnosis of diabetes, probable painful DPN as pain in both feet/legs and sensory signs in the feet/legs, and definite painful DPN as pain in both feet/legs and sensory signs in the feet/legs plus an abnormal IENFD or NCS (Supplementary Fig. 1). The symptoms and signs of neuropathy and pain reported in the DN4, MNSI, TCNS and UENS were not used to diagnose DPN and painful DPN.

The study was approved by the Regional Research Ethics Committee of Central Denmark Region (file number 1-10-72-130-16). The Danish National Committee on Health Research Ethics (file number S-20100082) approved the DD2 project. The Danish Data Protection Agency (file number 2008-58-0035) approved the DD2 project, and the study was registered at Aarhus University, internal notification number 62908-250. All patients and controls gave written informed consent.

STATISTICS

For the prevalence calculations we divided the patients into two strata based on the MNSIq and DN4q in the questionnaire study, (MNSIq ≥ 4 *and*/*or* DN4q ≥ 3 and those without) and by weighting the prevalences by the strata sizes we estimated the prevalence of definite DPN

and painful DPN with a 95% confidence interval (CI) [26]. An example of this calculation is described in supplementary Fig. 2.

Assuming clinical and demographic characteristics were monotonically increasing or decreasing when going from no DPN to definite DPN, we tested the hypothesis of no relationship using Spearman's rank order correlation. The correlation analyses were also stratified by sex.

We calculated sensitivity and specificity of the questionnaires (MNSIq and DN4q) and screening instruments (MNSI, TCNS, UENS) exclusively for the random sample of 165 patients as those calculations depend on patient sampling. Positive predictive values (PPVs) and negative predictive values (NPVs) and the receiver operating characteristic area (ROC) were calculated for the whole cohort of the examined patients, as they are independent of sampling. The gold standard (the comparator) was definite DPN and painful DPN. We performed the same calculations for at least probable (definite and probable) DPN and painful DPN as a gold standard. All results were presented with 95% exact binomial confidence intervals. McNemar's test for paired data was used to compare the prevalence of MNSIq ≥ 4 between the questionnaire survey study and the clinical study. McNemar's test was also used to compare proportions of patients with abnormal QST and IENFD (dichotomous), two different measures for small nerve fibers.

Study data were collected and managed using Research Electronic Data Capture (REDCap) hosted at Aarhus University. Double data entry was performed. For data analysis and figures, we used the STATA (version 14; StataCorp LP, College Station, TX) and R Core Team (2019).

RESULTS

Out of 5,514 eligible patients in the DD2 cohort who completed the questionnaire [15], 1,461 (26.5%) were invited for a clinical examination (Figure 1). Of those, 389 (26.6%) patients agreed to participate (Figure 1). There were no differences between responders and non-responders with respect to either demographic characteristics or symptoms of DPN and painful DPN reported in the questionnaire survey study (Supplementary Table 2). The time from participation in the questionnaire survey to the clinical study was median 1.29 (IQR 0.82; 1.71) years.

Of the 389 patients, 126 had definite DPN, of which 53 had painful DPN. A further 88 had probable, 53 possible, and 10 subclinical DPN, where NCS or skin biopsy results were abnormal in the absence of symptoms or signs (Figure 2). In addition, 49 had polyneuropathy

of other causes or other neurological or pain disorders indistinguishable from the symptoms of DPN and painful DPN (Figure 2), while 63 had no DPN.

Based on a clinical diagnosis of definite DPN and painful DPN in the cohort of 389 patients, the estimated prevalence of definite DPN and painful DPN in the questionnaire survey cohort of 5,514 patients, with a median time from diabetes diagnosis of 5.9 years (IQR 4.2; 7.1), was 22.7 % (95% CI: 17.5; 28.0) and 5.4% (95% CI: 3.5; 7.3) respectively. For probable and definite DPN the prevalence was 43.9 % (95% CI 37.3; 50.5) and for possible, probable and definite DPN it was 62.2% (95% CI 55.5; 68.8). For painful DPN the prevalence of at least probable painful DPN was 11.5% (95% CI 8.2; 14.9) and at least possible painful DPN 12.0% (95% CI 8.6; 15.3).

