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Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles

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Abstract

Patients with neuropathic pain are heterogeneous in etiology, pathophysiology, and clinical appearance. They exhibit a variety of pain-related sensory symptoms and signs (sensory profile). Different sensory profiles might indicate different classes of neurobiological mechanisms, and hence subgroups with different sensory profiles might respond differently to treatment. The aim of the investigation was to identify subgroups in a large sample of patients with neuropathic pain using hypothesis-free statistical methods on the database of 3 large multinational research networks (German Research Network on Neuropathic Pain (DFNS), IMI-Europain, and Neuropain). Standardized quantitative sensory testing was used in 902 (test cohort) and 233 (validation cohort) patients with peripheral neuropathic pain of different etiologies. For subgrouping, we performed a cluster analysis using 13 quantitative sensory testing parameters. Three distinct subgroups with characteristic sensory profiles were identified and replicated. Cluster 1 (sensory loss, 42%) showed a loss of small and large fiber function in combination with paradoxical heat sensations. Cluster 2 (thermal hyperalgesia, 33%) was characterized by preserved sensory functions in combination with heat and cold hyperalgesia and mild dynamic mechanical allodynia. Cluster 3 (mechanical hyperalgesia, 24%) was characterized by a loss of small fiber function in combination with pinprick hyperalgesia and dynamic mechanical allodynia. All clusters occurred across etiologies but frequencies differed. We present a new approach of subgrouping patients with peripheral neuropathic pain of different etiologies according to intrinsic sensory profiles. These 3 profiles may be related to pathophysiological mechanisms and may be useful in clinical trial design to enrich the study population for treatment responders.

Keywords: Neuropathic pain, Sensory signs, Clinical trials, QST, Epidemiology

1. Introduction

Neuropathic pain syndromes develop after a lesion or disease affecting the somatosensory nervous system.[22,58] Despite advances in understanding the complex neurobiology of pain, the pharmacological management of these syndromes remains insufficient and several promising drugs have failed in late-stage development.[21,35] Thus, there is a need to predict treatment responders both for clinical practice, in which even first-line treatments are beneficial in less than 50% of patients, and for clinical trial design, in which a negative outcome may be due to a low responder rate rather than uniform inefficacy of the treatment.

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Although all neuropathic pain disorders have a common denominator, ie, damage of the somatosensory nervous system, the underlying etiologies and pathogeneses of these damages are distinct. Furthermore, the patterns of sensory signs and symptoms that develop after neuropathy vary between the different etiologies and even between individual patients with neuropathies of the same etiology.[5,40] The expression of these sensory signs, the mosaic of hyperalgesia, allodynia, and sensory loss, which we call the individual somatosensory profile, reflects pathophysiological mechanisms in damaged and surviving afferent nerve fibers such as conduction block, ectopic impulse generation, peripheral sensitization, and central sensitization.[10]

Historically, neuropathic pain has been classified, investigated in clinical trials, and treated on the basis of the underlying etiology. However, recognising the heterogeneity of pain mechanisms other classification schemes might be more appropriate.[2,64] Thus, an entirely different strategy in which pain is differentiated on the basis of the underlying mechanisms has been proposed emphasizing the rationale for a treatment approach directed at mechanisms rather than diseases.[30,34,44,66]

Pathophysiological mechanisms of pain generation cannot be readily examined in patients. Nevertheless, the expression of some sensory signs can be related to mechanisms, eg, heat hyperalgesia to peripheral sensitization[36] and pinprick hyperalgesia to central sensitization.[6,55] Thus, the individual somatosensory profile may reveal some clues of pathophysiological dysfunctions of afferent processing.[5,40]

The aim of this investigation was to identify patient subgroups with distinct sensory profiles in a large sample of patients with neuropathic pain from a wide range of etiologies collected in 3 multinational research networks. Instead of testing previously published hypotheses of associations between sensory profiles and mechanisms, this large data set enabled us to apply hypothesis-free statistical segmentation methods. This way we explored the intrinsic patterning of sensory profiles in a representative spectrum of patients with peripheral neuropathic pain. The number and type of intrinsic patterns—if reproducible—can then be related back to pathophysiological and pharmacological mechanisms in future studies.

We used a standardized protocol of quantitative sensory testing (QST) in patients with peripheral neuropathic pain of different etiologies with the following aims:

(1) to describe and analyse typical patterns of sensory signs in more than 900 patients,
(2) to subgroup the patients on the basis of characteristic sensory profiles,
(3) to establish a sensory profile-based organizing principle of neuropathic pain, and
(4) to replicate the results in a second independent cohort of more than 200 patients.

