

The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy

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Abstract

Disabling neuropathic pain (NeuP) is a common sequel of diabetic peripheral neuropathy (DPN). We aimed to characterise the sensory phenotype of patients with and without NeuP, assess screening tools for NeuP, and relate DPN severity to NeuP. The Pain in Neuropathy Study (PiNS) is an observational cross-sectional multicentre study. A total of 191 patients with DPN underwent neurological examination, quantitative sensory testing, nerve conduction studies, and skin biopsy for intraepidermal nerve fibre density assessment. A set of questionnaires assessed the presence of pain, pain intensity, pain distribution, and the psychological and functional impact of pain. Patients were divided according to the presence of DPN, and thereafter according to the presence and severity of NeuP. The DN4 questionnaire demonstrated excellent sensitivity (88%) and specificity (93%) in screening for NeuP. There was a positive correlation between greater neuropathy severity ($r = 0.39$, $P < 0.01$), higher HbA1c ($r = 0.21$, $P < 0.01$), and the presence (and severity) of NeuP. Diabetic peripheral neuropathy sensory phenotype is characterised by hyposensitivity to applied stimuli that was more marked in the moderate/severe NeuP group than in the mild NeuP or no NeuP groups. Brush-evoked allodynia was present in only those with NeuP (15%); the paradoxical heat sensation did not discriminate between those with (40%) and without (41.3%) NeuP. The “irritable nociceptor” subgroup could only be applied to a minority of patients (6.3%) with NeuP. This study provides a firm basis to rationalise further phenotyping of painful DPN, for instance, stratification of patients with DPN for analgesic drug trials.

Keywords: Diabetes, Diabetic neuropathy, Neuropathic pain, Quantitative sensory testing, Chronic pain

1. Introduction

Diabetic peripheral neuropathy (DPN) is one of the most frequent complications of diabetes mellitus⁴⁶ and affects between 28% and 49% of patients.^{1,53} Between 25% and 50% of patients with DPN develop neuropathic pain (NeuP)^{1,8,50}—defined as pain arising as a consequence of a lesion or disease of the

somatosensory nervous system.^{26,48} This pain is typically localised to the feet but can spread proximally with more advanced disease.⁴⁷ Although analgesic agents exist for the symptomatic treatment of NeuP (including tricyclic antidepressants, serotonin–norepinephrine reuptake inhibitors and gabapentinoids),^{12,21} treatment is often inadequate and NeuP has a subsequent major deleterious impact on quality of life.^{17,24,50} Diabetic peripheral neuropathy is one of the commonest causes of NeuP in the community, and given the increasing prevalence of type 2 diabetes mellitus, it will become even more frequent in the future.⁵⁵

The pathophysiology of NeuP in DPN is complex and not fully understood. The initial inciting event is a “dying back” axonopathy principally affecting sensory neurons.¹² It is believed that these neurons become hyperexcitable as a consequence of altered gene expression and posttranslational modification of key ion channels.^{30,51} There are also changes with the central nervous system, both at the level of the spinal cord and brain at an anatomical and functional level leading to amplification of nociceptive processing.⁴⁵

In the last decade, significant advances in techniques to define somatosensory phenotype in the context of NeuP have been developed. These include questionnaires, such as DN4,⁶ painDETECT,²² and LANSS,⁴ designed to distinguish NeuP from other types of pain using patient-reported pain descriptors (and in some cases examination findings) and standardisation of quantitative sensory testing (QST) (for instance, the standardised protocol of the German Neuropathic Pain Network).³⁸

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Quantitative sensory testing assesses evoked sensory perception in response to a defined sensory stimulus. This can be used to test a range of sensory modalities including small-fibre sensory function (eg, thermal and pain thresholds) and large-fibre modalities (eg, vibration threshold).³⁸

Application of these techniques has shown that NeuP is a multidimensional entity, and there are distinct subgroups of patients who have particular patterns of sensory symptoms and signs. Such sensory phenotypes may reflect particular pathophysiological mechanisms.⁵¹ For example, certain patients have principally deafferentation with loss of sensory function, whereas others have evidence of preserved small-fibre function and associated hypersensitivity, a pattern termed the irritable nociceptor.^{16,20} An aspiration is to use patient stratification in regard to targeting therapy.² Sensory phenotyping may aid in the design of randomised controlled trials of analgesics, for instance, by enrolling patients according to the sensory phenotype that may be most responsive to a particular drug.^{9,41}

In the Pain in Neuropathy Study (PiNS), we aimed to characterise, in unprecedented depth, the somatosensory phenotype of patients with DPN. Our objectives were (1) to compare patients with and without NeuP to identify sensory abnormalities that were specific to NeuP, (2) to compare some of the existing commonly used screening tools for NeuP in these populations, and (3) to relate sensory phenotype to measures of DPN severity and HbA1c to investigate the relationship between NeuP and the underlying disease process.

2. Material and methods

2.1. Study design and patients

The PiNS is an observational cross-sectional multicentre study approved by the National Research Ethics Service of the United Kingdom (No.: 10/H07056/35). Most of the study participants were recruited from primary care practices in London and Oxford. Study participants were also recruited from diabetes clinics at Chelsea and Westminster Hospital (London), Sheffield Teaching Hospitals and Oxford University Teaching Hospitals; neurology clinics at King's College Hospital (London), and through advertisements. All study participants signed written consent before participating.

Patients with diabetes mellitus aged above 18 years with diagnosed DPN, or patients with symptoms and signs suggestive of DPN were included. Exclusion criteria were pregnancy, coincident major psychiatric disorders, poor or no English language skills, severe pain at recruitment from a cause other than DPN (to prevent potential confounding influence on pain reporting as well as psychological and quality-of-life reported outcomes), patients with documented central nervous system lesions, or patients with insufficient mental capacity to provide informed consent or to complete questionnaires.

The sensory phenotyping paradigm was aligned with a previous study of HIV polyneuropathy.³⁶ The study design consisted of a single clinical assessment appointment with questionnaires that were completed either before or after the appointment and then returned to the study centre by post. During the clinical assessment appointment, participants had detailed medical and drug histories documented that included sex, age, ethnicity, medical history, date of diabetes mellitus diagnosis, presence of a family history of neuropathy, presence of other potential causes of neuropathy (hypothyroidism, HIV, alcohol misuse, vitamin B12 deficiency, and neurotoxic drug exposure); smoking and alcohol consumption were assessed using UK Department of Health

methodology.²³ Basic clinical parameters were then measured for each participant (weight, height, and lying/standing blood pressures). Participants then underwent a structured neurological examination, a detailed QST assessment, nerve conduction studies, and skin biopsy. After the clinical assessment, the study investigators collected further drug, laboratory, and clinical investigation data from the clinical records. The data, where available, included detailed drug histories, nerve conduction study data, and the most recent routine haematological and biochemical parameters.

2.2. Structured neurological examination

A comprehensive structured upper and lower limb neurological examination was performed to detect clinical signs of a peripheral neuropathy.^{27,34} The examination included assessment of temperature, light touch and pinprick sensation, joint position proprioception, vibration perception, deep-tendon reflexes, muscle bulk, and motor power. Orthostatic hypotension, as a marker of autonomic neuropathy, was assessed by measuring lying and standing blood pressure in accordance with established protocols.¹⁴ Orthostatic hypotension was defined as either a 20 mm Hg reduction in systolic or a 10 mm Hg reduction in diastolic blood pressure assessed two minutes after standing.

2.3. Nerve conduction tests

Nerve conduction tests were performed with an Advance system (NeuroMetrix, Waltham, MA) and used conventional reusable electrodes. Sural sensory and peroneal motor nerve conduction studies were performed in one lower extremity. If both studies were normal,¹¹ no further tests were performed. If either test was abnormal, additional nerve conduction studies were performed that included ipsilateral tibial motor nerve; contralateral sural sensory nerve, peroneal motor or tibial motor nerves; or ulnar sensory, median sensory, and ulnar motor nerves in one upper extremity. The minimum case definition criterion for electrodiagnostic confirmation of DPN was an abnormality of any attribute of nerve conduction in 2 separate nerves, one of which was the sural nerve. Variables such as skin temperature, age, height, sex, and weight were measured and accounted for when interpreting nerve conduction tests. Our protocol was in line with those recommended by the American Academy of Neurology and American Association of Electrodiagnostic Medicine.¹⁹ Nerve conduction tests were not repeated if study participants had previous results.