The sensitivity of a MNSIq score ≥ 4 to detect definite DPN was 25.7% (95% CI 12.5; 43.3) and the specificity was 84.6% (95% CI 77.2; 90.3) (Table 2). The sensitivity of the DN4q score of ≥ 3 together with pain in both feet in detecting definite painful DPN was 80% (95% CI 44.4; 97.5) and the specificity was 89.9% (95% CI 83.6;94.3) (Table 2). Values for PPV, NPV and the TCNS, MNSI examination and the UENS are provided in Table 2. There was a positive correlation between clinical scores and increased certainty of the DPN diagnosis from controls without diabetes to definite DPN for the TCNS, UENS, and MNSI clinical examination. For healthy controls, and patients with no DPN and possible DPN, the median scores were similar (Figure 2).

Comparing the MNSIq answers from the questionnaire survey study with answers from the day of the clinical examination in the cohort of 389 patients, there was a change in mean sum scores of -0.31 SD (1.7), yet 37.2% changed status from MNSIq ≥ 4 to MNSIq < 4 and 14.3% the other way around ($P < 0.001$) (Supplementary Table 3).

In general, increasing certainty of the DPN diagnosis (from no DPN to definite DPN) was weakly correlated to different patient characteristics. The proportion of males increased from no DPN to definite DPN. Patients with definite DPN had higher BMI, weight, waist circumference, height, and systolic blood pressure compared to those with no DPN, which persisted for BMI, weight, and waist circumference after stratifying for sex. (Table 1 and supplementary Tables 4 and 5). Patients with definite DPN used more insulin or insulin analogs, antihypertensive, and analgesic drugs compared to the other groups, but the same was not true for other antidiabetics than insulin or insulin analogs or dietary treatment (Table 1).

In the cohort of 389 patients, 386 (99.2%) had results for IENFD and/or NCS. Of those, 31.4% had abnormal IENFD and 23.9% abnormal NCS (Supplementary Table 6). Of the 126

patients with definite DPN, 113 had results for both IENFD and NCS. Of those, 19 (16.8%) had abnormal NCS alone, 47 (41.6%) had abnormal IENFD alone, and 47 (41.6%) had both (data not shown). The agreement between two different small fiber measures, IENFD and cold and warm detection thresholds on the QST was poor. Of those with abnormal IENFD, 35.5% had abnormal cold and/or warm detection thresholds and vice versa, 47.8% of patients with abnormal cold and/or warm detection threshold had abnormal IENFD, $P = 0.007$ (Supplementary Table 7).

Compared to normative material [25] most patients had loss of both small and large nerve fiber function on the QST where patients with probable and definite DPN had the most pronounced sensory loss (Supplementary Fig. 3 and Supplementary Table 8).

DISCUSSION

In this study, we did a detailed examination of patients from a large nationwide population of Danish patients with recently diagnosed type 2 diabetes. We estimated the prevalence using the gold standard definition of DPN and painful DPN and assessed commonly used screening tools. We found that screening tools that included a clinical examination performed better in the diagnosis of DPN compared with questionnaires alone.

Our prevalence estimates showed that there was a decrease in the prevalence with higher certainty of the DPN diagnosis, from 62.2% with at least possible DPN, 43.9% with at least probable DPN and 22.7% with definite DPN. For definite painful DPN the prevalence was 5.4%. In comparison, the prevalence of possible DPN defined as MNSIq≥4 was 18% from the questionnaire survey study. The discrepancy between the prevalence of possible DPN using either the MNSIq or clinical evaluation may reflect the difference between a symptom-based questionnaire and clinical interview and examination. Interestingly, around 20% of the patients had probable DPN with either symptoms and/or signs of DPN, but with normal NCS and IENFD, possibly indicating early signs of nerve damage or neuropathy. For this large group there is a potential for prevention and counseling or intervention such as foot care, fall prevention and change of life style factors including weight loss [27, 28]. Our prevalence estimates of definite DPN (22.7%) and painful DPN (5.4%) were similar to previous estimates although the prevalence of painful DPN was lower than earlier estimates [29, 30]. The low prevalence could be explained by the good metabolic control in the group, short duration of diabetes and that we carefully excluded patients with other causes of the pain in the feet.