2. Materials and methods

2.1. Consortia

Three large multinational consortia collected phenotypic data of patients with peripheral neuropathic pain (test cohort): the German Research Network on Neuropathic Pain (DFNS), the EUROPAIN, and the NEUROPAIN collaboration. The gathered data comprised demographic, psychometric, and clinical data as well as results of a standardized quantitative sensory assessment that were captured in one joined central database of the DFNS.[40] Each study center used a computer-assisted program for data entry locally in each center (Neuroquast, Statconsult, Magdeburg, Germany). For data export into the central database, a special data export file was created, encrypted, and sent to the central database through e-mail. All centers and investigators underwent a strict quality assessment and certification process to allow future pooling of data across sites and countries.[39,63] A confirmatory analysis of heterogeneity between the participating centers in healthy subjects and patients painful neuropathies showed a high degree of homogeneity between the different centers, making it possible to analyze the database as a homogenous group.[62]

The DFNS (http://www.neuropathischer-schmerz.de) was established to investigate mechanisms and treatments of neuropathic pain and consists of 10 German centers. The study protocol was approved by the ethics committee of the University Hospital Kiel, Germany, and subsequently by the ethics committees of all participating centers. The EUROPAIN consortium (http://www.imieuropain.org) consists of academic study groups working on pain research from Germany, Denmark, and the United Kingdom, a Spanish SME and Europe's most active pharmaceutical companies working in the pain field. The ethics committees of each center approved the study protocol individually. The NEUROPAIN project is an investigator-initiated project (sponsored by Pfizer Ltd) consisting of several researchers in the field of neuropathic pain research within Europe (principle investigator [R.B.]) and aims to characterize subgroups of patients with neuropathic pain. The ethics committees of each participating center approved the study protocols individually.

2.2. Inclusion criteria

Patients with peripheral neuropathic pain of several etiologies (polyneuropathy [PNP], peripheral nerve injury [PNI], postherpetic neuralgia [PHN], and radiculopathy [RAD]) were included (**Table 1**).

2.2.1. German Research Network on Neuropathic Pain

Patients were included when the following criteria for each respective diagnosis were fulfilled:

(1) polyneuropathy: according to the clinical criteria published by England et al.[18] Peripheral nerve injury: presence of somatosensory signs in the innervation territory of the injured nerve according to clinical examination and/or sensory neurography. Postherpetic neuralgia: presence of neuropathic pain for more than 3 months in the affected area after healing of the acute herpes zoster rash. Radiculopathy: history of nerve root damage and consistent neurological findings.

Table 1
Patient characteristics.

	Original data set	Validation data set	*P*
Age, y	58 ± 14	57 ± 14	0.834
Female, n (%)	429/902 (48)	97/233 (42)	0.106
Pain			
Current	6.0 ± 3.1	5.9 ± 2.1	0.275
Duration <1 y	193/902 (21%)	39/233 (17%)	0.116
Duration >5 y	201/902 (22%)	46/233 (21%)	0.402
Aetiology			<0.001
Polyneuropathy	512/902 (57%)	113/233 (48%)	
Peripheral nerve injury	227/902 (25%)	110/233 (47%)	
PHN	88/902 (10%)	10/233 (4%)	
Radiculopathy	75/902 (8%)	—	

P values are given for the chi-square approximate test or analysis of variance.
PHN, postherpetic neuralgia.

2.2.2. Europain and Neuropain consortia

The main inclusion criterion was recurrent or ongoing peripheral neuropathic pain with a pain intensity ≥3 (Numerical Rating Scale, 0-10). Special inclusion criteria for each diagnosis and type of pain were as follows:

polyneuropathy: pathological nerve conduction studies or pathologically decreased vibration detection threshold (VDT) at 2 of 4 sites (<5/8) at the lower limb,[33,42] which could not be explained by another disease or pain with PNP-type of location and evidence of small fiber neuropathy based on skin punch biopsy, laser-evoked potentials, or bedside thermal testing, which could not be explained by another disease.

Peripheral nerve injury: history of traumatic nerve injury of the distal upper or lower limb and sensory motor abnormalities confined to the innervation territory of the injured nervous structure.

Postherpetic neuralgia: unilateral zoster rash in the facial or thoracic area with postzoster scarring, hypopigmentation, or hyperpigmentation in the affected dermatome or sensory deficit in the area of the previous zoster rash determined by bedside testing.