2.4. Skin biopsy for intraepidermal nerve fibre density assessment

The determination of intraepidermal nerve fibre density (IENFD) from skin biopsy samples is a validated and sensitive diagnostic tool for the assessment of small-fibre neuropathies, including diabetic neuropathy.²⁹ Punch biopsies of the skin were performed immediately after the completion of QST. Skin biopsies were not taken from participants on warfarin or those who had other contraindications to skin biopsy. Biopsy samples were taken in accordance with the consensus document produced by the European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the utilisation of skin biopsy samples in the diagnosis of peripheral neuropathies.²⁹ Subcutaneous anaesthesia was achieved with lidocaine (1%, 1–1.2 mL) before the biopsy was taken under sterile conditions. Skin biopsies were taken 10 cm proximal to the lateral malleolus with a disposable 3-mm punch biopsy circular

blade (Stiefel Laboratories Inc, GSK Plc, Brentford, United Kingdom). The biopsy was fixed in fresh periodate-lysine-paraformaldehyde (2%) for 12 to 24 hours. Tissue was then washed in 0.1 M phosphate buffer and stored for 2 to 3 days in 15% sucrose in 0.1 M phosphate buffer. After embedding in O.C. T. (Fisher Scientific UK Ltd, Loughborough, United Kingdom), the tissue was snap-frozen and stored at -80°C .

Sections with 50- μm thickness were cut on a cryostat, and immunohistochemistry for protein gene product 9.5 was performed on free-floating sections using the immunoperoxidase method. Samples were selected at random. Staining was performed on 24-well plates allowing reagents complete penetration of floating samples. For the bright-field method, samples were washed with TBS and placed on a 5% hydrogen peroxide solution on ethanol, followed by blockade of nonspecific protein binding with 4% normal donkey serum, 0.5% milk, and 0.1% Triton X in TBS. Primary antibody rabbit anti-protein gene product 9.5 Ab (1:15,000; Ultraclean Ltd, Yarmouth, Isle of Wight, United Kingdom) was incubated overnight at room temperature. After rinsing the samples with TBS, secondary Biotinylated Goat Anti-Rabbit IgG Antibody (1:400; Vector Laboratories, Burlingame, CA) was used followed by addition of VECTASTAIN ABC Kit (Vector Laboratories) for 1 hour. Samples were washed and transferred to the DAB Peroxidase Substrate Kit, 3,3'-diaminobenzidine (Vector Laboratories) until a visible stain emerged. Samples were rinsed with distilled water and progressively dehydrated with ethanol. Samples were mounted using DPX mounting media. Protein gene product 9.5-immunoreactive nerve fibres crossing the basal membrane of the epidermis were counted under a $\times 40$ objective, and a measurement of the length of the sample was also obtained. The IENFD was assessed using a double bright-field microscope at $\times 40$ magnification using established counting rules and expressed as fibres per millimetre of epidermal length. IENFD was considered abnormal if it was below the fifth centile for age- and sex-matched healthy controls.²⁸

2.5. Quantitative sensory testing

Somatosensory phenotypes were determined according to a previously published protocol of the German research network of neuropathic pain (DFNS).³⁸ The investigators (A.C.T., S.R.P., and J.D.R.) underwent a formal training in conducting the DFNS QST protocol at Mannheim University using healthy volunteers. Cold and warm detection thresholds as well as cold and heat pain thresholds and thermal sensory limen (including paradoxical heat sensations) were established using a ThermoTest (Somedic, Hörby, Sweden). We also tested mechanical detection and pain thresholds and mechanical pain sensitivity, allodynia, pressure pain thresholds (PPTs), wind-up ratio (WUR), and vibration detection thresholds. Participants were familiarized with the testing procedure on the dorsum of the forearm before all parameters were measured over the dorsum of both feet (S1 dermatome). Pressure pain thresholds were recorded over the arch of the foot, and vibration detection thresholds were tested over the medial malleolus.

Quantitative sensory testing data were entered into the data analysis system Equista provided by the DFNS. Equista transformed the raw QST data into z-scores thus normalising for age, sex, and the body location of testing.^{31,39} Positive z-scores denote gain of function, whereas negative z-scores denote loss of function. The sample size was determined according to the warm detection threshold data for patients with diabetes.⁴⁰ This calculation revealed that a minimum sample size of 34 was

required per group for a power of >0.8 (difference in means 2.0; SD 4.3; $\alpha = 0.05$).

We calculated warm and cold sensibility indices to determine the pain sensitivity range in which thermal sensations were felt.²⁵ The warm sensibility index (WSI) was defined as: (warm pain detection threshold – warm threshold)/(warm pain detection threshold – reference temperature). The cold sensibility index (CSI) was defined as: (cold pain detection threshold – cold threshold)/(cold pain detection threshold – reference temperature).

2.6. Neuropathy screening tools, symptom, and function questionnaires

A set of questionnaires assessing the presence of pain, pain intensity, pain distribution, and the psychological and functional impact of pain were either completed before the appointment or after the appointment and then returned to the study centre by post. We briefly describe the tools used. Detailed descriptions of the tools can be found in supplementary methods (available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

Patients were asked to keep a pain intensity diary for 7 days, recording pain at 9 AM and 9 PM daily on an 11-point scale, with 0 being no pain and 10 the worst pain imaginable. Study participants also completed a body map highlighting the distribution of any pain experienced. The surface area of NeuP was calculated from the body areas highlighted on the body map. Areas of pain were highlighted on a computer-generated image of a standardised body map, and the surface area of NeuP was subsequently quantified. The pain diary was used to assess the presence and severity of painful diabetic neuropathy.

The Toronto Clinical Scoring System (TCSS)¹⁰ was used as a screening tool for DPN and correlates with diabetic neuropathy severity. The Douleur Neuropathique en 4 Questions (DN4)⁶ was used as a screening tool for NeuP. The PainDETECT²² was used as a self-administered screening tool for NeuP. The Brief Pain Inventory (BPI)⁴⁴ includes pain severity and pain interference scores that were used to assess the severity any type of pain (nonneuropathic and neuropathic) experienced and the impact of the pain on activities of daily living. The Neuropathic Pain Symptom Inventory (NPSI),⁷ a self-administered questionnaire, was used to evaluate NeuP symptoms including evoked pain, spontaneous pain, paroxysmal pain, and dysesthesias. The Pain Catastrophizing Scale (PCS)³⁵ was used to assess the cognitive process by which pain is appraised. The Depression Anxiety Positive Outlook instrument (DAPOS)³⁷ was used to measure mood and anxiety. The Pain Anxiety Symptom Scale 20 (PASS-20)³³ was used to assess pain-related anxiety. Survey of Autonomic Symptoms (SAS)⁵⁴ was used to measure the presence and impact of autonomic symptoms. The Insomnia Severity Index (ISI)³ was used to measure the study participant's perception, subjective symptoms, and the consequences of any sleep disturbances. The Short-Form 36 of the MOS Outcomes Study (SF-36)⁵² was used for the assessment of health-related quality of life.

2.7. Definition of painful diabetic neuropathy

All study participants enrolled had diabetes mellitus. Study participants were screened for a “possible” clinical neuropathy defined as a combination of symptoms or signs of neuropathy including any one or more of the following: neuropathic symptoms (decreased sensation, positive sensory symptoms, eg, burning, aching pain) mainly in the toes, feet, or legs;

decreased distal sensation; decreased/absent ankle reflexes, and the DPN was then confirmed by abnormalities on either the nerve conduction studies or IENFD⁴⁶ (**Figure 1**). Only study participants with a confirmed DPN proceeded to NeuP subtyping.

The presence of chronic NeuP caused by peripheral DPN was determined at the time of the clinical assessment and was in line with the IASP definition of NeuP, ie, “pain caused by a lesion or disease of the somatosensory system” (**Figure 1**) and the IASP/NeuPSIG grading system was used.⁴⁸

The assessment of each study participant therefore satisfied the following criteria:

1. Pain with a distinct neuroanatomically plausible distribution, ie, pain symmetrically distributed in the extremities—completion of body map and clinical history.
2. A history suggestive of a relevant lesion or disease affecting the peripheral or central somatosensory system—diagnosis of diabetes mellitus and a history of neuropathic symptoms including decreased sensation, positive sensory symptoms, eg, burning, aching pain mainly in the toes, feet, or legs
3. Demonstration of the distinct neuroanatomically plausible distribution by at least 1 confirmatory test—presence of clinical signs of peripheral neuropathy, ie, decreased distal sensation or decreased/absent ankle reflexes

4. Demonstration of the relevant lesion or disease by at least 1 confirmatory test—abnormality on either the nerve conduction tests or IENFD.

