

A Standardized Clinical Evaluation of Phenotypic Diversity in Diabetic Polyneuropathy

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Abstract

Diabetic polyneuropathy (DPN) is a major cause of neuropathic pain and a frequent target condition in analgesic treatment trials. Differences in the clinical symptoms and signs associated with DPN suggest distinct pathophysiological mechanisms underlying nerve damage and dysfunction that are likely to have therapeutic relevance. The aim of this study was to develop a tool for the bedside assessment of painful neuropathies such as DPN that captures the diversity of phenotypes. Sixty-one patients with type 2 diabetes (DM2) and painful neuropathy, 19 patients with painless DPN, 25 patients with DM2 but no clinical evidence of neuropathy and 20 healthy control subjects completed a structured interview (47 items) and a standardized physical examination (39 items). After analyzing critical features of pain and painless symptoms and examining the outcome of physical tests of sensory function, we determined the principal components of the phenotypic variance among patients. Increased sensitivity to mechanical or thermal stimuli and, to a lesser extent, the sensory quality of pain or paresthesia were the most discriminating elements of DPN phenotypes. Correlation patterns of symptoms and signs indicated the involvement of functionally distinct nerve fiber populations. We combined interview questions and physical tests identifying these differences in a shortened assessment protocol that we named Standardized Evaluation of Pain and Somatosensory Function (StEPS). StEPS generates a phenotypic profile of patients with neuropathy. Separate intensity ratings for spontaneous painful symptoms and pain evoked by standard stimuli support a detailed documentation of neuropathic pain and its response to analgesic treatment.

1. Introduction

Patient stratification by phenotype is increasingly recognized as a promising approach to providing pain relief with greater precision and reducing outcome variation in analgesic treatment trials [5]. Diabetic polyneuropathy (DPN) is one of the leading causes of neuropathic pain and a frequent target condition in studies of neuroprotective or analgesic treatment. Up to 54% of patients with type 1 diabetes and 45% of patients with type 2 diabetes develop a clinical neuropathy [24,63]. DPN, which involves multiple somatic and autonomic nerves and produces symptoms and signs in a distal symmetrical distribution, is the most common manifestation [15]. The phenotype of DPN varies substantially. The majority of patients present with a variety of sensory deficits, whereas pain occurs in 16% to 21% of the patients [1,19]. Mild autonomic impairment can be detected in many patients with type 2 diabetes [39], although stringent criteria of autonomic neuropathy yield a lower prevalence of 22% [64]. Loss of motor function is less frequent [3]. The cause of this phenotypic diversity is unclear. Age, diabetes duration, poor glycemic control and vascular disease contribute to the overall risk of DPN but do not explain differences in symptoms and signs [56,63]. Nor do pathological findings in nerve or skin biopsies necessarily match the clinical phenotype. For example, deficits in temperature discrimination correlate inconsistently with the intraepidermal loss of temperature-sensitive C or A δ nerve fibers [21], and degenerative changes in nociceptive nerve fibers do not explain the manifestation of pain [17,59].

Phenotypic variance in DPN is likely to result from a combination of genetic and pathophysiological factors [9,15,60,62]. A thorough standardized characterization of DPN's clinical features will be necessary to identify these factors and determine their potential correlation with treatment response [61]. Strategies for the assessment of the neuropathic phenotype should ideally provide comprehensive information about all relevant disorders of nerve function and be suitable for implementation in research trials as well as clinical practice. However, most questionnaires for DPN are deliberately short and designed to screen large study populations for key symptoms of the disease or physical impairment, not phenotypic variance [14,23,26,53]. Measures of nerve conduction, quantitative sensory testing (QST) and autonomic reflexes generate detailed information about nerve fiber function,

but these investigations demand time, technical expertise and equipment that is typically only found in specialized neuromuscular centers [30,37,38,55].

Here we have taken an alternative approach and developed a structured interview and standardized physical examination for the bedside evaluation of sensory function and pain in DPN. We prospectively compared patients with type 2 diabetes and healthy control subjects, and conducted a principal component analysis (PCA) to determine which combinations of clinical symptoms and signs distinguish DPN phenotypes. Previous studies of neuropathic pain included subjects with diverse neurological disorders, generating information that may not entirely apply to DPN [11,42,50], especially painless variants of the disease [6,12,29]. To our knowledge, this is the first specific analysis of phenotypic variance in DPN that evaluates sensory loss and pain with a broad array of standardized clinical measures.

2. Methods

The study was performed at Massachusetts General Hospital in Boston, Massachusetts. Partners Human Research Committee, which serves as the institutional review board of Massachusetts General Hospital, approved the research (Protocol 2009P-001449). All patients gave written informed consent.

2.1 Study participants

We prospectively recruited 20 healthy control (HC) subjects and 164 patients diagnosed with type 2 diabetes mellitus (DM2) according to the criteria established by the American Diabetes Association [2]. Patients gave authorization to confirm the diagnosis with their treating physician. All subjects were older than 18 years. Probable DPN as defined by the Toronto Diabetic Neuropathy Expert Group was independently diagnosed by two experienced neurologists (WSD, DAC) and a trained physician assistant (ACB). The diagnosis required a combination of ≥ 2 neuropathic symptoms or signs including paresthesia, pain, sensory deficits in a distal symmetric distribution and unequivocally decreased or absent ankle reflexes [55]. Records of previous nerve conduction studies, skin or nerve biopsies were

reviewed when available to confirm the diagnosis. Patients with DPN were classified into those with (P) or without chronic pain (NoP) defined as persistent or recurrent pain for ≥ 3 months [58]. If pain was present, its intensity had to be ≥ 4 on a numerical rating scale (NRS) from 0 (no pain) to 10 (maximum imaginable pain) in the week before enrollment. This threshold was chosen to match the minimum pain intensity required for inclusion in many analgesic treatment trials [22,28]. All subjects with DPN-P fulfilled the criteria for definite neuropathic pain [57]. Subjects were excluded if they had another neurological or painful disorder, infection, severe medical or psychiatric illness, or if they were unable to give written informed consent. Previously prescribed analgesic treatment was allowed to continue.

2.2 Diabetic neuropathy scores

We determined the Diabetic Neuropathy Symptom (DNS) score, which documents unsteady gait, pain, paresthesia and numbness. The highest DNS score is 4 [43]. We also calculated the symptoms and sensory tests scores of the Toronto Clinical Neuropathy Scoring System (CNS). The symptoms score rates pain, numbness, tingling, weakness, ataxia and upper-limb symptoms. The sensory tests score is calculated based on the presence of abnormal responses to pinprick, temperature stimulation, light touch or vibration, or failure to recognize changes in the position of the big toe. The highest symptoms score is 6, the highest sensory tests score is 5 [13].

2.3 Assessment of sensory symptoms and signs

The investigators evaluating symptoms and signs were blind to the diagnosis of diabetes or polyneuropathy. They were specifically trained in the evaluation protocol to ensure consistent test application and interpretation.

We designed the assessment of sensory symptoms and signs as an extension of the Standardized Evaluation of Pain (StEP), which we recently validated for the diagnosis of radicular back pain [50]. Symptoms were recorded in a structured interview (47 items). Questions addressed the physical location of pain, its temporal characteristics, sensory quality and dependence on external stimuli. If pain evoked by stimuli such as touch or cold was reported, we asked whether the pain was limited to

the presence of the stimulus or persisted beyond the exposure (aftersensation). Subjects indicated the intensity of each aspect of their pain on an NRS from 0 to 10. Other interview items explored the presence of numbness or positive painless sensations (paresthesia) such as tingling or itch.