Radiculopathy: pain in the L5 and/or S1 dermatome and positive straight leg raising test or sensory deficit within the matching dermatome or diminished Achilles tendon reflex for S1 lesions and magnetic resonance imaging of the lumbar spine confirming nerve root impairment by a herniated intervertebral disk or electromyography showing denervation in the L5 or S1 territory.

2.3. Exclusion criteria

Patients with trigeminal neuralgia, central neuropathic pain, and complex regional pain syndromes were excluded because it is believed that the underlying pathophysiological mechanisms are distinct from classical peripheral neuropathic pain etiologies. Further exclusion criteria were age <18 years, missing informed consent, communication problems, pain treatment by topical local anaesthetics for ≥7 days in the last 4 months or by topical capsaicin in the last 6 months, other pain locations with pain intensities ≥6 on ≥15 d/mo, other severe systemic or focal diseases of the central nervous system, spinal canal stenosis, peripheral vascular disease, pending litigation, major cognitive or psychiatric disorders, and treatment with an effect on neuropathic pain for any conditions except the inclusion criterion. By the latter criterion, we intended to assure that pain was the leading diagnosis and not depression. Because patient selection was done by each individual center, we do not know how many patients were excluded for this reason. Data sets were excluded in case of incomplete records (eg, no precise diagnosis documented, more than one QST variable missing in the affected area, no information about age, sex, or other demographic data) (**Fig. 1**).

All subjects signed written informed consent according to the Declaration of Helsinki for participation in the respective study and for transfer of the study records into the central database. The ethics committee of each center approved the study protocol individually. The study is reported according to the STROBE statement. Several centers contributed to more than one consortium, which contributed to uniform clinical standards across consortia.

2.4. Quantitative sensory testing and questionnaires

To assure process quality of QST, the investigators of each center underwent standardized training courses for the performance of QST.[63] The standardized protocol of DFNS was used for QST as described in detail previously.[51,62]

Quantitative sensory testing was conducted at the most painful site within the affected body area (test area) and the mirror-image contralateral area (control area). In cases of PNP, the cheek was assessed as the control area. The procedure started with a brief demonstration of each test in an area not to be included in the actual QST assessment, followed by QST of the control area and then QST of the test areas.[4]

The QST assessed the function of small and large afferent fibers. The standardized assessment contained 13 different thermal and mechanical tests. The following parameters were tested: thermal detection thresholds for the perception of cold (cold detection threshold [CDT]) and warmth (warm detection threshold [WDT]), paradoxical heat sensation (PHS) during the procedure of alternating warm and cold stimuli (TSL), thermal pain thresholds for cold (cold pain threshold [CPT]) and hot stimuli (heat pain threshold [HPT]), mechanical detection thresholds (MDT) for touch and vibration (VDT), mechanical pain sensitivity (MPS) including thresholds for pinprick (mechanical pain threshold [MPT]) and blunt pressure (pressure pain threshold [PPT]), a stimulus–response–function for pinprick sensitivity (MPS) and dynamic mechanical allodynia (dynamic mechanical allodynia [DMA]), and pain summation to repetitive pinprick stimuli (wind-up ratio [WUR]). For all parameters, negative (loss of function) and positive (gain of function) phenomena were assessed.

In the DFNS, the German version of the Center for Epidemiological Studies—Depression (CES-D[48]) was used for assessment of depression, in Neuropain, the Hospital Anxiety and Depression Scale (HADS[71]). Within the DFNS, the Neuropathic Pain Scale (NPS[25]) was used, in Europain and Neuropain, the Neuropathic Pain Symptom Inventory (NPSI[9]). Two items are highly comparable in these questionnaires, describing the stabbing and burning quality of spontaneous pain.

2.5. Statistical analyses

2.5.1. Z transformation and quantitative sensory testing profiles

In a control group of normal volunteers,[39,47,51] cold pain, HPTs, and VDTs as well as the numbers of PHSs during the TSL procedure were normally distributed. All other parameters were normally distributed in log space and were transformed logarithmically before statistical analysis. To compare individual QST data of patients or of a group of patients with age- and sex-matched control data, standard normal distributions of the patient data were calculated for each individual QST variable (z transformation, exception PHS and DMA). The calculation was based on measurements in 180 healthy controls.[51] Z scores of zero represent a value corresponding precisely to the mean of the healthy control cohort, z scores above "0" indicate a gain of function when the patient was more sensitive to the test stimuli compared with controls (hyperaesthesia or hyperalgesia), whereas z scores below "0" indicate a loss of function referring to a lower sensitivity of the patient (hypoaesthesia or hypoalgesia). Paradoxical heat sensation and DMA normally do not occur in healthy subjects. Thus, z transformation was not possible for these parameters because one would divide by zero. For PHS and DMA percentages are plotted against original data: occurrences of PHS (0-3), log numerical ratings scale for DMA (0-100), and are inserted on the right side of the sensory profile (**Fig. 2**).