Only study participants with chronic NeuP present for at least 3 months were included in the NeuP groups. Study participants with non-NeuP in the extremities, such as musculoskeletal pain of the ankle, were included in the no NeuP group. The severity of NeuP was calculated as the mean of the pain scores obtained from the 7-day pain intensity diary. The study participants were thereafter allocated into 3 groups based on the mean pain intensity score: 0: no NeuP, 0.1 to 3.9: mild NeuP, and 4 to 10: moderate/severe NeuP.¹⁸

2.8. Statistical analysis

SPSS Statistics Version 21 (IBM) and GraphPad Prism were used for statistical analysis. The QST z-scores were compared across the 3 groups with 1-way ANOVA (LSD post hoc test), or between 2 groups with unpaired t-tests. The QST z-score data were expressed as mean \pm 95% CI. All other data were tested for normality with the D’Agostino-Pearson normality test and by visual inspection of their distribution. All other data were not normally distributed and reported as median with interquartile range (IQR). Data were compared across the 3 groups with the

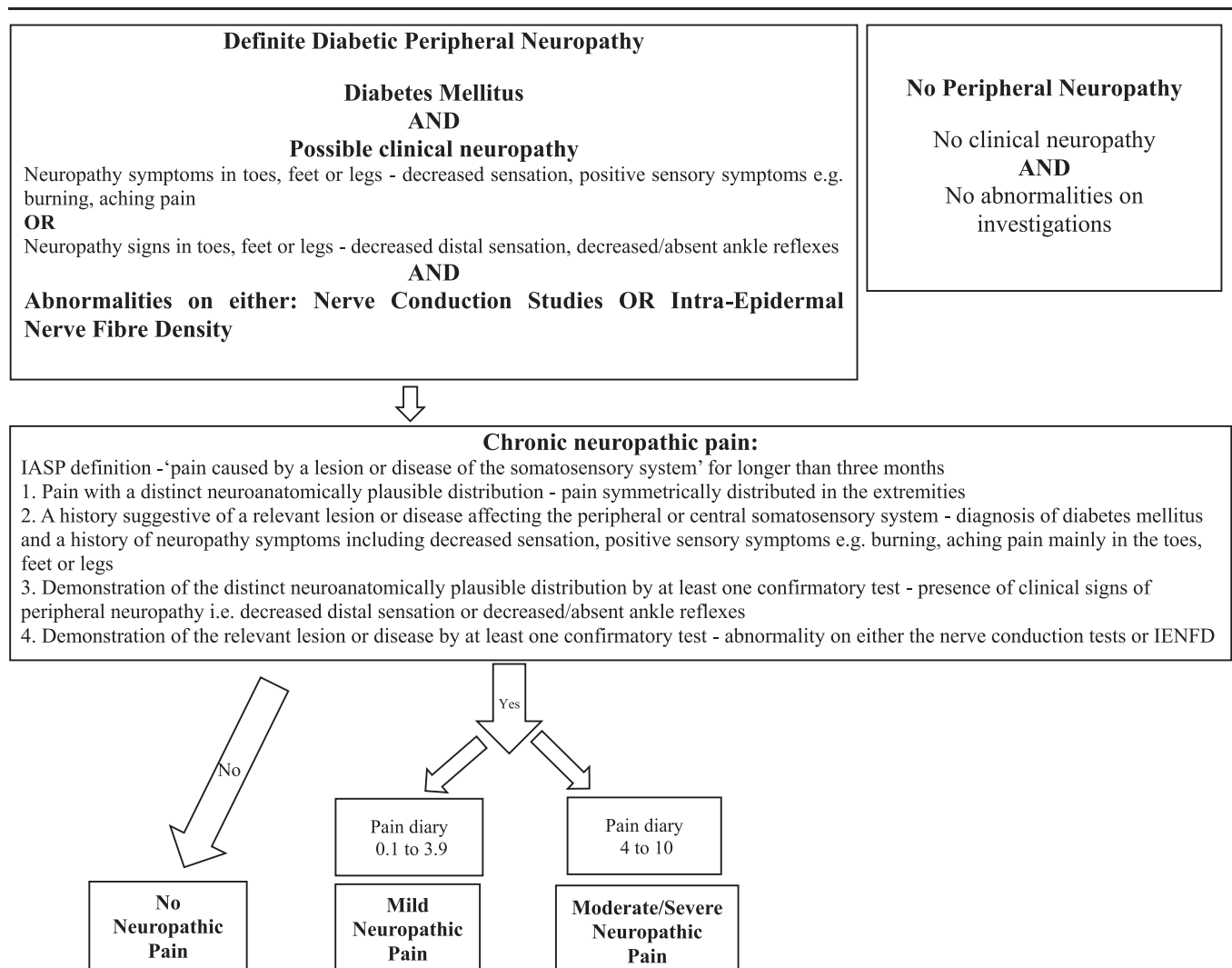


Figure 1. Flow diagram of study participant recruitment and the criteria used to subdivide the study participants into the different subgroups.

Kruskal–Wallis test (Dunn post hoc test), or between 2 groups with the Mann–Whitney U test. Spearman correlation analyses were performed to explore associations between histological parameters, QST findings, neurophysiological data, and symptom and functional outcomes. Categorical data were analysed with χ^2 test of association. Significance was set at $P = 0.05$.

3. Results

3.1. Study participants

A total of 209 patients were assessed. We recruited 191 study participants with DPN (9 study participants were excluded because they did not complete their 7-day pain intensity diaries). A smaller group, as a result of targeted recruitment for DPN, of 18 study participants were found not to have DPN according to our criteria. All study participants were clinically assessed by one of the study investigators (A.C.T., J.D.R., and P.R.S.). Study participants with DPN were divided into groups according to the severity of their NeuP: 80 participants had no NeuP, 41 had mild NeuP, and 70 had moderate/severe NeuP.

3.2. Demographics and pharmacotherapy use

All study participants had a diagnosis of diabetic mellitus. Most of the study participants were aged above 60 years and were white, and two thirds of the participants were men (Table 1). There were no significant differences between the different groups in terms of sex, ethnicity, body mass index, waist–hip circumference. Most of the study participants (91.1%) had type 2 diabetes mellitus, in line with population prevalence. Study participants across the 3 groups were diagnosed with diabetes for a similar duration. There were 17 study participants who had type 1 diabetes mellitus and 12 participants with DPN NeuP. It is therefore not possible to comment on variations of somatosensory phenotype between type 1 and type 2 diabetic participants, as the size of the type 1

cohort is too small to make a meaningful comparison. However, a significant finding is that the median (IQR) duration of diabetic treatment was different between the 2 groups: 31.8 (28.4) years for type 1 diabetes mellitus and 13.3 (12.4) years for type 2 diabetes mellitus (Mann–Whitney U test, $P < 0.01$). The participants with moderate/severe NeuP were slightly younger and had poorer diabetic control (demonstrated by a significantly higher HbA1c, results available in 199 (95%) of study participants) compared with those with DPN with no NeuP (Table 1). HbA1c correlated with NeuP severity ($r = 0.21$, $P < 0.01$), and although the association is not strong, it is statistically significant.

There was increased reported analgesic use in study participants with NeuP (Table 2). Those with the moderate/severe NeuP reported greater use of the serotonin–norepinephrine reuptake inhibitors (SNRIs) duloxetine and pregabalin. Although the mild NeuP reported greater use of analgesics, the choice of analgesic did not differ compared with the study participants with no NeuP. Study participants with DPN with no NeuP were prescribed antidepressants or antiepileptics classically used for NeuP. The reasons for the use of amitriptyline were either as a night time sedative for sleeping difficulties or for pain (not necessarily neuropathic) that was unrelated to their DPN. Gabapentinoids were prescribed for suspected NeuP unrelated to their DPN, and it should be noted that these patients did not have a history of painful DPN that was relieved completely by their treatment. We have included a summary of all reported drug use (Supplementary Table 1, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

3.3. Pain questionnaires, pain diary, and body maps

Study participants with painful DPN scored higher on screening tools, the DN4 and painDETECT, designed to detect pain with neuropathic characteristics (Table 3). Our gold standard for the definition of NeuP was the IASP/NeuPSIG

Table 1
Summary of key demographic details and blood results.

	No NeuP	Mild NeuP	Moderate/severe NeuP	P
Number of participants	80	41	70	
Age, y	68.6 (61.1–73.4)	68.5 (61.2–73.3)	64.6 (54–70.5)	0.03
Male	53 (66.3%)	33 (80.5%)	43 (61.4%)	0.11
Ethnicity				0.14
White European	78 (97.4%)	41 (100%)	61 (87.1%)	
African origin	1 (1.3%)	0 (0%)	4 (5.7%)	
Asian	1 (1.3%)	0 (0%)	1 (1.4%)	
Mixed ethnicity	0 (0%)	0 (0%)	2 (2.9%)	
Other	0 (0%)	0 (0%)	2 (2.9%)	
HbA1c, % [mmol/mol]	7.3 (6.6–8.1) [56 (49–65)]	7.5 (6.8–8.9) [59 (51–74)]	7.8 (6.9–8.9)* [62 (51–72)]	0.05
Type 2 diabetes	75 (93.8%)	37 (90.2%)	62 (88.6%)	0.53
Duration of diabetes, y	14.1 (9.8–21.8)	13.55 (8.0–23.2)	14.2 (7.6–21)	0.74
Standing BP	143/80 ($\pm 19/12$)	143/77 ($\pm 20/11$)	137/76 ($\pm 18/14$)	0.14
Lying BP	144/78 ($\pm 18/10$)	146/77 ($\pm 17/11$)	145/79 ($\pm 16/11$)	0.74
Orthostatic hypotension	10 (13%)	8 (20%)	15 (22%)	0.31
BMI, kg/m ²	30.1 (26.2–35)	31.1 (27.1–37.1)	31.1 (26.7–35.9)	0.82
Waist–hip circumference ratio	0.96 (0.91–1.04)	0.96 (0.90–1.04)	0.99 (0.92–1.04)	0.21

Data shown as median (interquartile range) and analysed by the Kruskal–Wallis, Dunn multiple comparison test. Categorical data were analysed by the χ^2 test of association, and values and percentages are shown. Symbols reflect the differences between the respective groups.