The physical examination (39 items) consisted of a standardized bedside evaluation of sensory signs and a brief assessment of peripheral autonomic functions. Quantitative tests of autonomic and motor functions are reported separately. We used two von Frey filaments (North Coast Medical) to examine the reaction to punctate mechanical stimulation at low (2 g) or high strength (26 g). To test the response to dynamic mechanical stimulation, we moved a soft brush (SENSELab 05; Somedic) at a constant speed of 3–5 cm/s over the skin without changing direction. The response to pressure was tested with a rubber-tipped algometer (Wagner Instruments), applied for 10 s at steady low (2 kg/cm²) or high force (6 kg/cm²). For pinprick-evoked pain, we used a medium-size unbeveled pin. Warm (40°C, 45°C) and cold (20°C, 5°C) stimuli were produced with a pen-shaped Peltier thermode of 1.25 cm diameter (NTE-2A; Physitemp Instruments). Each temperature was applied for 10 s. We waited at least 1 min before changing the test temperature. The sense of vibration was assessed with a standard tuning fork (128 Hz) placed over the first metatarsophalangeal joint. Proprioception was evaluated by examining the sense of position and passive movement at the interphalangeal joint of the big toe. To test for temporal summation, the stronger of the two von Frey filaments (26 g) was applied repeatedly, at a rate of 1–2 Hz for 30 seconds. The test was considered positive if a painless response turned painful or evoked pain increased in its intensity during the stimulation. With the exception of temporal summation, each of the test stimuli was applied 4 times and the result considered positive when ≥ 3 stimulations produced a consistent response. If pain was provoked, we asked the patient to rate the intensity of the pain using an NRS. All tests were performed on the dorsum of the foot and compared to responses on the dorsum of the hand or the forearm, if DPN involved the hands.

We also compared the sensitivity and specificity of pain detection with the Douleur Neuropathique en 4 Questions (DN4) screening tool for neuropathic pain [10]. DN4 items were integrated into the standardized evaluation of sensory symptoms and signs so that neither the patients nor the investigators

were able to differentiate interview or examination items belonging to the DN4.

2.4 Statistical analysis

General subject characteristics were compared across groups in a one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Frequency distribution of sensory symptoms and signs was analyzed with Fisher's exact test. We performed a principal component analysis (PCA) to ~~identify correlated symptoms and signs of DPN, evaluate and reduce the number and dimensions of phenotypic features, identify correlated symptoms and signs and determine the level of contribution of these variables to the phenotypic variance.~~ The analysis allowed us to reduce the complexity of the phenotypic variance between subjects to the principal components of differences in their responses to interview questions and physical test outcomes. We included the phenotyping results of all patients with DM2 in this analysis, considering diabetes without polyneuropathy (DM2 w/o DPN) as reference condition. Items with high ~~PCA-component~~ loadings were included in an abridged version of the assessment protocol. To determine the effectiveness of the full assessment and this reduced set of items in capturing sensory deficits and pain, we added the responses in each set of variables to a series of logistic regression models and compared the results against the independent neurological classification of the patients. Based on the separation between subgroups of patients, we generated receiver operating characteristic (ROC) curves and calculated the area under the curves (AUCs) to assess accuracy. All analyses were performed using the Statistical Analysis System software program (SAS Institute). Venn diagrams were generated with the Venn Diagram Plotter (Pacific Northwest National Laboratory).

3. Results

3.1 Subject characteristics

Table 1 lists demographic and clinical characteristics of the 105 patients with DM2 and 20 HC subjects included in the study. The median duration of diabetes in patients with DPN-P (10 years) or DPN-NoP (15 years) was longer than in patients w/o DPN (5 years; $P < 0.05$), although 7 patients w/o DPN were

diagnosed with DM2 more than 10 years ago. Hemoglobin A1c (HbA1c) values did not significantly differ between patient groups. As would be expected, patients with DPN-P had higher DNS and Toronto CNS symptom scores than patients with DPN-NoP ($P < 0.001$). The majority of patients with DM2 had a family history of diabetes. Half of the patients with DPN-P further reported a family member with neuropathy, a larger proportion than that of patients with DPN-NoP or DM2 w/o DPN ($P < 0.001$).

3.2 Neuropathic phenotypes

The outcome of the standardized assessment was in good agreement with the independent neurological triage of patients with and without DPN and patients with neuropathic pain. Relative to age-adjusted reference values [41], the assessment revealed increased pain sensitivity in 68 subjects and a loss of sensory function in ~~86~~81 subjects. Sensory deficits were also found in 3 HC subjects and 3 patients classified as having DM2 w/o DPN (Fig. 1).

Patients with DPN-P described both pain located in the skin and deeper (Fig. 2). Intermittent episodes of spontaneous or stimulus-dependent pain were more common than continuous pain, which was reported by 34% of the patients. Sharp, stinging or pins and needles were the most frequent pain descriptions. Burning pain, often regarded as a pathognomonic symptom of neuropathic pain, was reported by 64% of the patients. Paresthesia occurred in virtually all patients with DPN-P and 74% of patients with DPN-NoP. Tingling was the predominant type of paresthesia. Patients with DPN-P further described paresthesia of other sensory qualities, for example itching or warm, which were rare in DPN-NoP ($P \leq 0.015$) (Fig. 2). The overall pattern of sensory deficits in DPN-P and DPN-NoP was similar, although the responses to some mechanical stimuli were more often decreased in patients with a painful neuropathy. For example, the detection of brush movement was reduced in 39% of patients with DPN-P versus 5% of patients with DPN-NoP ($P = 0.004$) (Fig. 3).

Increased excitability of nerve fibers whose terminals are spared from degenerative changes or regenerating can be a critical modulator of neuropathic pain [17,27,35,36]. We identified 7 patients with DPN-P who, based on their physical examination, appeared to have intact small-fiber function. They

accurately responded to pinprick, were able to discriminate cold and hot temperatures, and did not exhibit abnormal sweating [21,55]. All of these patients described stinging pain or a sensation of painful needles and pins. Burning (43%) or cold pain (14%) was less frequent compared to patients with deficits in small fiber function, whereas temporal summation (43%), pinprick (57%) and heat hyperalgesia (71%) were more common. We also identified 5 patients with DPN-P who did not show clinical signs of large-fiber dysfunction. These patients were able to detect light punctate stimuli (2 g), brush movement and vibration, and had intact proprioception. Interestingly none of these patients described a continuous presence of pain (Fig. 2). Light punctate or dynamic mechanical stimulation did not evoke a painful response in patients with preserved large-fiber function (Fig. 3).

3.3 Correlations between symptoms and signs

Numbness was a common symptom among all patients with DPN. Numbness is typically associated with a deficit in touch sensitivity [32]. Physical tests revealed indeed a decreased detection of mechanical stimuli in the majority of patients reporting numbness. However, some patients experienced numbness in the absence of a clinically manifest touch deficit. Six patients showed only a reduced sense of vibration. Seven patients had decreased responses only in nociceptive tests and in 4 of these patients, only the responses to noxious cold or hot temperature were affected (Fig. 4A). Rather than indicating a particular sensory deficit, numbness may signal more generally a disruption of the normal pattern of afferent input.

We evaluated the relation between symptoms and physical test outcomes further by conducting a PCA. Painful responses to light mechanical stimulation (2 g) showed good correlation with a history of mechanical allodynia ($R = 0.44$). Rather unexpected was the weak correlation of painful responses to brush movement ($R = 0.24$). Patients reporting increased sensitivity to pressure-evoked pain, for example, from a tight shoe, also responded with pain to the application of calibrated pressure in the physical examination ($R = 0.40$), whereas mechanical stimulation at lighter force was painless (Fig. 4B). Patient reports of thermal allodynia equally correlated with painful responses to cold or warm stimuli in the physical tests (Fig. 4C). In addition, heightened temperature sensitivity appeared to influence the

sensory character of pain and paresthesia. Patients with cold-evoked pain described pain ($R = 0.44$) or paresthesia ($R = 0.38$) of a cold quality more often than burning pain ($R = 0.09$) or warm paresthesia ($R = 0.20$) (Fig. 4C).

Deficits in sensory function correlated across mechanical test modalities, whereas stimulus-dependent pain appeared to be more specific. For example, allodynia in response to light punctate stimulation was strongly associated with pain evoked by brush movement ($R = 0.59$) but distinct from pinprick hyperalgesia ($R = 0.11$ and -0.02 , respectively) (Fig. 5A). Painful responses to cold or warm stimuli also showed greater temperature specificity than deficits in temperature discrimination (Fig. 5B).