By this procedure, sensory profiles of an individual patient or a group of patients can be displayed graphically on one common

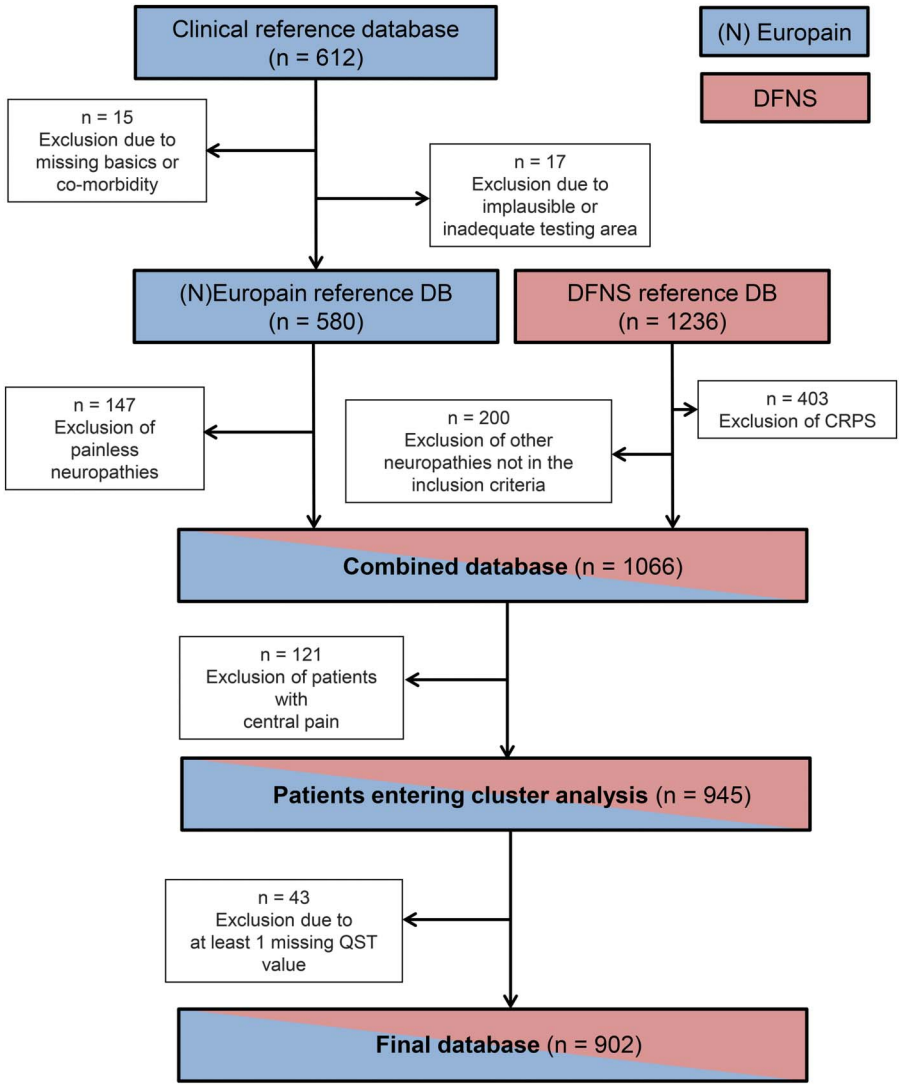

Figure 1. CONSORT flowchart for test data set. For cluster analysis of sensory profiles in patients with peripheral neuropathic pain, databases from 3 consortia were combined: German Research Network on Neuropathic Pain (DFNS) (shaded in red), IMI-Europain, and Neuropain (shaded in blue). CRPS, complex regional pain syndrome; DB, database.

scale of sensory gain or loss as well as the 95% confidence interval for healthy subjects.