In this study, we used the Toronto classification for defining possible, probable and definite DPN. This hierarchical system is reflected in an increase of median scores in both TCNS, UENS and MNSI clinical examination when going from possible to probable and to definite DPN. In QST parameters, patients with probable and definite DPN presented with similar degree of sensory loss of both small and large fiber measures. Taken together, these findings indicate that while a hierarchical system in classifying neuropathy is useful, there is also a substantial overlap between these different groups.

To our knowledge, the performance of the MNSIq in detecting definite DPN has not been evaluated in a cohort of solely type 2 diabetes patients or in patients with recently diagnosed diabetes. We found a sensitivity of the MNSIq ≥ 4 to detect definite DPN of 25.7%, a specificity of 84.6%, - a PPV of 42.9, and an NPV of 74.7%. Herman and colleagues [31] found a sensitivity of 40% and a specificity of 92%, a PPV of 69% and an NPV of 78% for MNSIq ≥ 4 for DPN in young (mean age 47 years) type 1 diabetes patients without severe complications or concurrent diseases. The duration and type of diabetes could also account for some of the differences. The performance of the MNSI examination part, TCNS and UENS was worse than previously reported in patients with longstanding type 1 diabetes for the MNSI and in patients with impaired glucose tolerance and early neuropathy for the TCNS and UENS [12, 32], but they performed better than the MNSIq, although MNSIq had the highest specificity. It is possible that better performances would have been obtained if other criteria for DPN were used. However, there is currently no evidence from this or other studies indicating better gold standards than the present to classify patients with painful or non-painful DPN. The combination of presence of pain in both feet and a positive DN4q score performed well, supporting the use of these screening questionnaires for detection of painful DPN.

Small fiber involvement was assessed using structural measures with IENFD and functional measures with determination of cold, warm detection thresholds and thermal sensory limen. The overall agreement between the functional and structural measures was weak, which is not surprising. In a previous meta-analysis we have shown that there is in general weak correspondence between structural and functional measures [33]. The reason for this lack of, or at best weak relationship between small fiber function and small nerve fiber count penetrating the skin from dermis into epidermis is not known. One reason might be that in DPN there is both degeneration and regeneration of nerve fibers [1] and it is currently unknown to what extent regenerating fibers may contribute to both the structural and functional measures [33].

There was low consistency in the MNSIq answers from the time of questionnaires survey study to the clinical study. This may be explained by fluctuations in DPN symptoms, the time difference between the two assessments, and the low sensitivity of the MNSIq, and suggests that questionnaires should not stand alone in the screening and diagnosis of DPN.

We used widely accepted gold standards for the diagnosis of definite DPN and definite painful DPN. We did not collect data on other diabetes complications than DPN in this study, these data are described in detail for the DD2 cohort elsewhere [34, 35]. We performed a structured neurological examination and obtained a detailed history in order to exclude other causes of polyneuropathy, however we did not verify the findings with e.g. B12 vitamin deficiency by blood samples. Even though we based the diagnosis of DPN on the bedside neurological examination together with history and NCS/IENFD, we cannot rule out incorporation bias, as some of the measures (i.e. reflexes and vibration measures) were also included in the scoring of the clinical neuropathy scales. Because we included patients from a known, representative population of 5,514 type 2 diabetes patients, we were able to do stratified sampling based on the questionnaire responses Therefore, our sample size of 389, combined with a thorough examination provided strong estimates of the prevalence with relatively narrow confidence intervals. Lastly, with 389 responders from a total of 1461 invited, a selection bias is possible although there were no differences in clinical characteristics between the groups (supplementary table 2) and the questionnaire study cohort from which we sampled was representative of recently diagnosed Danish type 2 diabetes patients [15, 34].

In conclusion, DPN in patients with recently diagnosed diabetes is common, with one in five patients having confirmed DPN, and almost half having at least symptoms and/or signs of DPN. However, painful DPN was not as common as previously reported. Neuropathy scales including both questions and clinical examination, or clinical examination alone, were more accurate in the detection of definite DPN than questionnaires alone. Therefore, it is better to use the gold standard, even in epidemiological studies if possible.