* $P < 0.05$ between no neuropathic pain (NeuP) and moderate/severe NeuP.

BMI, body mass index; BP, blood pressure.

Table 2**Summary of analgesic use.**

	No NeuP	Mild NeuP	Moderate/severe NeuP	P
Number of participants	80	41	70	
Analgesic use				
Participants reported analgesic drug use	24 (30.0%)	23 (56.1%)*	55 (78.6%)†‡	<0.01
Paracetamol	12 (50.0%)	8 (34.8%)	25 (45.5%)	0.55
NSAIDS	4 (16.7%)	3 (13.0%)	15 (27.3%)	0.30
Antidepressants				
Tricyclic antidepressants (amitriptyline, nortriptyline, imipramine)	10 (41.7%)	11 (47.8%)	13 (23.6%)	0.07
SNRI (duloxetine)	1 (4.2%)	1 (4.3%)	17 (30.9%)†‡	<0.01
Gabapentinoids				
Gabapentin	2 (8.3%)	5 (21.7%)	13 (23.6%)	0.28
Pregabalin	2 (8.3%)	0 (0%)	18 (32.7%)§	<0.01
Topical lidocaine patches	0 (0%)	0 (0%)	3 (5.7%)	0.27
Tramadol	2 (8.3%)	1 (4.3%)	12 (21.8%)	0.08
Opioids				
Weak (codeine)	8 (33.3%)	6 (26.1%)	18 (32.7%)	0.82
Strong (fentanyl patches, oral morphine)	1 (4.2%)	0 (0%)	6 (10.9%)	0.39

Data shown as values and percentages and analysed by χ^2 test of association.

Symbols reflect the differences between the respective groups.

* $P < 0.01$ between no NeuP and mild NeuP.

† $P < 0.01$ between no neuropathic pain (NeuP) and moderate/severe NeuP.

‡ $P < 0.05$ between mild NeuP and moderate/severe NeuP.

§ $P < 0.05$ between no neuropathic pain (NeuP) and moderate/severe NeuP.

|| $P < 0.01$ between mild NeuP and moderate/severe NeuP.

NSAIDS, nonsteroidal anti-inflammatory drugs; SNRI, serotonin–norepinephrine reuptake inhibitor.

grading system.⁴⁸ Using this as a reference, the sensitivity of DN4 and painDETECT was 88.3% and 61.3%, respectively, and the specificity of DN4 and painDETECT was 92.5% and 91.7%, respectively. The DN4 and painDETECT, correlated well with the mean score obtained from the 7-day pain intensity diary (**Fig. 2**). Some study participants with DPN and no NeuP still experienced pain in their feet or legs, such as musculoskeletal pain of the ankle or leg cramps, that was unrelated to their DPN. The DN4 and painDETECT quantified the NeuP characteristics of this nonneuropathic lower limb pain (**Table 3**).

A similar proportion of study participants with mild NeuP and moderate/severe NeuP reported NeuP in their hands; however, those with moderate/severe NeuP experienced NeuP over a greater surface area of their hands ($P < 0.05$, Mann–Whitney U

test) (**Fig. 3C**). There were no differences in either the frequency or surface area distribution of NeuP over the leg between study participants with mild NeuP and moderate/severe NeuP (**Fig. 3C**). Using the NPSI, study participants with moderate/severe NeuP reported greater severity of symptoms across all parameters of the NPSI compared with those with mild NeuP (**Table 3**).

Study participants in all groups did report pain of non-neuropathic origin, and the distribution of the pain is shown as blue areas in **Figure 3C**. In the majority of cases, the non-NeuP related to nonspecific lower back pain and musculoskeletal pain of the hip, knee, or ankle. The high frequency of such non-NeuP highlights the importance of detailed pain assessment in clinical trials of analgesic agents designed to ameliorate NeuP in diabetes mellitus.

Table 3**Summary of neuropathic pain (NeuP) screening scores.**

	No NeuP	Mild NeuP	Moderate/severe NeuP	P
API	NA	2.1 (1.4–3.4)	6.5 (5.2–7.6)	
DN4	1.5 (0–3)	5 (4–6.5)*	6 (5–7)†	<0.01
painDETECT	2 (0–6)	12 (7–17)*	18 (11–23)†‡	<0.01
BPI pain severity	1.3 (0–3.2)	2.8 (2–3.8)§	6 (4.5–7.3)†	<0.01
NPSI total	NA	1.6 (1.0–2.5)	4.0 (2.5–9.4)	<0.01
NPSI deep spontaneous pain	NA	0 (0–1.5)	2.5 (0–5)	<0.01
NPSI superficial spontaneous pain	NA	1 (0–3.5)	7 (3–8)	<0.01
NPSI evoked spontaneous pain	NA	0.7 (0–1.7)	2.3 (0–5.4)	<0.01
NPSI paraesthesiae	NA	2 (1–4.3)	5.8 (2.4–8)	<0.01
NPSI paroxysmal pain	NA	2 (0–4)	4.3 (1–7.5)	<0.01

Data shown as median (interquartile range) and analysed by the Kruskal–Wallis, Dunn multiple comparison test.

Symbols reflect the differences between the respective groups.

* $P < 0.01$ between no NeuP and mild NeuP.

† $P < 0.01$ between no NeuP and moderate/severe NeuP.

‡ $P < 0.05$ between mild NeuP and moderate/severe NeuP.

§ $P < 0.05$ between no NeuP and mild NeuP.

|| $P < 0.01$ between mild NeuP and moderate/severe NeuP.

API, 7-day pain diary; BPI, Brief Pain Inventory; NPSI, Neuropathic Pain Symptom Inventory.

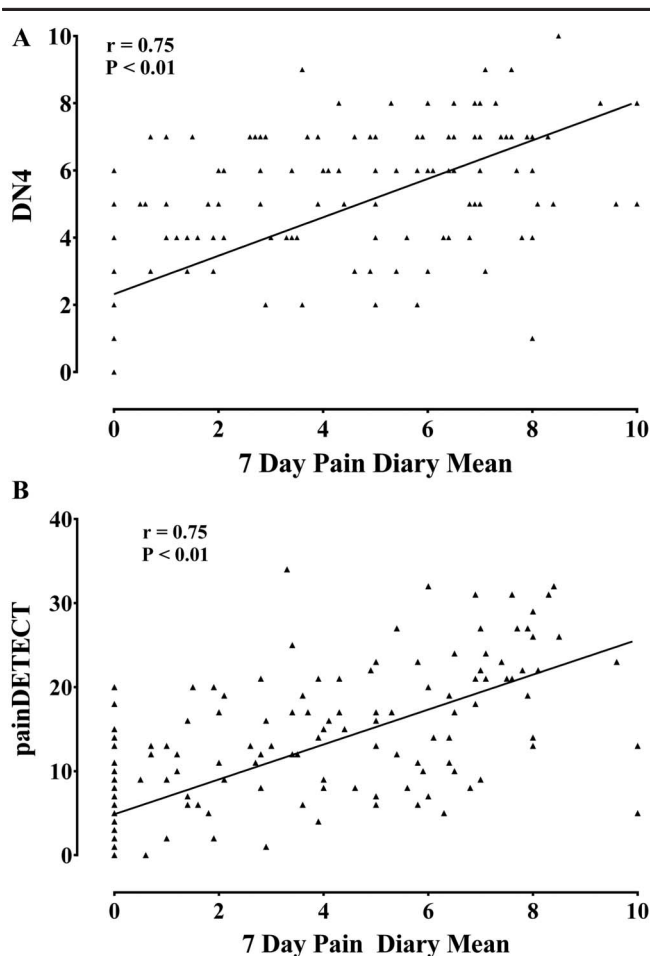


Figure 2. Scatter plot of (A) DN4 scores, (B) painDETECT scores, against 7-day pain intensity diary mean. All screening scores were highly correlated with the 7-day pain diary mean (Spearman correlation).

3.4. Structured neurological examination, nerve conduction studies, and intraepidermal nerve fibre density

The TCSS total score was significantly higher in study participants with NeuP compared with no NeuP (**Fig. 3A** and Supplementary Table, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>). The study participants with a NeuP reported more frequent sensory symptoms such as paraesthesiae and numbness, which was also of greater intensity. Patients with painful neuropathy also had a more severe DPN on clinical examination. There was greater proximal spread of clinical signs as evidenced by a higher Medical Research Council (MRC) sensory sum score (Supplementary Table 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>). The TCSS and MRC sensory sum scores correlated with NeuP intensity (**Fig. 4**). The nerve conduction studies (Supplementary Table 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>) and IENFD (**Fig. 3** and Supplementary Table 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>) did not discriminate between study participants with and without a NeuP. Sural sensory nerve responses were absent in 54% of study participants with diabetic neuropathy; and in study participants with preserved responses, the amplitudes were reduced with slowing of conduction. Peroneal motor response was absent in 23% of study participants with diabetic neuropathy; and in study participants with preserved responses, the amplitudes were reduced with slowing.