3.4 Principal components of the phenotypic variance

PCA reduces the complexity of multivariate outcomes to principal components and calculates the loading (weight) of each variable on these components. Loss of sensory function and pain hypersensitivity determined the first two principal components of phenotypic variance in the physical tests (Fig. 6A). Thermal test responses scored higher on the first component, whereas responses to punctate mechanical stimulation or pinprick dominated in the second component (Fig. 6B, C). These two components accounted for 16% and 12%, respectively, of the variance. A third component (7%) was defined by differences in the temporal summation of repeated mechanical stimulation and autonomic signs (data not shown). Across 11 components, which combined accounted for 70% of the variance between physical signs, we identified a decrease in the detection of warm temperature (40°C), a reduced response to pinprick, loss of proprioception, abnormal sweating and trophic changes of the skin as indicators of DPN with consistently high weights. Heat hyperalgesia (45°C) and temporal summation of mechanical stimuli were signs of increased pain sensitivity with high loadings on the components. We also identified tests that contributed little to the phenotypic variance. For example, a painful response to stimulation with the von Frey filament of 2-g strength was strongly correlated to pain evoked by the filament of 26-g strength (Fig. 6). Hence we considered the test redundant.

Differences in the experience of pain caused by a certain body position or physical activity such

as walking, and the sensitivity to cold or warm temperatures marked the first principal component of neuropathic symptoms (Fig. 7). Patient reports of pain evoked by these external factors were responsible for 39% of the variance. The sensory quality of pain or paresthesia was a prominent factor on the second principal component, which accounted for 6% of the phenotypic variance (data not shown). Across 9 symptom components, which combined accounted for 70% of the phenotypic variance, we found high weights for the presence of continuous versus episodic pain, the experience of cold-evoked pain, a burning quality of pain, and paresthesia characterized by a cold or pulling sensation. On the other hand, symptoms such as throbbing pain or pain that felt like an electric shock did not add substantially to the differentiation of phenotypes.

3.5 Standardized Evaluation of Pain and Somatosensory Function (StEPS)

We combined items with high weights on the principal components and items such as tingling paresthesia or numbness, which were common symptoms across all phenotypes of DPN, in an abridged assessment tool that we named Standardized Evaluation of Pain and Somatosensory Function (StEPS). StEPS includes 20 interview questions (plus a global intensity rating of current pain) and 20 physical tests (see Supplemental Digital Content 1). ~~We combined items with high loadings on the principal components in a reduced set of interview questions and physical tests and named this abridged assessment tool Standardized Evaluation of Pain and Somatosensory Function (StEPS) (see Supplemental Digital Content 1).~~ StEPS generates a brief but informative phenotypic profile of sensory neuropathy that can be completed within 20 minutes. The intensity of spontaneous pain and painful responses to external stimuli are rated independently to document their specific contribution to the patient's experience of pain. To compare the diagnostic performance of StEPS with that of the original 47-item interview and the 39-item physical examination, we conducted a receiver operating characteristic (ROC) analysis. StEPS clearly distinguished DPN-P (area under the ROC curve 1.00; CI 0.99, 1.00) and DPN-NoP (0.89; CI 0.79, 0.99) from DM2 w/o DPN (Fig. 8). StEPS also differentiated DPN-P from DPN-NoP with high sensitivity and specificity (0.98; CI 0.96, 1.00). Numbness, tingling paresthesia, a decreased response to

pinprick and a reduced sense of vibration were among the most sensitive and specific symptoms or signs, respectively, of neuropathy. Deep or superficial pain, a stinging or burning pain quality, episodic pain and painful aftersensations were among the strongest indicators of DPN-P (see Table S1, Supplemental Digital Content 2). The diagnostic accuracy of StEPS was equivalent to that of the full assessment protocol (Fig. 8). Sensitivity and specificity of the pain diagnosis matched or exceeded those of the DN4 questionnaire (see Table S2, Supplemental Digital Content 23).

4. Discussion

We developed a novel phenotyping tool for painful peripheral neuropathy based on an analysis of ~~interindividual~~ differences in the clinical manifestation of DPN. StEPS utilizes a standardized bedside assessment of symptoms and signs to reveal deficits in sensory functions and determine the essential characteristics of neuropathic pain. Instead of measuring a global average pain score, StEPS ~~obtains~~ records separate pain ratings to evaluate, for example, the specific intensity of dynamic mechanical or cold allodynia. Thus treatment response can be evaluated with greater precision and examined for potential associations with the clinical phenotype. StEPS generates more detailed information than screening tools and questionnaires that are primarily designed to distinguish neuropathic from nociceptive pain [8]. Physical tests included in the phenotyping protocol examine concrete peripheral nerve functions as opposed to assessing the severity of neuropathy by measuring the impairment of daily activities. Designed for a bedside evaluation of patients, StEPS offers a pragmatic and affordable alternative to QST that will be easier to implement in clinical trials with large numbers of patients or multiple participating centers.

Our phenotyping strategy ~~included-comprised~~ two complementary components, a structured patient interview and a physical examination involving standardized test stimuli. Patient descriptions of the sensory quality of pain or paresthesia provided clues as to which subtypes of neurons were affected by DPN-P. Consistent with previous findings [6], stinging ~~or pain and painful~~ pins and needles were the most common characterizations of pain, an observation that corresponds to previous findings (Baron,

~~2009, 19592166~~. Psychophysiological studies have shown that pain of this quality originates in A δ fibers, which are typically activated by punctate stimuli such as pinprick [40,65]. Burning pain, which is elicited by the activation of heat-sensitive C fibers [44,49], was present in approximately two thirds of the patients with DPN-P but less common in a subgroup of patients with clinically intact small-fiber function.

Several sensory qualities of paresthesia were almost exclusively reported by patients with DPN-P. These included painless warm or cold sensations, which may indicate an increased activity of thermosensitive C or A δ fibers, and itch. Itch is produced by a heterogeneous population of nerve fibers, some of which may also be nociceptive. The association with DPN-P may therefore reflect a heightened excitability of polymodal afferents that signal both itch and pain [7]. However, although the sensory characteristics of pain and paresthesia appeared to indicate the ~~differential~~ involvement of different nerve fiber populations, ~~their~~ the overall contribution of these symptoms to the phenotypic variance ~~in DPN~~ was small (6%). Patients typically used multiple attributes to describe their pain and paresthesia, which reduced the selectivity of these characteristics, ~~compared to differences in the sensitivity to pain-provoking stimuli (39%).~~

~~Numbness was the most frequent painless symptom. Patients in this study reported numbness more often than participants in previous surveys (Baron, 2009, 19592166; Otto, 2003, 12507713). Unlike positive sensations such as tingling paresthesia, numbness is usually associated with a loss of touch detection. Mechanosensitivity was reduced in the majority of patients who described numbness of their feet. However, in some patients only the sense of vibration or the response to pinprick was decreased, whereas the detection of light mechanical stimuli was intact. Eight patients did not show a mechanosensory deficit in the physical tests. We did not measure exact thresholds of mechanical stimulus detection. Consequently, we can only draw conclusions on the relation between numbness and clinical deficits in mechanosensitivity that would have been recognizable in the bedside tests we performed. On the other hand, QST studies confirm that some patients with polyneuropathy experience numbness or “discomfort” in their hands and feet in the absence of touch deficits (Hershman, 2011, 21128110). Numbness even occurs in conditions of chronic non-neuropathic pain, which has led to the~~

~~hypothesis that plasticity in central sensory pathways contributes to its development [Geber, 2008, 18423989]. More research is needed to resolve the relation between the perception of numbness and disturbances in the normal pattern of afferent input.~~

~~Experience of evoked pain in response to mechanical or thermal stimuli and painful~~
~~after sensations defined the largest proportion of phenotypic differences in the interview. The analysis of~~
~~physical test outcomes confirmed that stimulus-dependent pain distinguished better between patient~~
~~phenotypes than sensory loss. However, the majority of patients with DPN-P exhibited sensory deficits~~
~~including those indicative of small fiber degeneration [Cheng, 2013, 23685187; Devigili, 2008, 18524793].~~
~~Sensory loss was actually more frequent in DPN-P than DPN-NoP. Mechanosensory~~ Sensory ~~deficits~~
correlated across different test modalities, whereas painful responses, for example, ~~to the~~ punctate
stimulation with von Frey filaments, brush movement or pinprick ~~hyperalgesia~~ showed greater specificity.
~~We found a similar pattern of correlated sensory deficits but distinct painful reactions when we tested the~~
~~response to thermal stimuli.~~ This pattern matches the findings of a recent meta-analysis by Freeman and
colleagues, who revisited QST results from four randomized clinical trials involving patients with painful
DPN, polyneuropathy associated with human immunodeficiency virus (HIV) infection, nerve trauma or
central poststroke pain. In this study, increased discriminatory increases in the detection thresholds for
mechanical or thermal stimuli were common and highly correlated, whereas allodynia and hyperalgesia
were more discriminating differed between stimulus modalities [29]. Studies-Previous investigations that
combined QST, skin biopsies and nerve conduction tests provide further evidence that degenerative
changes in DPN usually affect several types of nerve fibers, leading to a explaining the broad spectrum
of deficits with little variation between patients [51]. ~~Evoked pain distinguishes between patient~~
~~phenotypes better than sensory loss.~~