Author contributions

S.S.G. designed the study, collected data, performed the statistical analyses, drafted the manuscript, contributed to the discussion, and approved the final manuscript. M.I. designed the study, collected data, drafted the manuscript, contributed to the discussion, and approved the final manuscript. A.G.K., T.K., P.K. and D.H.C. collected data, contributed to the discussion and revised the manuscript critically and approved the final manuscript. N.B.F.,

S.H.S., H.T., T.S.J., D.L.B, S.K.N, N.T.A and T.K. designed the study, revised the manuscript critically, contributed to the discussion, and approved the final manuscript. R.W.T., J.S.N, H.A. and B.C. revised the manuscript critically, contributed to the discussion and approved the final manuscript.

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Table 1. Patient characteristics of the 330 diabetes patients with no DPN, possible DPN, probable DPN, and definite DPN and the correlation between characteristics and the DPN groups.

	No DPN	Possible DPN	Probable DPN	Definite DPN	r_s/ *P*
N	63	53	88	126	
Female sex, n (%)	31 (49.2)	28 (52.8)	37 (42.1)	41 (32.5)	-0.15/ 0.006
Age (years)	64.1 (58.3; 70.7)	64.6 (57.3; 70.9)	67.7 (59.1; 72.4)	67.2 (58.0; 72.3)	0.10/0.064
Duration of diabetes (years)	5.5 (4.2; 6.7)	5.9 (4.2; 7.1)	5.8 (4.0; 7.0)	6.1 (4.5; 7.4)	0.08/0.14
BMI (kg/m²)	30.0 (27.5; 34.3)	29.4 (26.7; 35.9)	29.8 (26.5; 33.5)	32.8 (29.2; 37.6)	0.19/ <0.001
Height (cm)	170.0 (162.0; 178.0)	169.0 (163.0; 177.0)	174.5 (165.0; 180.0)	177.0 (168.8; 181.6)	0.23/<0.001
Weight (kg)	91.8 (75.7; 101.0)	88.0 (76.5; 102.6)	88.3 (77.2; 102.5)	105.5 (86.9; 114.8)	0.28/<0.001
Waist circumference (cm)	104.8 (95.4; 113.1)	104.0 (96.8; 114.3)	104.3 (94.6; 114.9)	113.3 (104.3; 123.8)	0.28/<0.001
Systolic blood pressure (mmHg)†	138.0 (126.0; 145.0)	135.0 (129.0; 144.5)	136.0 (123.0; 148.0)	143.0 (131.0; 151.3)	0.14/0.010
Diastolic blood pressure (mm/Hg)†	82.0 (75.0; 91.0)	82.0 (75.5; 90.0)	82.0 (74.3; 89.0)	83.0 (78.0; 89.0)	0.02/0.66
HbA_{1C} (mmol/mol)	49.0 (45.0; 53.0)	49.0 (45.0; 53.0)	49.0 (43.5; 57.0)	50.0 (45.0; 58.0)	0.09/0.10
Total cholesterol (mmol/L)	4.2 (3.7; 4.6)	4.1 (3.6; 4.8)	4.1 (3.5; 4.7)	3.9 (3.3; 4.5)	-0.07/0.22
Triglycerides (mmol/L)	1.8 (1.3; 2.7)	2.0 (1.4; 2.6)	1.6 (1.3; 2.4)	2.0 (1.5; 2.9)	0.08/0.16
Alcohol (>7/14 units/week female/male) ‡	5 (7.9)	7 (13.2)	9 (10.2)	14 (11.1)	0.02/0.71
Current smoker	9 (14.3)	9 (17.0)	16 (18.4)	17 (13.6)	-0.02/0.73
Physical activity (≥3 times/week)§	20 (40.0)	14 (30.4)	39 (47.6)	41 (34.2)	-0.03/0.60
Treatment with:					
Antidiabetics other than insulin §§	51 (81.0)	48 (90.6)	81 (92.1)	109 (86.5)	0.03/0.61
Insulin or insulin analogs	7 (11.1)	4 (7.6)	5 (5.7)	32 (25.4)	0.19/<0.001
No other than dietary	10 (15.9)	3 (5.7)	7 (8.0)	13 (10.3)	-0.03/0.59
Antihypertensives	42 (66.7)	37 (69.8)	56 (63.6)	100 (79.4)	0.11/0.043