There were no electrophysiological differences between participants with and without NeuP (Supplementary Table 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>). The IENFD measured in a biopsy taken at 10 cm above the lateral malleolus was markedly reduced among all study participants with a peripheral neuropathy and did not correlate with the mean pain scores and was not significantly different between groups (**Fig. 3** and Supplementary Table 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

3.5. Quantitative sensory testing

Quantitative sensory testing is a means of assessing sensory phenotype, and differences in QST parameters may give insight into pathophysiological mechanisms. All study participants underwent QST of the feet using the protocol developed by the DFNS.

The data for thermal QST parameters for study participants with DPN are summarised in **Figure 5A**. The mean z-scores for all thermal parameters, apart from the cold detection threshold in participants with moderate/severe NeuP, fell within the normative range of the DFNS healthy control data, although data from individual participants did fall outside the normative range. Between-group comparisons revealed significant differences in the loss-of-function direction, and the shift towards thermal hypoaesthesia was greatest in the study participants with moderate/severe NeuP. The only thermal parameter that showed no differences across all 3 groups was cold pain thresholds as most study participants reached the cut-off temperature indicating a “flooring effect”. The mean z-scores for all thermal parameters for the study participants without DPN all fell within the normative range and were significantly higher across parameters when compared with study participants with DPN (Supplementary Figure 1, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

The WSI and CSI are measures for the range of thermal sensitivity. There were clear reductions in the WSI and CSI between study participants with no DPN and those with DPN (Supplementary Figure 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>). However, WSI did not discriminate between patients with and without NeuP. In contrast, CSI showed small differences between the study participants with moderate/severe NeuP and the study participants with no or mild NeuP (Supplementary Figure 2, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

The data for mechanical QST parameters for participants with DPN are summarised in **Figure 5B**. The mean z-score for mechanical detection threshold (MDT) in study participants with moderate/severe painful diabetic neuropathy was reduced (ie, indicating hyposensitivity) when compared with the DFNS healthy control cohort and was significantly lower than the study participants with no NeuP. Mean z-score values for the VDT when compared with the DFNS normative data revealed a loss of function for the VDT with no difference across the 3 groups. Mean z-scores of all other mechanical parameters fell within the normative range of the DFNS healthy control data. The mean z-scores of the mechanical parameters for the study participants without DPN all fell within the normative range, and apart from WUR and PPT, were significantly higher across parameters when compared with study participants with DPN (Supplementary Figure 1, available online as Supplemental Digital Content at <http://links.lww.com/PAIN/A226>).

The frequency of abnormal QST values outside 2 SD of the mean for DFNS healthy control data is shown in **Figure 5C**. The overall pattern for thermal parameters is similar to the mean z-scores, with

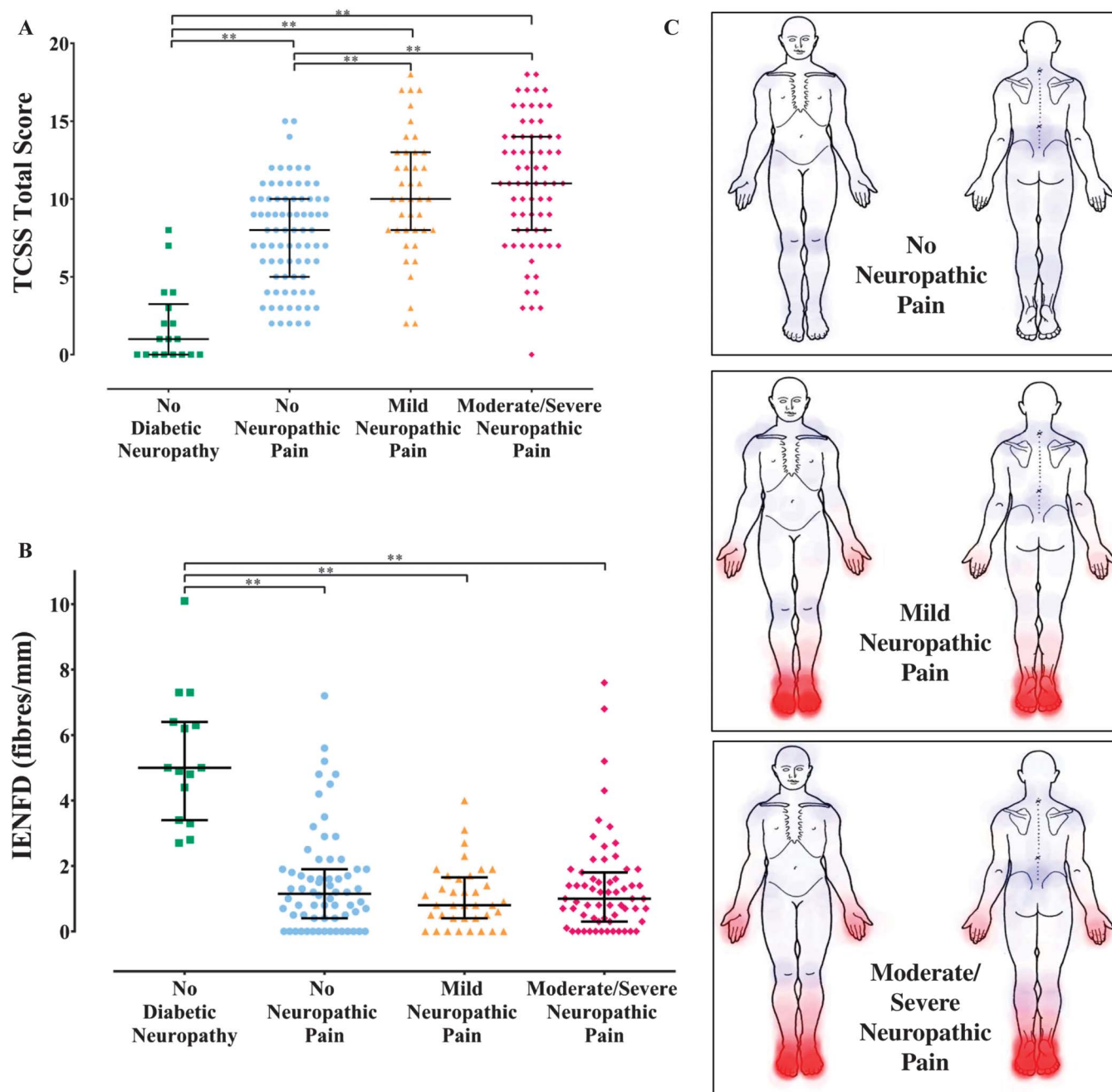


Figure 3. (A) Scatter plot and median (interquartile range [IQR]) of Toronto Clinical Scoring System (TCSS) scores for study participants with no peripheral diabetic neuropathy, and diabetic neuropathy with no neuropathic pain (NeuP), mild NeuP, moderate/severe NeuP. Kruskal–Wallis, Dunn multiple comparison test: $**P < 0.01$. (B) Scatter plot and median (IQR) of intraepidermal nerve fibre density (IENFD) from the distal leg for study participants with no peripheral diabetic neuropathy, and diabetic neuropathy with no NeuP, mild NeuP, moderate/severe NeuP. Intraepidermal nerve fibre densities were determined for 182 (87%) study participants. Kruskal–Wallis, Dunn multiple comparison test: $**P < 0.01$. (C) Heat maps obtained from the 7-day pain diary demonstrating the areas of NeuP (in red) and non-NeuP (in blue) within the study participants with diabetic neuropathy and no NeuP, mild NeuP, and moderate/severe NeuP.

the highest frequency of loss of function in the study participants associated with moderate/severe painful diabetic neuropathy. For the mechanical parameters, all 3 groups showed a high frequency of loss of function across VDT, mechanical pain threshold, and MDT. Significant difference was observed for MDT, with a higher percentage of study participants showing a loss of function with moderate/severe NeuP compared with participants with no NeuP. Overall, a smaller frequency of study participants showed loss of function for mechanical pain sensitivity, with more study participants showing a loss of function with moderate/severe NeuP compared with no NeuP. Statistically significant gain of function was not

observed across the thermal and mechanical parameters, apart from dynamic mechanical allodynia.