Low specificity of sensory deficits was one factor contributing to the relatively weak separation
between DPN-P and DPN-NoP when the outcome of the physical tests alone was considered. Painful
responses to light touch, brush movement, moderately cold or warm temperature were found exclusively
in patients with DPN-P and provided for a better distinction. However, fewer than half of the patients with

DPN-P in our study exhibited mechanical or thermal allodynia. For example, punctate mechanical allodynia was present in 7% of patients with DPN-P. This proportion was smaller than that observed in a previous examination of 35 patients with DPN [45], but comparable to the percentage of patients who described moderate or severe pain in response to the stimulation with von Frey filaments in four larger QST investigations [29]. ~~The frequency of hyperalgesia evoked by pinprick or cold was also similar to that in previous QST studies of DPN (Freeman, 2014, 24472518).~~ In order to achieve high sensitivity and specificity for the identification of neuropathic pain phenotypes, physical tests need to be combined with an interview. Otherwise, critical aspects of the phenotype will be missed: spontaneous pain, the sensory quality of pain or paresthesia, and the patient's experience of evoked pain during daily activities, which standard stimuli in a test setting can only incompletely reproduce.

A phenotype marked by ~~the combination of~~ continuous pain, a stinging pain quality, hyperalgesia in response to pinprick or heat, increased pain sensitivity to pinprick, pressure and heat, and temporal summation suggested heightened excitability of nociceptive nerve fibers in a patient subgroup with clinically intact small-fiber function. Similar associations of ongoing pain and mechanical or thermal hyperalgesia have been reported in patients with postherpetic neuralgia or chronic pain after nerve trauma [33,47]. Preserved skin innervation and thermal thresholds support the concept that “irritable” nociceptive afferents are causing pain in patients with these clinical and histopathological characteristics [48]. DPN certainly involves additional mechanisms of spontaneous and evoked pain, because similar symptoms and signs can be observed in patients with impaired small-fiber functions.

~~Based on~~We used a PCA to determine correlation patterns of symptoms and signs and identify the principal components of phenotypic diversity in DPN.~~, we identified those items of the structured interview and the physical examination that best captured the phenotypic diversity of DPN. We combined these items in StEPS to create~~ We combined interview items and physical tests with high weight on these components to create StEPS. StEPS is a bedside tool for the assessment of painful neuropathy whose application does not require major technical equipment or advanced expertise. Separate intensity ratings for painful symptoms and signs allow measuring changes in the pain phenotype over the course of the

disease and evaluating the response to treatment in greater detail than global pain scores. Some of the items in StEPS are also contained in screening tools for neuropathic pain such as DN4 or painDETECT [10,31]. These items reflect core features of neuropathic pain that ~~contribute to the~~also apply to phenotypes of DPN, for example, a burning ~~character-quality~~of pain or pain evoked by brush movement [8]. StEPS is more comprehensive than the Neuropathic Pain Symptom Inventory and other questionnaires designed for self-administration that have previously been used to cluster patients with neuropathic pain based on combination patterns of sensory abnormalities [6,11,29]. The inclusion of physical tests in StEPS increases the depth ~~and specificity~~ of the phenotype characterization and enables the validation of patient-reported painful stimuli [61]. Standardization of the assessment procedure is essential. Dyck and colleagues have demonstrated the limits of evaluating DPN without a defined protocol, even when patient interview and examination are performed by an experienced physician [25]. StEPS does not yield quantitative information about thresholds of sensory discrimination or pain. This is the domain of QST [4,52]. However, QST relies entirely on the recording of stimulus-evoked responses. The technique does not assess spontaneous pain or paresthesia, which constitute relevant components of DPN phenotypes. As a consequence, QST alone is not always sufficient to distinguish painful from painless neuropathies [21,34,54].

The phenotypic diversity of DPN reflects differences in the vulnerability of distinct nerve fiber populations to hyperglycemia-~~induced damage and other pathogenetic factors of neuropathy in DM2~~. It furthermore suggests differences ~~between patients in the pathophysiological mechanisms responsible for neuropathic pain in the mechanisms responsible for the development of pain, even among patients who, based on the extent of sensory deficits, exhibit similar levels of neuropathy~~ [18,62]. Identifying pain mechanisms in patients is challenging, but clinical symptoms and signs can provide surrogate markers of the underlying pathophysiological processes ~~to that can~~ guide treatment decisions. Correlations between sensory abnormalities and analgesic treatment response have, for example, been reported for oxcarbazepine, topical clonidine and botulinum toxin A [16,20,46]. StEPS facilitates the collection of structured phenotypic information. Compared to QST, a bedside tool like StEPS is less expensive and

easier to administer in large treatment trials or genetic studies with multiple participating centers. Phenotypic characteristics of patients can be established in clinical practice without the need to invest in extensive training or major equipment. [The sSensitivity of StEPS to treatment response and its utility to phenotype patients with neuropathies other than DPN will be tested in future studies.](#) We hope that this pragmatic phenotyping strategy is going to advance the development of tailored pharmacotherapeutic approaches to neuroprotection and pain treatment.

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Conflict of interest statement

J. Scholz has received consultation fees from GlaxoSmithKline. J. B. Davis and S. N. Tate are former employees and shareholders of GlaxoSmithKline.

List of Supplemental Digital Content

Supplemental Digital Content 1. Standardized Evaluation of Pain and Somatosensory Function (StEPS). pdf

[Supplemental Digital Content 2. Table listing the area under the ROC curves for individual StEPS items in the distinction between patients with diabetes who did not develop a neuropathy \(DM2 w/o DPN\) and patients with painful \(P\) or painless \(NoP\) variants of DPN. docx](#)

Supplemental Digital Content [23](#). Table comparing the sensitivity and specificity to identify DPN-P of the full version of the assessment protocol, StEPS and DN4. docx

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Figure legends

Figure 1. Standards for the Reporting of Diagnostic Accuracy (STARD) flowchart. ~~Numbers marked with an asterisk (*) include 60 subjects who exhibited both increased pain sensitivity and sensory deficits.~~

Figure 2. Frequency distribution for selected neuropathic symptoms. We used Fisher's exact test to calculate the statistical significance of differences between diabetic polyneuropathy (DPN) with (P) and without pain (NoP). *P* values of significant differences are shown only when symptoms were present in either patient group. Separate frequency charts show the symptoms of 7 patients with DPN-P who, based on their physical examination, had intact small-fiber functions (response to pinprick, temperature discrimination, sweating) and 5 patients with DPN-NoP who had clinically intact large-fiber functions (detection of light punctate stimuli, brush movement or vibration, senses of position and passive movement; see also Figure 3).

Figure 3. Selected outcomes of the standardized physical tests. *P* values from Fisher's exact test are shown for significant differences between diabetic polyneuropathy (DPN) with (P) and without pain (NoP) when clinical signs were present in either patient group. Seven patients with DPN-P had intact small-fiber functions (response to pinprick, temperature discrimination, sweating). We also identified 5 patients with DPN-P who had intact large-fiber functions (detection of light punctate stimuli, brush movement and vibration, senses of position and passive movement). Temporal summation (1) denotes an initially painless response that turned painful during repeated stimulation. Temporal summation (2) indicates that the first stimulus was painful, but pain intensity increased during repeated stimulation.