Cholesterol lowering drugs	48 (76.2)	45 (84.9)	69 (78.4)	102 (81.0)	0.02/0.70
Anticoagulants ¶	28 (44.4)	24 (45.3)	39 (44.3)	64 (50.8)	0.05/0.34
Analgesics ¶¶	16 (25.4)	16 (30.2)	31 (35.2)	57 (45.2)	0.16/0.004
Asymptomatic DPN	-	33 (62.3)	13 (14.8)	28 (22.2)	-

Data are shown as medians (interquartile range) or *n* (%).Clinical characteristics are provided for the 330 patients with either possible, probable or definite DPN, not including subclinical DPN and other causes of neuropathy, neuropathy symptoms or pain.

†The recommended goal for hypertension treatment in patients with diabetes in Denmark is a systolic blood pressure of 120-130 mmHg and a diastolic blood pressure of 70–80 mmHg. ‡The maximum amount of alcohol units per week recommended by the Danish Health Authority. §On average, how often are you physically active per week. §§Antidiabetics other than insulin: metformin, dapagliflozin, liraglutide, sitagliptin, glimepiride, glibendamide, empagliflozin; ¶Anticoagulants: acetylsalicylic acid, clopidogrel, warfarin, and novel oral anticoagulants; ¶¶Analgesics**:** paracetamol, NSAIDs, TCAs, gabapentin, pregabalin, SNRIs, tramadol, codeine, and morphine. Missing data; <0.01%, except for physical activity with (32) 9.7% missing.

r_s=Spearman's Rho. - : not applicable. TCA: tricyclic antidepressants. NSAID: nonsteroidal anti-inflammatory drugs. SNRI: serotonin-noradrenaline reuptake inhibitors. HbA_{1C}: glycated hemoglobin.

Table 2. Diagnostic accuracy of questionnaires and clinical scoring instruments with different cutoff points to detect definite DPN and the DN4q and pain in both feet to detect definite painful DPN.

	MNSIq ≥3*	MNSIq ≥4*	MNSI q≥5*	DN4≥3 and pain in both feet**	TCNS > 5*	UENS> 3*	MNSI clinical* examination > 2
Definite DPN* Definite painful DPN**							
Sensitivity	37.1 (21.5;55.1)	25.7 (12.5;43.3)	25.7 (12.5;43.3)	80.0 (44.4;97.5)	62.9 (44.9;78.5)	82.9 (66.4;93.4)	74.3 (56.7;87.5)
Specificity	73.1 (64.6;80.5)	84.6 (77.2;90.3)	88.5 (81.7;93.4)	89.9 (83.6;94.3)	74.6 (66.2;81.8)	50.0 (41.1;58.9)	68.5 (59.7;76.3)
Correctly classified	65.5	72.1	75.2	89.2	72.1	57.0	69.7
ROC area	0.55 (0.46;0.64)	0.55 (0.47;0.63)	0.57 (0.49;0.65)	0.85 (0.72;0.98)	0.69 (0.60;0.78)	0.66 (0.59;0.74)	0.71 (0.63;0.80)
Positive predictive value	41.0 (34.2;47.9)	42.9 (35.1;51.1)	46.2 (37.0;55.6)	43.3 (33.6;53.3)	52.7 (45.2;60.2)	44.7 (38.5;51.0)	48.0 (41.0;55.1)
Negative predictive value	77.7 (70.8;83.5)	74.7 (68.6;80.1)	73.7 (68.0;78.9)	97.0 (94.2;98.7)	85.5 (80.0;90.0)	91.0 (84.9;95.3)	84.9 (78.9;89.7)
At least probable DPN* At least probable painful DPN**							
Sensitivity	40.5 (29.3;52.6)	27.0 (17.4;38.6)	24.3 (15.1;35.7)	72.7 (49.8;89.3)	-	-	-
Specificity	80.2 (70.6;87.8)	90.1 (82.1;95.4)	93.4 (86.2;97.5)	95.2 (89.9;98.2)	-	-	-
Correctly classified	62.4	61.8	62.4	91.9	-	-	-
ROC area	0.60 (0.53;0.67)	0.59 (0.53;0.65)	0.59 (0.53;0.64)	0.84 (0.74;0.94)	-	-	-
Positive predictive value	66.7 (59.9;73.0)	67.9 (60.0;75.2)	73.1 (64.2;80.8)	69.2 (59.4;77.9)	-	-	-
Negative predictive value	58.7 (51.1;66.0)	53.6 (47.0;60.2)	53.0 (46.8;59.0)	93.3 (89.6;96.0)	-	-	-