3.6. Allodynia, paradoxical heat sensation, and irritable nociceptor subtype

The presence of dynamic brush-evoked allodynia would suggest aberrant central processing contributing to NeuP in these patients. This was an example of a QST finding that was specific to NeuP as it was only observed in participants with NeuP, **Figure 3C**—7 (17%) in mild NeuP and 10 (14%) moderate/severe

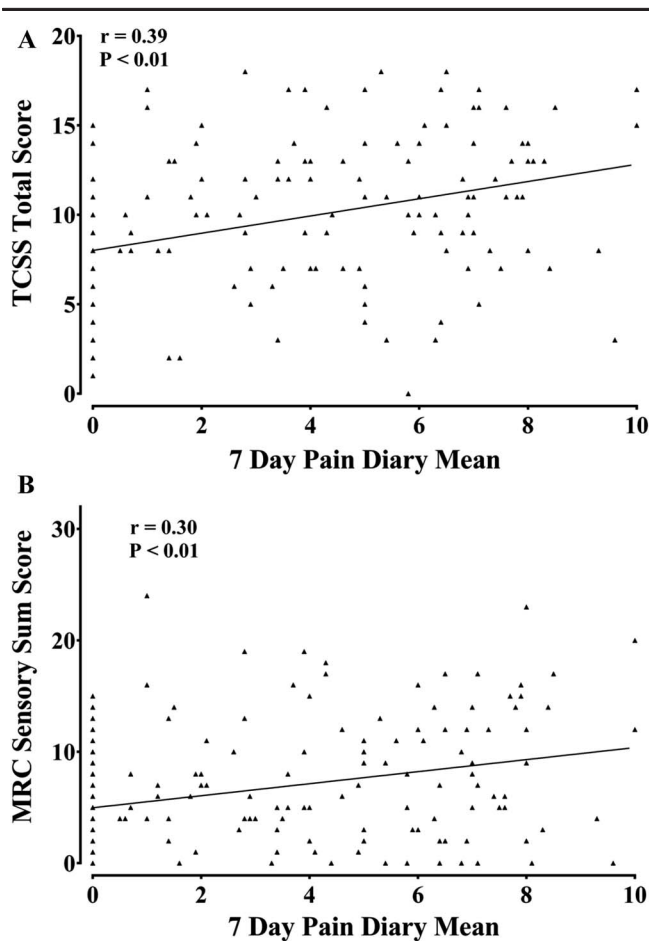


Figure 4. Scatter plot of (A) Toronto Clinical Scoring System (TCSS), and (B) MRC sensory sum score scores, against the 7-day pain intensity diary mean showing a correlation of clinical neuropathy severity and neuropathic pain intensity (Spearman correlations).

NeuP. The participants with allodynia did not differ from those in whom allodynia was not elicited across demographic data, clinical measurements, nerve conduction studies, IEFND and psychological problems, sleep disturbance, and health-related quality of life. A significantly higher number of study participants reported evoked pain, ie, allodynia, 39.8% on the NPSI than the 15% of study participants with NeuP who were found to have clinical evidence of dynamic brush-evoked allodynia (**Fig. 6**).

Paradoxical heat refers to the perception of heat in response to cooling and has been reported across a diverse range of peripheral neuropathies.³² Paradoxical heat sensations were observed across all groups with DPN, and the proportions were not statistically different, 41.3% without and with 40% with NeuP. Unlike allodynia, it was not discriminatory between patients with NeuP and those without NeuP. It can therefore not be seen as a “positive” sensory sign but likely represents loss of function. We assessed the presence of paradoxical heat sensation in only those who retained thermal perception; therefore, when compared with those who did not report paradoxical heat sensations, they retained thermal perception and had milder neuropathy. The concept of the “irritable nociceptor” is of a sensory phenotype with preserved small-fibre function (cold, warm, and pinprick sensitivity) together with hyperalgesia. Applying preexisting criteria¹⁶ for the definition of the irritable nociceptor, we found this to be a rare subtype in DPN. The irritable nociceptor subtype was observed in only 4.9% of participants with mild NeuP, and

7.1% of participants with moderate/severe NeuP. The participants with irritable nociceptor subtype did not differ from those without the irritable nociceptor subtype across demographic data, clinical measurements, NCS, IENFD, psychological morbidity, sleep disturbance, and health-related quality of life.

3.7. The relationship between clinical examination, quantitative sensory testing, and intraepidermal nerve fibre density

Quantitative sensory testing thermal and mechanical parameters, apart from WUR, correlated well with the clinical scores when considering all the study participants, ie, including those with and without DPN (**Table 4**). The clinical scores, TCSS and MRC sensory sum score, correlated negatively with QST z-scores. Therefore, the higher the clinical scores, ie, more severe the DPN, the greater the loss of sensation on QST parameters. The IENFD positively correlated with QST thermal and mechanical parameters, apart from WUR, among all the study participants, ie, including those with and without DPN. Therefore, preserved epidermal innervation correlates with preserved sensory function across the QST parameters.

3.8. Psychological well-being and quality-of-life measures

Study participants with moderate/severe NeuP reported statistically significant higher scores for the ONLS, PCS, DAPOS, PASS-20, ISI, BPI Pain interference, and SF-36 when compared with study participants with no NeuP or mild NeuP (**Table 5**). Therefore, among the study participants with moderate/severe NeuP, the rates of physical disability, pain catastrophization, depression, anxiety, clinical insomnia, and poorer quality of life were significantly higher.

4. Discussion

We have undertaken detailed sensory phenotyping using patient-reported pain descriptors, sensory examination, and QST in a large cohort of patients with diabetic neuropathy, predominantly in those with type 2 diabetes mellitus. This sensory phenotype has then been compared in patients with and without NeuP and related to detailed assessment of neuropathy severity. Key findings are that the DN4 questionnaire performed extremely well as a screening instrument for NeuP in this population, the presence (and severity) of NeuP was associated with more advanced DPN and higher HbA1c, and QST revealed hyposensitivity across a range of small-fibre and large-fibre mediated sensory modalities, and this sensory loss was most marked in patients with moderate/severe NeuP compared with patients with mild or no NeuP. Only a minority of patients had sensory gain signs, and very few patients with NeuP would be classified in the “irritable nociceptor” group.

Our diagnosis of DPN and NeuP was based on a combination of clinical probability followed by confirmatory investigations. We have used the NeuPSIG grading system⁴⁸ as a gold standard to define patients with chronic NeuP. Such pain lasted longer than 3 months of duration, in a neuroanatomically plausible distribution (symmetrical pain in the extremities), accompanied by a history of a disorder affecting the somatosensory nervous system (confirmed DPN), associated with abnormal sensory signs and a confirmatory test showing a lesion of the somatosensory nervous system (reduced IENFD or abnormal nerve conduction studies). Compared with this gold standard, the patient-reported symptom-based approach of the DN4 questionnaire performed extremely well in identifying patients with NeuP and performed better than another commonly used screening tool for NeuP, the