Figure 4. Correlations of symptoms and signs. (A) Venn diagrams show the association of numbness with deficits in the detection of innocuous mechanical stimulation (light blue), proprioceptive deficits (purple) or decreased responses to noxious stimulation (orange). Diagram sections contain the numbers

of patients with the respective combination of numbness and signs. (B, C) The correlation of symptoms and signs was examined further in a principal component analysis (PCA). Circles are scaled to reflect the proportion of patients exhibiting the respective combination of symptoms or signs. Pearson correlation coefficients are indicated based on the color key. (B) Correlation between the outcomes of selected bedside tests and patient reports of pain evoked by light touch or pressure. For example, 21 (84%) of 25 patients reporting allodynia and 27 (79%) of 34 patients with increased pressure sensitivity experienced painful aftersensations, $R = 0.52$ and 0.57 , respectively. (C) Correlation between the sensory character of pain or paresthesia, responses to thermal stimuli and patient reports of cold or warmth-evoked pain. Eleven (65%) of 17 patients reporting cold-evoked pain described a painful cold sensation ($R = 0.44$), compared to 8 patients (47%) describing pain of a burning quality ($R = 0.09$). Twelve patients (71%) also described cold paresthesia ($R = 0.38$), whereas none of the patients reported the experience of warm paresthesia.

Figure 5. Correlation matrix of sensory deficits and pain provoked by (A) mechanical or (B) thermal test stimuli. Circle size reflects the proportion of patients exhibiting the respective combination of test outcomes. Pearson correlation coefficients are represented by magenta (negative) and green (positive) colors.

Figure 6. Principal components contributing to the variance of clinical signs among patients. A principal component analysis (PCA) revealed the major dimensions of phenotypic differences among patients with DM2. Each dot on the scatter plots s in this figure represents one of the 39 test items in the physical examination. Scales indicate the loading (weight) of items on the respective component. (A) ~~Sensory deficits (white dots), painful responses (dark grey dots) and autonomic signs (light gray dots) are projected onto the first two principal components.~~ Sensory deficits (white) and painful responses (dark grey) determined the first two dimensions of phenotypic differences, with painful responses having greater weight on Component 1, and sensory deficits bearing greater absolute weight on Component 2.

(B) Responses to thermal stimuli produced the greatest differences on ~~the first component~~Component 1, which accounted for 16% of the total variance in physical test outcomes. Pink dots identify responses to cold, red dots responses to warm or hot stimuli. (C) Responses to punctate mechanical stimuli dominated on the second principal component, which contributed 12% to the overall variance in clinical signs. Test outcomes after stimulation with von Frey filaments are shown in light orange, responses to pinprick in dark orange.

Figure 7. Principal component analysis (PCA) of neuropathic symptoms. Each dot represents one of the 47 items in the interview. The ~~first-leading~~ symptom component was defined by differences in the experience of evoked pain. Light blue dots mark painful reactions to different physical stimuli. Dark blue dots indicate the reported duration of pain relative to the stimulus exposure. This component alone accounted for 39% of the total variance of symptoms.

Figure 8. Diagnostic performance of the Standardized Evaluation of Pain and Somatosensory Function (StEPS). Receiver operating characteristic (ROC) curves indicate the sensitivity and specificity of StEPS to identify patients with painful (P) or painless (NoP) diabetic polyneuropathy (DPN). Diagnostic accuracy of StEPS is compared to that of the full 47-item interview and 39-item physical examination. Numbers denote areas under the curves (AUCs).

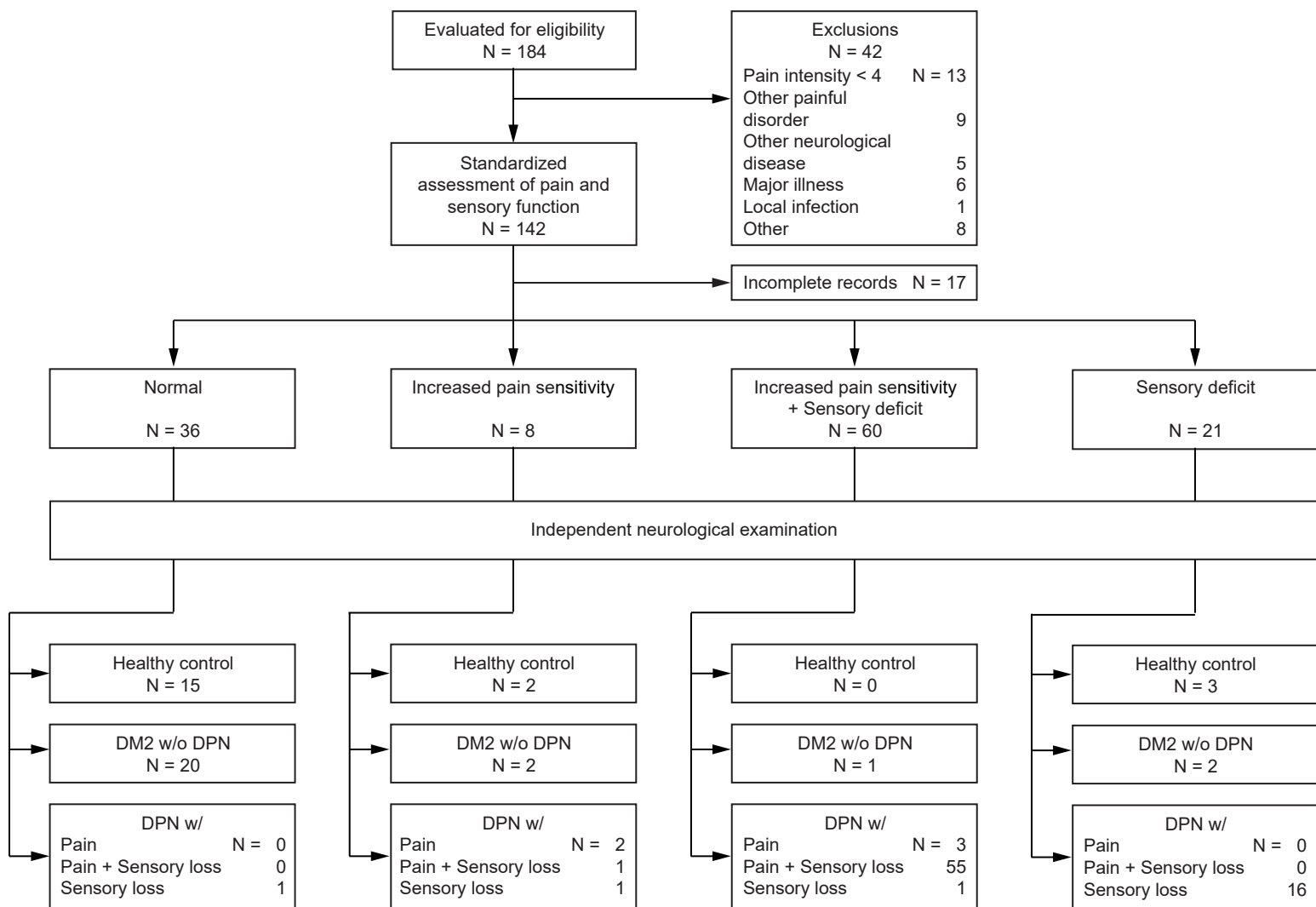
Table 1**Demographic and clinical characteristics of the study participants.**