Data are % with 95% confidence interval. The questionnaires (MNSIq and DN4q) were filled in on the day of the clinical examination. The MNSIq consists of 13 questions and the DN4q of seven. There were 17 with missing data on the DN4q. The sensitivity and specificity were calculated in the sample of randomly selected patients (165) and positive and negative predictive values for all 389 patients. Gold standard (the comparator) were patients with definite DPN/painful DPN or at least probable DPN/painful

DPN compared to those without definite DPN/painful DPN or without at least probable DPN/painful DPN. MNSIq: Michigan neuropathy screening instrument questionnaire. DN4q: douleur neuropathique en 4 questions. TCNS: Toronto Clinical Neuropathy Scoring System. UENS: Utah Early Neuropathy Score. ROC area: receiver operating characteristic area (sensitivity + specificity) /2. - : not applicable.

Figure legends.

Figure 1. Flowchart of patient inclusion and patient groups/diagnosis according to the Toronto criteria [8] and the NeuPSIG grading of neuropathic pain [7].

†Questionnaires were sent to 6,726 patients enrolled in the DD2 cohort by February 2016. ‡Reminders were sent to 72.7% of those not responding to the first invitation (because of study completion, not all received a reminder) and 7.1% confirmed to participate. §Of the131 that refused to participate, *n* = 71 gave reasons: had no time, energy, or was not interested, (*n* = 54), other acute or chronic diseases than diabetes (*n* =13), living abroad (*n* = 2) and geographical distance (*n* = 2). §§Reasons for exclusion: psychiatric disease (*n* = 1), dementia and other cognitive problems (*n* = 5), and language difficulties (*n* = 2).
¶Subclinical DPN: Patients with no symptoms or signs of DPN and abnormal NCS (*n* = 5) or IENFD (*n* = 5).
¶ ¶Other causes of polyneuropathy: Chemotherapy- or alcohol-induced polyneuropathy, chronic inflammatory demyelinating polyneuropathy, sarcoidosis, vitamin B12 deficiency, infection with human immunodeficiency virus, and psoriasis arthritis. Other diseases causing pain or neurological symptoms: Spinal stenosis, arthritis (osteo-, psoriasis, borrelia-), herniated disc, multiple sclerosis, fibromyalgia, peripheral arterial disease, stroke, transversal myelitis, sequela from trauma and operations (in the back and feet), pes planus transverse, progressive supranuclear palsy, and restless leg syndrome.
DD2: Danish centre for strategic research in type 2 diabetes. IENFD: intraepidermal nerve fiber density
NCS: nerve conduction studies. DPN: diabetic polyneuropathy.
In the group of 389 patients, skin biopsies for IENFD were not taken for 55/389, and NCS were not conducted on 9/389. Three patients had neither IENFD nor NCS results and could therefore only be classified as maximum probable DPN.

Figure 2. The median TCNS, UENS and MNSI examination scores and the correlation between the DPN groups inclusive controls without diabetes and the scores.