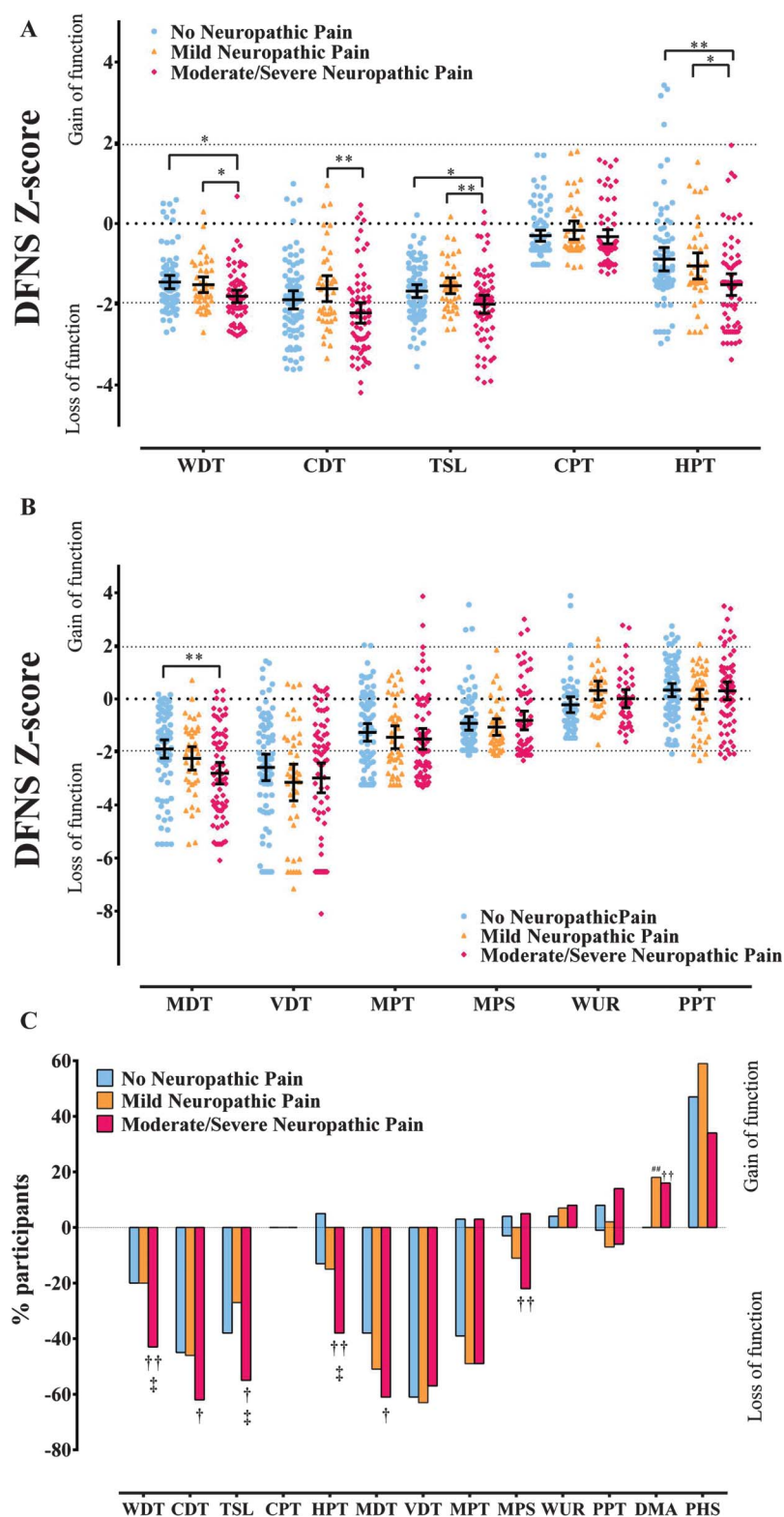


Figure 5. (A) Scatter plot and mean \pm 95% CI of z-scores for thermal quantitative sensory testing (QST) parameters in study participants with no neuropathic pain (NeuP), mild NeuP, and moderate/severe NeuP. One-way analysis of variance (ANOVA), least significant difference (LSD) post hoc test: * $P < 0.05$; ** $P < 0.01$. (B) Scatter plot and mean \pm 95% confidence interval of z-scores for mechanical QST parameters in study participants with no NeuP, mild NeuP, and moderate/severe NeuP. One-way ANOVA, LSD post hoc test: ** $P < 0.01$. (C) Loss and gain of sensory function. Comparison of study participants with no NeuP, mild NeuP, and moderate/severe NeuP who have QST values outside the 95% confidence interval of the German research network of neuropathic pain reference database. The y-axis shows the percentage of patients in each group with 'gain' of sensory function plotted upwards and 'loss' of sensory function plotted downwards. Data analysed with χ^2 test of association: † $P < 0.05$, †† $P < 0.01$ between no NeuP and moderate/severe NeuP, ††† $P < 0.01$ between no NeuP and mild NeuP, †††† $P < 0.05$ between mild NeuP and moderate/severe NeuP. CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

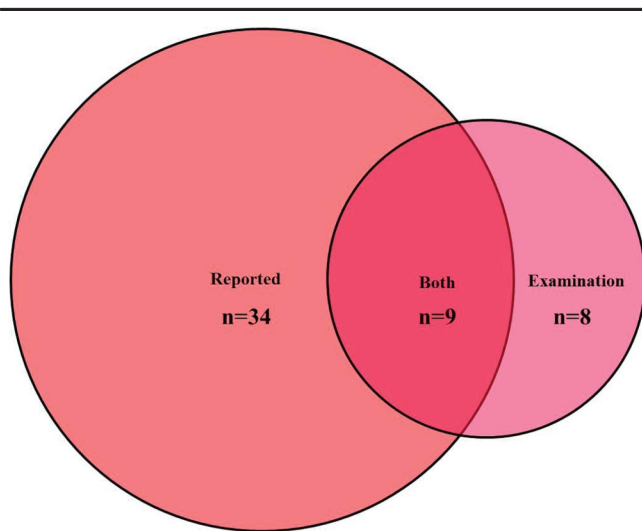


Figure 6. Venn diagram demonstrating the number of participants with brush-evoked allodynia reported only on questionnaires, dynamic mechanical allodynia only on examination, participants with both reported allodynia and allodynia on examination.

PainDETECT. One previous study has studied the DN4 in DPN⁴³; however, there has never been a direct comparison between these 2 questionnaires in this population.

Patients with NeuP were grouped into mild (mean pain numerical rating scale >0 and <4) and moderate/severe (mean pain numerical rating scale ≥4). Patients with moderate/severe NeuP had greater spatial distribution of pain than those with mild NeuP. The use of analgesics paralleled the severity of NeuP with greater use of pregabalin and duloxetine in the moderate/severe group (reassuringly as these are drugs that are recommended for use in the NeuP).^{12,21} Study participants with more severe NeuP reported higher scores for anxiety, depression, poorer overall health, and poorer quality of sleep compared with the study participants with no NeuP and mild NeuP. It is unclear from our study whether these comorbidities are predisposing factors or complications of the NeuP.

The DFNS QST protocol has been increasingly used to categorise the somatosensory profile of patients with NeuP in a standardised manner. The PiNS is the first study to adopt the DFNS protocol to specifically assess DPN, and importantly, we have compared the sensory profile of subjects with and without NeuP. In our cohort, the QST pattern of diabetic neuropathy was consistent with loss of function in sensory modalities mediated by both large and small sensory fibres. Furthermore, the study participants with moderate/severe NeuP demonstrated the greatest loss of function compared with the study participants with mild NeuP or no NeuP. Diabetic NeuP would therefore be

predominantly classified under the “deafferentation” phenotype. Interestingly, PPTs, as a measure of deep structure nociceptive innervation, were not impaired in DPN and did not correlate with NeuP suggesting that the deeper pain fibres are relatively intact.

Two broad sensory profiles that have emerged in the literature have been termed the “irritable nociceptor” profile with preserved small-fibre function (cold, warm, and pinprick sensitivity) together with hyperalgesia and a “deafferentation” profile that is dominated by sensory loss. Because very few DPN subjects have preserved small-fibre function (compared with postherpetic neuralgia), very few subjects would be classified within the “irritable nociceptor” group in our cohort. Some patients did however demonstrate signs of sensory gain (often in combination with hyposensitivity). Dynamic mechanical (brush-evoked) allodynia was the only evoked “gain of function” parameter that discriminated between study participants being present in 15% of DPN subjects with NeuP and absent from those without NeuP. The presence of dynamic brush-evoked allodynia would suggest aberrant central processing of sensory inputs in these patients. We could not find any demographic or clinical parameters that were specific to patients with brush-evoked allodynia (for instance, there was no evidence that these patients had less denervation)⁴⁹; however, we were limited by the relatively small size of this group. The discrepancy between reported brush-evoked allodynia and clinical evidence of allodynia is an interesting paradox that will not be easily answered. Most of the study participants report allodynia in the evening, whereas we examined the study participants during the day. However, there is no evidence that allodynia varies during the day. There were a significant number of study participants who exhibited allodynia on clinical examination but did not report it, and questionnaires would not capture this group of patients. Paradoxical heat sensation (in which a cooling stimulus is perceived as being hot) was relatively common in all DPN groups but did not differ for those with and without NeuP, and this sign is probably as much a “negative” as a “positive” sensory sign.

A previous article studying peripheral neuropathy of multiple aetiologies also described a “deafferentation phenotype” in most of the subjects.³² Sensory profiling of NeuP has demonstrated distinct groups of patients with particular patterns of sensory dysfunction that can be seen across different aetiologies of NeuP. That is not to say however that sensory profile does not differ depending on aetiology. In contrast to DPN, patients with HIV-associated polyneuropathy, phenotyped using a protocol very similar to the PiNS, do not exhibit dynamic mechanical allodynia and do exhibit an increase in PPTs.³⁶ Therefore, although HIV and diabetic neuropathy both fall under the “deafferentation” profile, there are clear differences between the different diseases.

Many studies have recorded assessment of NeuP in DPN, but there are limited studies on the relationship between NeuP

Table 4
Summary of correlations between quantitative sensory testing z-scores, and IENFD and clinical scores.

	WDT	CDT	TSL	CPT	HPT	MDT	VDT	MPT	MPS	WUR	PPT
IENFD (fibres/mm)	0.40*	0.39*	0.35*	0.39*	0.23*	0.31*	0.35*	0.50*	0.54*	−0.02	0.29*
MRC sensory sum score	−0.49*	−0.46*	−0.47*	−0.39*	−0.46*	−0.75*	−0.61*	−0.61*	−0.45*	0.10	−0.30*
TCSS total score	−0.39*	−0.40*	−0.39*	−0.30*	−0.44*	−0.63*	−0.54*	−0.37*	−0.26*	0.10	−0.17†
TCSS examination subscore	−0.34*	−0.42*	−0.37*	−0.35*	−0.40*	−0.60*	−0.55*	−0.38*	−0.24*	0.02	−0.19*

Symbols reflect the significant differences between the respective groups.

Spearman correlation.

* $P < 0.01$.

† $P < 0.05$.

CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; IENFD, intraepidermal nerve fibre density; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; TCSS, Toronto Clinical Scoring System; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

Table 5**Summary of scores for quality life measures, depression, anxiety, insomnia, and autonomic symptom abnormalities.**

	No NeuP	Mild NeuP	Moderate/severe NeuP	P
ONLS	1 (0-2)	2 (0-3)	3 (1-4)*†	<0.01
BPI pain interference	0.7 (0-2.9)	2.1 (1.1-3.3)	5.5 (4-7.8)*‡	<0.01
PCS rumination	2 (0-7)	3 (1-7)	8 (4-13)*‡	<0.01
PCS magnification	1 (0-3)	2 (0.5-4)	4 (2-8)*‡	<0.01
PCS helplessness	2 (0-7)	3 (1-5.5)	11 (6-16)*‡	<0.01
PCS total	6 (0-16)	8 (5-14.5)	24 (11.3-38.8)*‡	<0.01
DAPOS depression	6 (5-9.5)	6 (5-9)	8 (5-15)*†	<0.01
DAPOS anxiety	3 (3-4)	3 (3-6)	5 (3-8)*†	<0.01
DAPOS positive outlook	11.5 (9-14)	11 (9.5-13)	10 (7-12)*†	<0.01
PASS cognitive	3 (0-6)	5 (2-8.5)	11.5 (6-19.3)*‡	<0.01
PASS escape-avoidance	3 (0-6)	5 (2-9.5)	10 (3.5-14.5)*‡	<0.01
PASS fear	0 (0-2)	2 (0-5.5)	6 (1.8-14.3)*‡	<0.01
PASS physiological anxiety	0 (0-1.8)	0 (0-4)	3 (0-9)*‡	<0.01
PASS total	6 (0-16)	16 (6.5-24)§	32 (15.5-55.5)*‡	<0.01
SAS symptom score	3 (2-4)	3 (2-5)	5 (3-7)*†	<0.01
SAS total impact score	6.5 (3-10)	9 (5-13.5)	12 (7-19)	<0.01
ISI total score	7 (1.3-11.8)	8 (4-12.3)	16 (9-22)*†	<0.01
Clinical insomnia	12 (16.7%)	7 (18.4%)	32 (54.2%)*‡	<0.01
SF-36 physical functioning	60 (40-80)	60 (30-85)	25 (10-55)*‡	<0.01
SF-36 role physical	75 (25-100)	50 (0-100)	0 (0-50)*†	<0.01
SF-36 bodily pain	64 (51-84)	52 (41-62)	22 (21.5-41)*‡	<0.01
SF-36 general health	50 (35-65.8)	55 (35-67)	30 (15-45)*‡	<0.01
SF-36 vitality	55 (40-70)	50 (30-65)	40 (25-50)*	<0.01
SF-36 social functioning	100 (75-100)	87.5 (62.5-100)	37.5 (25-62.5)*‡	<0.01
SF-36 role emotional	100 (67-100)	100 (33-100)	33 (0-100)*‡	<0.01
SF-36 mental health	84 (68-92)	76 (65-88)	60 (48-76)*‡	<0.01

All data presented as median (interquartile range). Data were analysed by the Kruskal–Wallis, Dunn multiple comparison test, or Mann–Whitney U test.

Symbols reflect the differences between the respective groups.

* $P < 0.01$ between no neuropathic pain (NeuP) and moderate/severe NeuP.

† $P < 0.05$ between mild NeuP and moderate/severe NeuP.

‡ $P < 0.01$ between mild NeuP and moderate/severe NeuP.

§ $P < 0.05$, between no NeuP and mild NeuP.

BPI, Brief Pain Inventory; DAPOS, Depression, Anxiety, and Positive Outlook Scale; ISI, Insomnia Severity Index; ONLS, Overall Neuropathy Limitations Scale; PASS-20, short form of Pain Anxiety Symptoms Scale; PCS, Pain Catastrophizing Scale; SAS, Survey of Autonomic Symptoms; SF-36, short form 36 of the MOS Outcomes Study.

and DPN severity and none in a cohort of this size. Using 2 clinical measures of neuropathy severity, TCSS and MRC sensory sum scores were both higher in the mild and moderate/severe NeuP groups and correlated with the severity of the NeuP. The clinical scores were consistent with the greater sensory loss in these groups revealed by QST. Therefore, DPN severity correlates with NeuP consistent with other NeuP conditions in which the more severe the injury to the somatosensory system, the higher the likelihood of NeuP. The IENFD is known to be reduced in DPN⁴⁰ and was used as a measure of loss of small fibres from the skin. We did not find a correlation between IENFD and the severity of NeuP in our cohort. Published studies on this point have been conflicting with some groups reporting an inverse correlation between IENFD and pain in DPN⁴² and others reporting no significant relationship.¹⁵ It is something of a paradox that on using QST to assess the functional integrity of small fibres, we saw greater hyposensitivity in small-fibre modalities in the DPN inversely correlating with NeuP; however, the measure of structural integrity of small fibres did not differ. The most likely reason for this is the flooring effect of IENFD, which was measured at a distal site and was found to be markedly reduced both in NeuP and no NeuP groups.

The presence and severity of NeuP correlates with poorer diabetic control in our patient cohort as reflected by higher HbA1c in the NeuP group. It has long been established that poorer diabetic control is a strong predictor for the development of diabetic neuropathy, and a number of putative mechanisms have been shown to contribute to hyperglycaemic induced nerve injury.^{12,13}

The higher HbA1c in the moderate/severe NeuP group is consistent with the more severe DPN in this cohort. Interestingly, metabolites such as methylglyoxal produced as a consequence of hyperglycaemia can lead posttranslational modifications of ion channels such as Nav1.8⁵ directly resulting in hyperexcitability and NeuP.

Our findings have implications for the conduct of clinical trials of analgesic agents for the treatment of NeuP in DPN. The DN4 is an effective screening tool for NeuP in DPN. Many patients with DPN have pain of nonneuropathic origin (for instance musculoskeletal pain). This non-NeuP needs to be considered, and particular attention should be paid to the location of the pain and exactly what pain is being rated by patients. Distinct patterns of sensory dysfunction are noted on QST, although the most common finding is hyposensitivity to sensory stimuli. A minority of patients did show sensory gain signs; however, the term “irritable nociceptor” could only be applied to 6.3% of painful DPN. Given the rarity of this “irritable nociceptor” group, it is unlikely to be helpful in stratifying patients with painful DPN, in contrast to some recent clinical trials in peripheral neuropathy of diverse causes.¹⁶

In summary, the PiNS has provided evidence in a well-characterised DPN cohort that NeuP is related to neuropathy severity. The sensory profile of patients with DPN with NeuP was distinct from those patients without NeuP showing greater hyposensitivity to sensory stimuli across a range of sensory modalities. The sensory profile was not uniform in the NeuP group, and an appreciable minority of patients also demonstrated positive sensory signs such as dynamic allodynia; however, very few patients would meet the criteria for the “irritable nociceptor” group. This study provides us with a firm basis on which to

rationalise further phenotyping of painful diabetic neuropathy, for instance to optimise clinical trial outcomes. Future prospective studies will be needed to evaluate how sensory phenotype evolves over time in relation to DPN and NeuP.

Conflict of interest statement

D. L. H. Bennett received consultancy fees, outside the remit of submitted work, from Abide paid to the University of Oxford. A. S. C. Rice has no directly relevant interests to declare for this study. He is an owner of Share options in Spinifex Pharmaceuticals (acquired by Novartis in July 2015). In the last 36 months, Imperial College has received funds from Pfizer and Astellas to support research in ASCR's laboratory. A. S. C. Rice undertakes consultancy and advisory board work for Imperial College Consultants—in the last 36 months, this has included remunerated work for: Spinifex, Abide, Astellas, Neusentis, Merck, Medivir, Mitsubishi, Aquilas, Asahi Kasei, Relmada, Novartis, and Orion. S. Tesfaye reports grants and personal fees from NeuroMetrix, personal fees from Astellas Pharma, personal fees from Worwag Pharma, nonfinancial support from Impeto Medical, during the conduct of the study; personal fees from Pfizer, personal fees from Eli Lilly, outside the submitted work. The other authors have no conflicts of interest to declare.

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Author contributors: A. C. Themistocleous and J. D. Ramirez were involved in data collection, data interpretation, data analysis, literature review, manuscript preparation including figures and writing, prepared the first draft of the manuscript and contributed equally to the article. P. R. Shillo and D. Selvarajah contributed to

data collection. JGL created the PINS database and contributed to data analysis and data interpretation. C. Orengo contributed to data analysis, data interpretation. S. Tesfaye contributed to study design, data analysis, data interpretation. A. S. C. Rice contributed to securing funding (London Pain Consortium), study design, data analysis, data interpretation, literature review, and oversaw the conduct of the study at the Chelsea and Westminster site. D. L. H. Bennett assumed overall responsibility for the study, secured funding, and contributed to study design, data analysis, data interpretation, literature review, manuscript preparation including figures, and writing, and prepared the first draft of the manuscript. Andreas C. Themistocleous and Juan D. Ramirez contributed equally to the work. All authors approved the final report.

Appendix A. Supplemental Digital Content

Supplemental Digital Content associated with this article can be found online at <http://links.lww.com/PAIN/A226>.

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