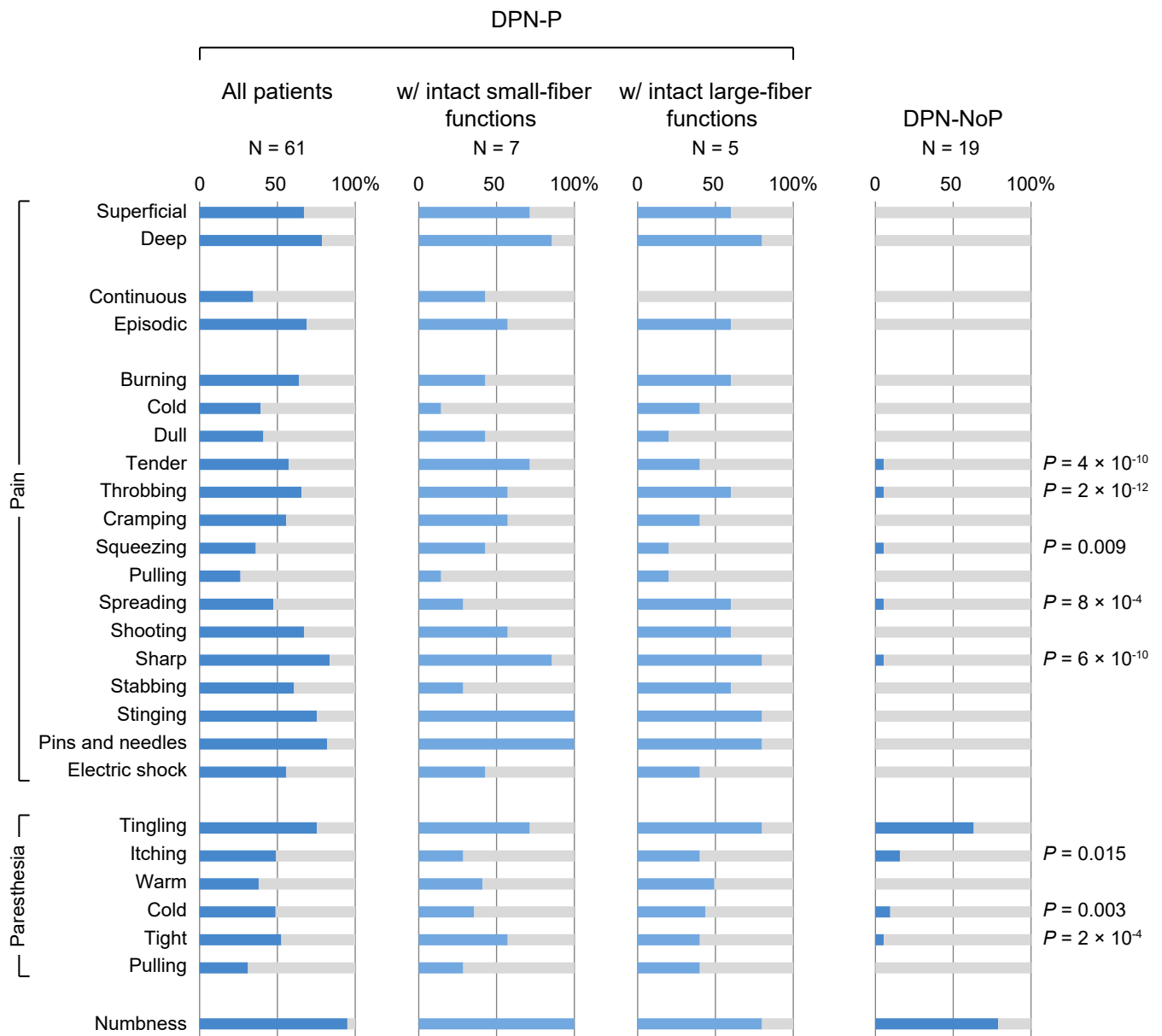
	DPN		DM2 w/o DPN	HC	<i>P</i>
	P	NoP			
N	61	19	25	20	
Age, median Y (Q1, Q3)	54 (49, 59)	57 (50, 63)	55 (50, 59)	61 (56, 66)	< 0.05*
Women, N (%)	25 (41)	4 (21)	6 (24)	7 (35)	< 0.01
Duration of diabetes, median Y (Q1, Q3)	10 (6, 13)	15 (5, 20)	5 (4, 13)		< 0.05*
HbA1c, median % (Q1, Q3)	7.3 (6.4, 8.4)	7.6 (7.1, 8.1)	7.5 (6.3, 8.9)		NS
DNS score, median (Q1, Q3)	3 (3, 4)	2 (1.5, 2)	0 (0, 0)	0 (0, 0)	< 0.001*
Toronto CNS scores					
Symptoms, median (Q1, Q3)	3 (3, 5)	2 (1.5, 2)	0 (0, 0)	0 (0, 0)	< 0.001*
Sensory tests, median (Q1, Q3)	3 (2, 4)	2 (1.5, 4)	1 (0, 1)	1 (0, 1)	< 0.001*
Duration of pain, median Y (Q1, Q3)	4 (2, 7)				
Pain intensity (NRS), median (Q1, Q3) [†]	7 (5.0, 8.0)				
Family history of diabetes, N (%)	48 (79)	17 (89)	23 (92)	7 (35)	< 0.001
Family history of neuropathy, N (%)	32 (52)	5 (26)	4 (16)	0 (0)	< 0.001

* ANOVA followed by Tukey's test for multiple comparisons revealed significant differences in the age of patients with DPN-P and HC subjects, and in the diabetes duration of patients with DPN-NoP and DM2 w/o DPN. DNS and Toronto CNS symptom scores were significantly different between all subgroups of patients with DM2, Toronto CNS sensory tests scores between DPN-P or DPN-NoP and DM2 w/o DPN.

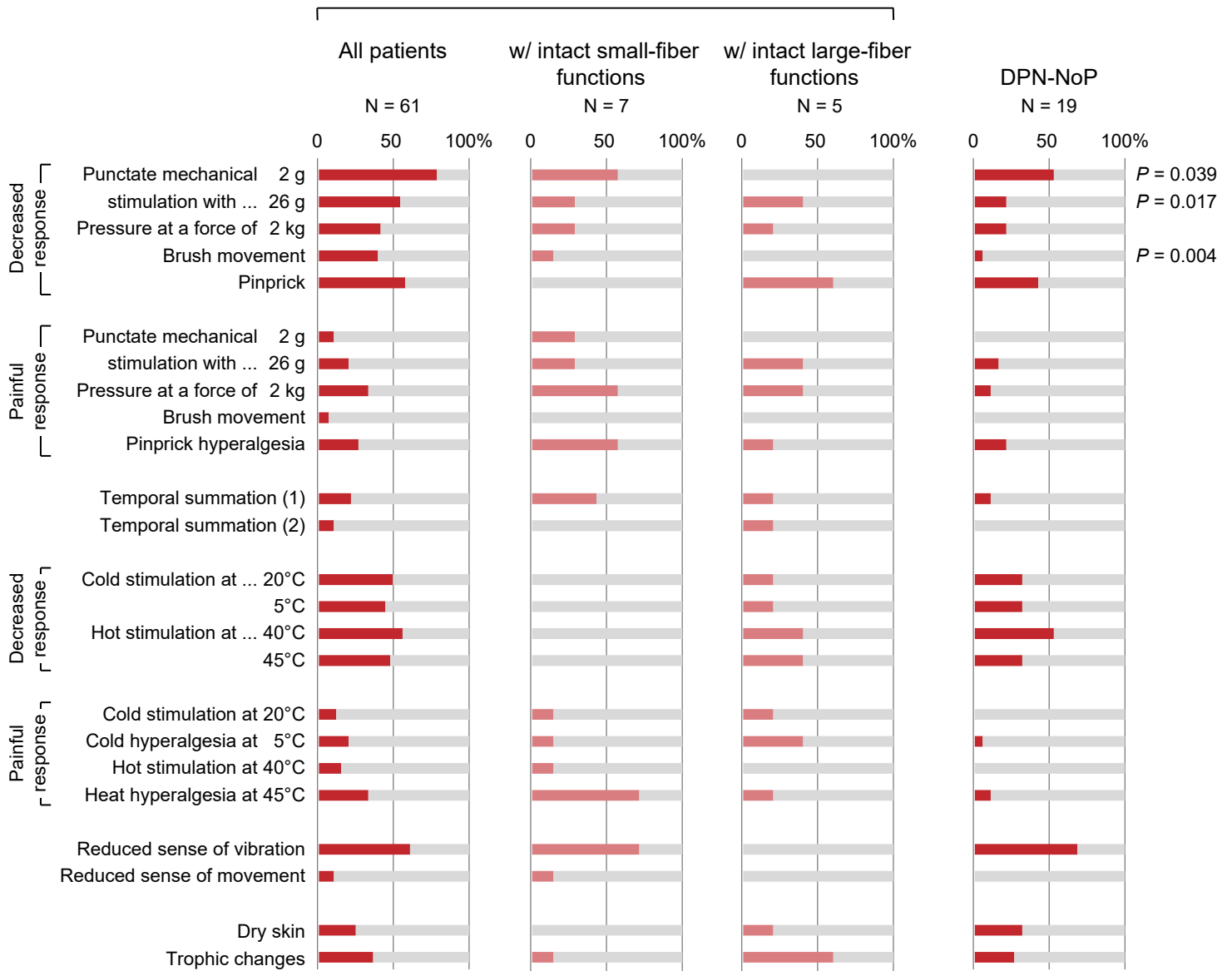
† Global pain intensity in the week prior to enrollment.