TCNS: r_s: 0.79, $p < 0.001$, UENS r_s: 0.73, $p < 0.001$, MNSI r_s 0.61, $p < 0.001$
TCNS: Toronto Clinical Neuropathy Scoring System: 0-5 = no neuropathy, 6-8 = mild neuropathy, 9-11 = moderate neuropathy, >11 = severe neuropathy (highest possible score is 19). UENS: the Utah early neuropathy scale (highest possible score is 42). MNSI: Michigan neuropathy screening instrument, examination part (highest possible score is 10). r_s=Spearman's Rho. DPN: diabetic polyneuropathy.

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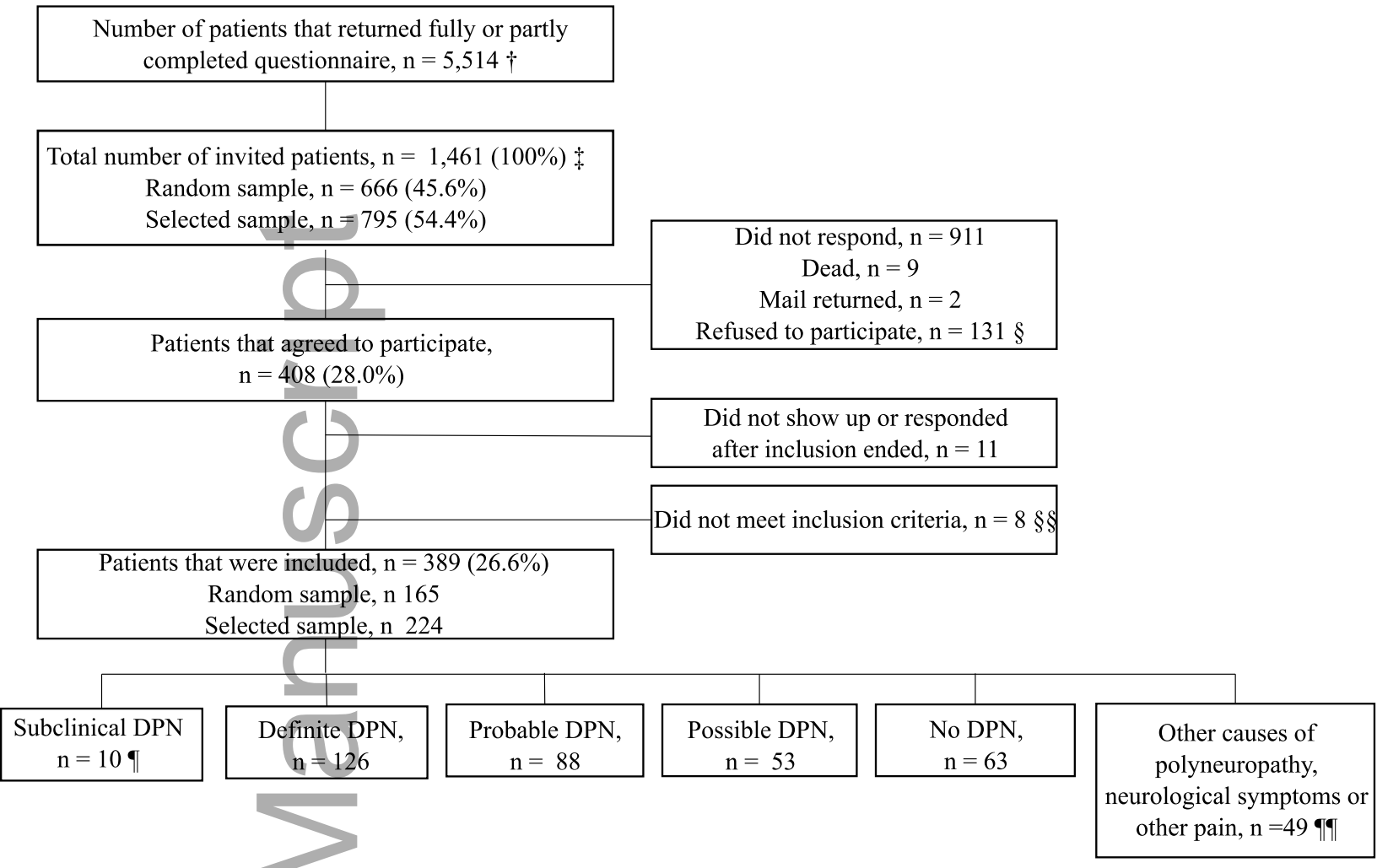

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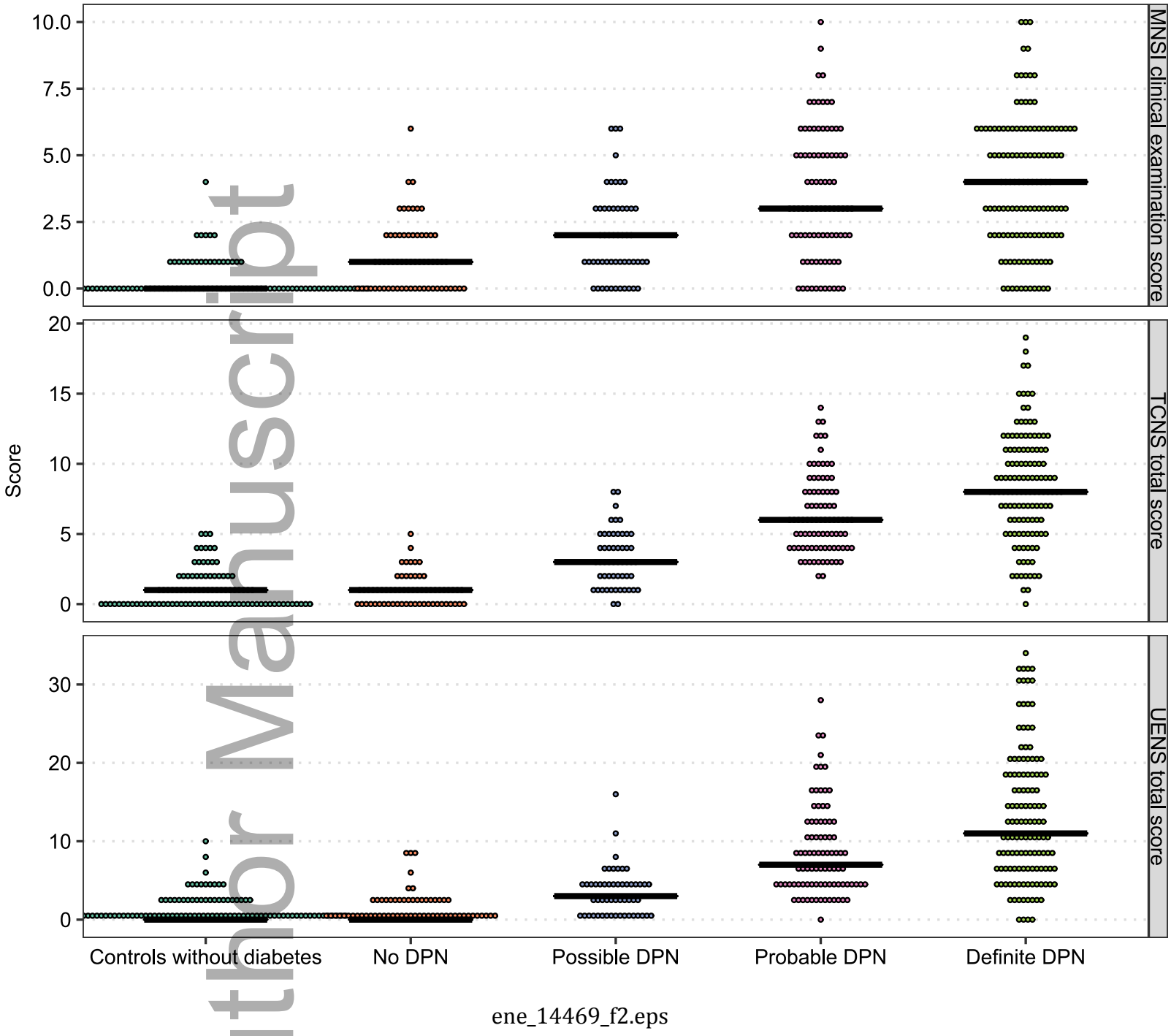

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