CNS, Clinical Neuropathy Scoring System; DM2, type 2 diabetes mellitus; DNS, Diabetic Neuropathy Symptom score; DPN, diabetic polyneuropathy; HbA1c, hemoglobin A1c; Hx, history; NoP, no pain; NRS, numerical rating scale from 0 to 10; NS, not significant; P, pain; Q1 and Q3, first and third quartiles; Y, years.

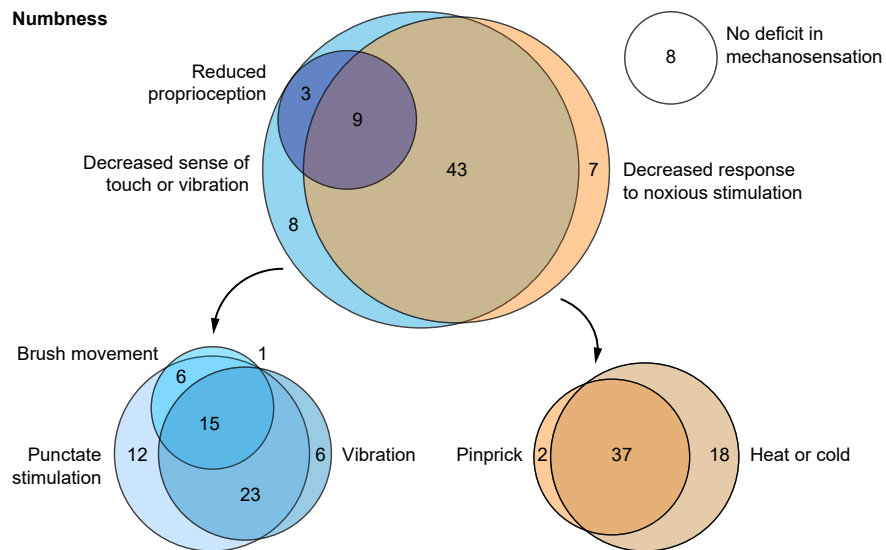




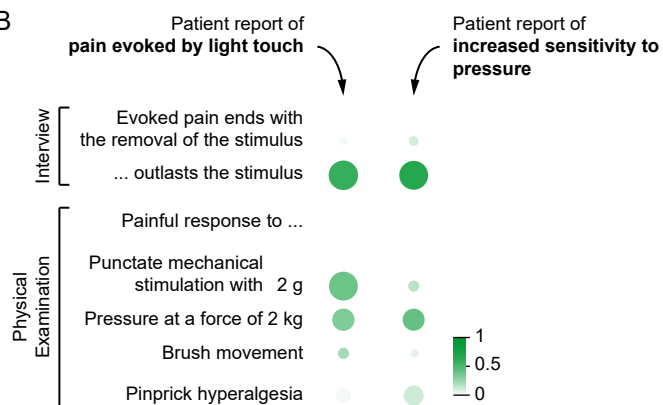
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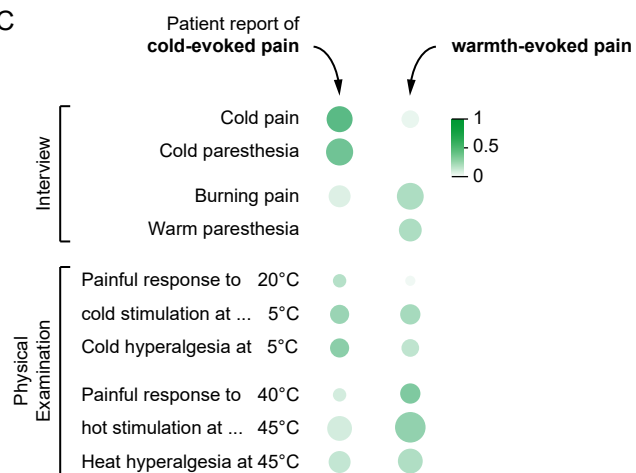
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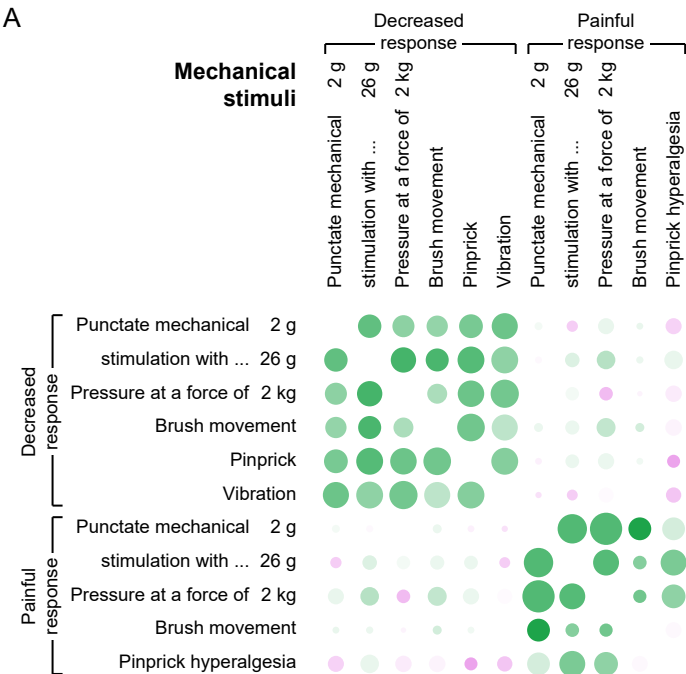
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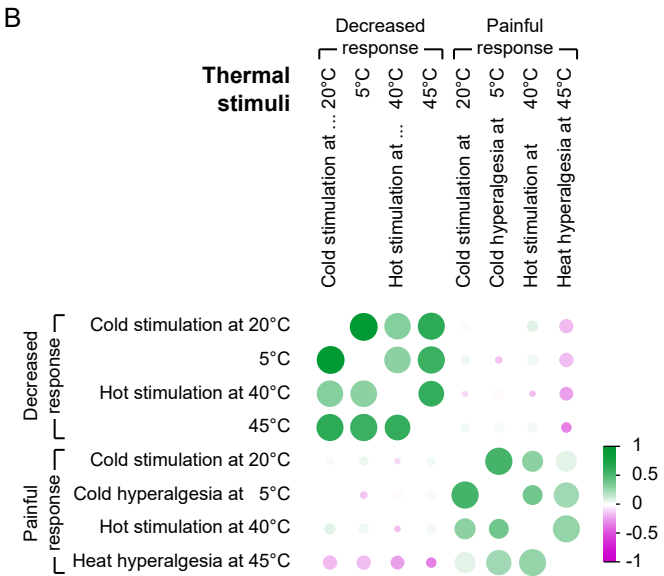
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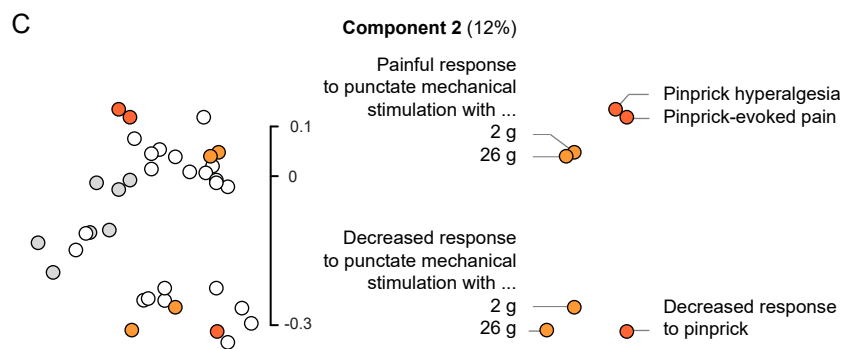
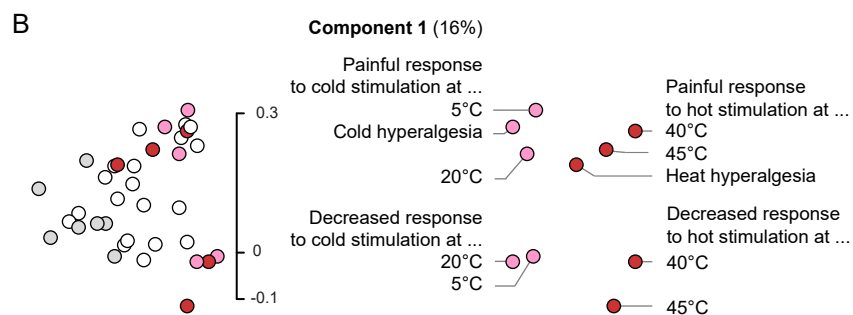
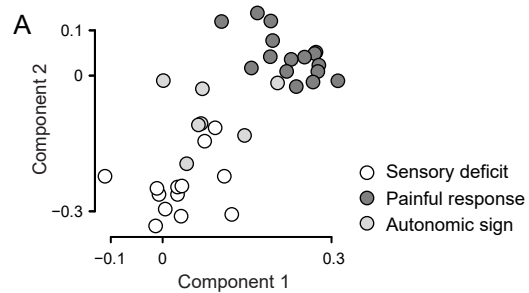


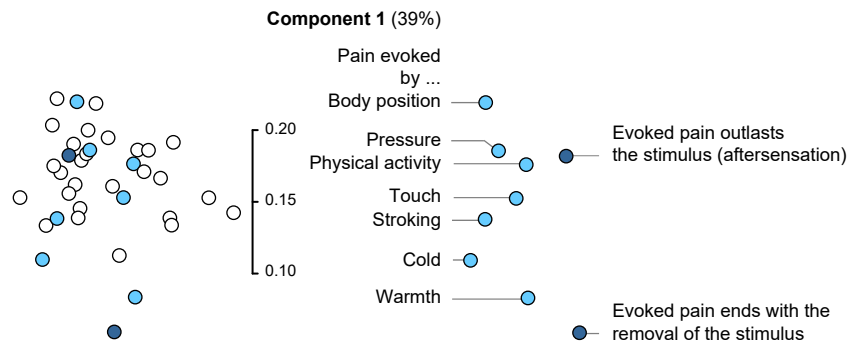
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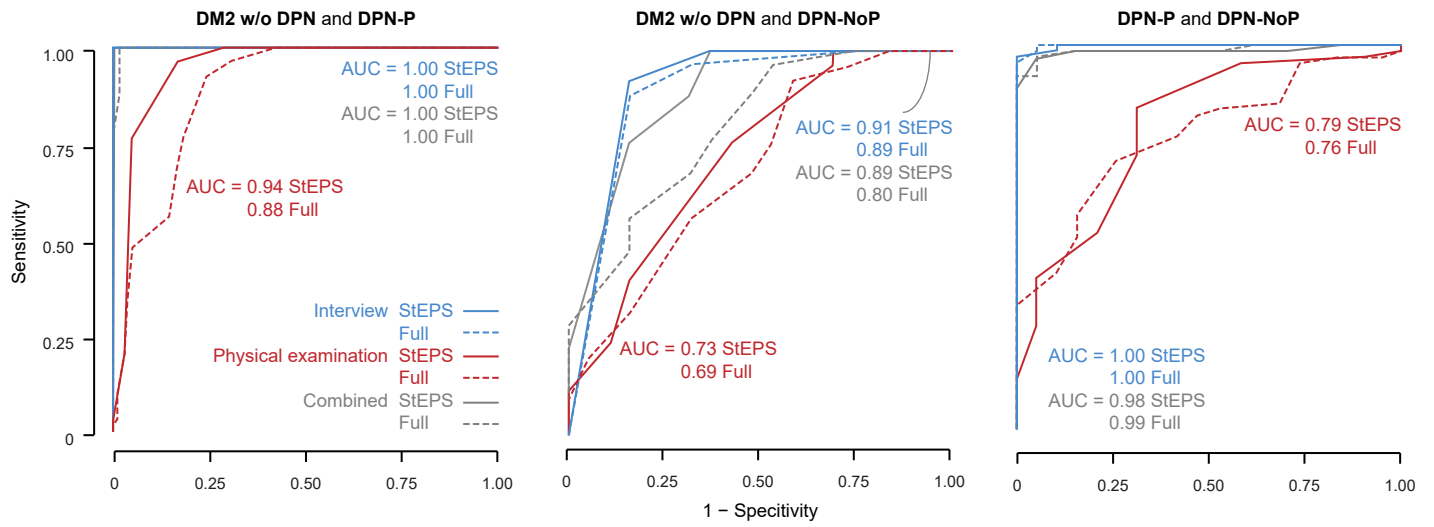
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Distinction between ...



Supplemental Table 1**Area under the ROC curves for individual StEPS items in the distinction between ...**

DM2 w/o DPN and DPN-P		DM2 w/o DPN and DPN-NoP	
Item	AUC	Item	AUC
Interview		Interview	
Numbness	0.95 (0.91, 1.00)	Numbness	0.87 (0.77, 0.98)
Deep pain	0.90 (0.85, 0.95)	Tingling paresthesia	0.82 (0.70, 0.93)
Stinging pain	0.88 (0.83, 0.94)	Physical examination	
Tingling paresthesia	0.88 (0.83, 0.94)	Reduced sense of vibration	0.78 (0.66, 0.91)
Superficial pain	0.85 (0.79, 0.91)	Decreased response to pinprick	0.71 (0.60, 0.82)
Episodic pain	0.85 (0.79, 0.91)		
Evoked pain outlasts the stimulus (aftersensation)	0.83 (0.77, 0.89)		
Burning pain	0.83 (0.77, 0.89)		
Pain caused by pressure	0.79 (0.72, 0.85)		
Cramping pain	0.78 (0.72, 0.84)		
Cold paresthesia	0.75 (0.69, 0.82)		
Itching paresthesia	0.73 (0.66, 0.81)		
Pain triggered by touch	0.72 (0.66, 0.78)		
Pain provoked by stroking	0.71 (0.65, 0.78)		
Cold pain	0.70 (0.64, 0.77)		
Physical examination			
Decreased response to pinprick	0.80 (0.73, 0.86)		
Decreased response to punctate mechanical stimulation with 2 g	0.78 (0.68, 0.88)		
Reduced sense of vibration	0.75 (0.65, 0.84)		

AUCs ≥ 0.7 are listed in descending order. Values in brackets denote the 95% confidence interval.

AUC, area under the curve; DM2, type 2 diabetes mellitus; DPN, diabetic polyneuropathy; NoP, no pain; P, pain; ROC, receiver operating characteristic; StEPS, Standardized Evaluation of Pain and Somatosensory Function.

Supplemental Table 2**Continued****DPN-P and DPN-NoP**

Item	AUC
Interview	
Deep pain	0.90 (0.85, 0.95)
Stinging pain	0.88 (0.83, 0.94)
Superficial pain	0.85 (0.79, 0.91)
Episodic pain	0.85 (0.79, 0.91)
Burning pain	0.83 (0.77, 0.89)
Pain caused by pressure	0.79 (0.72, 0.85)
Cramping pain	0.78 (0.72, 0.84)
Evoked pain outlasts the stimulus (aftersensation)	0.78 (0.68, 0.87)
Pain provoked by stroking	0.71 (0.65, 0.78)
Cold pain	0.70 (0.64, 0.77)
Cold paresthesia	0.70 (0.61, 0.80)

Supplemental Table 2

Sensitivity and specificity to identify DPN-P

	Standardized evaluation of pain and somatosensory function		DN4	
	Full version	StEPS	10 Items	7 Items
Sensitivity, %	100 (94, 100)	100 (94, 100)	85 (74, 93)	90 (80, 96)
Specificity, %	89 (79, 95)	98 (92, 100)	98 (92, 100)	95 (87, 99)
PPV, %	90 (80, 96)	98 (91, 100)	98 (90, 100)	95 (86, 99)
NPV, %	100 (94, 100)	100 (94, 100)	88 (78, 94)	91 (82, 97)

Values in brackets denote the 95% confidence interval.

DN4, Douleur Neuropathique en 4 Questions [1]; DPN-P, painful diabetic polyneuropathy; NPV, negative predictive value; P, pain; PPV, positive predictive value.

References

- [1] Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, Cunin G, Fermanian J, Ginies P, Grun-Overdyking A, Jafari-Schluep H, Lanteri-Minet M, Laurent B, Mick G, Serrie A, Valade D, Vicaud E. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* 2005;114(1-2):29-36.