2.5.2. Subgrouping of patients by cluster analysis

A cluster analysis was performed to unravel different and distinguishable subgroups of patients who are characterized by typical QST profiles. The 11 z-transformed QST variables (WDT, TSL, CPT, HPT, PPT, MPT, MPS, WUR, MDT, CDT, and VDT) were the primary basis for the analysis. In addition, PHS was transformed to a binary 0/2-variable showing absence (coded as 0) or presence (coded as +2) of pathological values; this puts PHS into similar metrics as the 11 z-transformed variables where 1.96 SD above or below the reference data mean of $z = 0$ is considered abnormal, and PHS is abnormal except for the lower extremity in older males. Dynamic mechanical allodynia occurred in a wide range of intensity values. By comparing the log-intensity scores with the impact of DMA on the quality of life of the patients, it was useful to use 3 different intensity levels. According to these observations, DMA was transformed to a 0/2/3-variable representing no DMA (coded as 0), DMA with average pain ratings below 1 (coded as +2), and DMA with average pain ratings between 1 and 100 (coded as +3). Accordingly, all 13 variables had a similar metric of means and variances, and we could use the squared Euclidian distance as the distance measure giving equal weight to all QST variables.

Because our data set is not computationally challenging, we used the widely known clustering algorithm k-means as the primary hypothesis-free analysis tool that divides the data set into a predetermined number of k clusters.[38] The transformed DMA and PHS variables were included into this procedure, because the Euclidian distance is a meaningful distance measure for a dichotomous or trichotomous variable. To make the cluster analysis completely hypothesis-free, we did not make any a-priori assumptions about the expected number of clusters. Instead, we performed k-means analyses for k ranging from 2 to 10 and used a series of well-established quality criteria from differing mathematical background to determine the optimum number of clusters:

(1) As a measure of fragmentation of the k-means solution for a given number of k clusters, mean silhouette width per cluster and the number of negative silhouette widths were used to

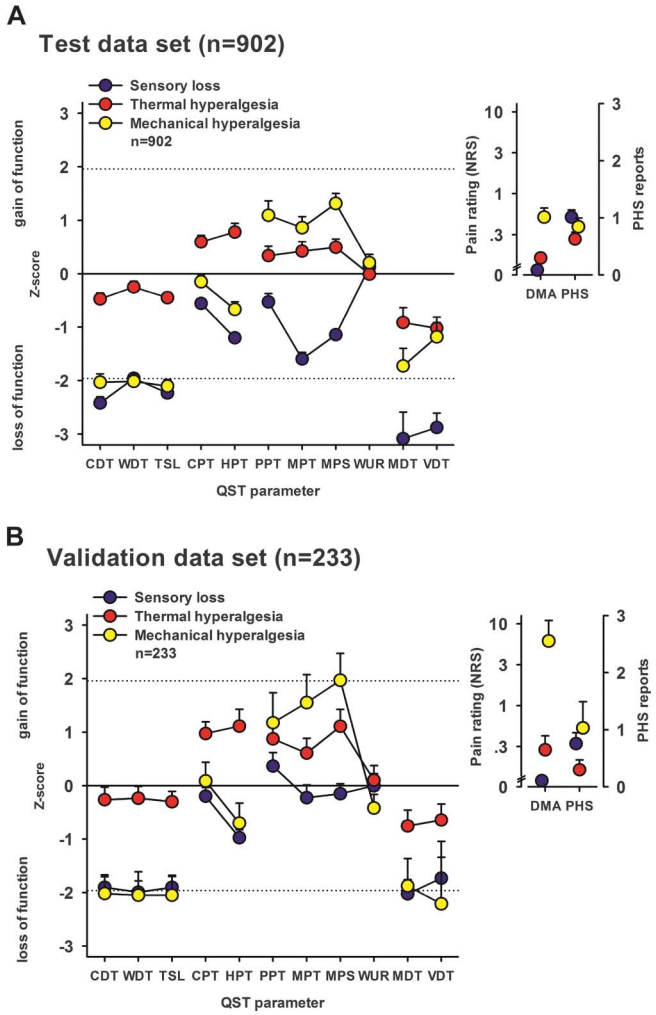

Figure 2. Sensory profiles of the 3-cluster solution for test and replication data sets. Sensory profiles of the 3 clusters presented as mean z scores ± 95% confidence interval for the test data set (n = 902, A) and the validation data set (n = 233, B). Note that z transformation eliminates differences due to test site, sex, and age. Positive z scores indicate positive sensory signs (hyperalgesia), whereas negative z values indicate negative sensory signs (hypoaesthesia and hypoalgesia). Dashed lines: 95% confidence interval for healthy subjects ($-1.96 < z < +1.96$). Note that if the mean of a cluster is within the shaded area, this does not imply that it does not differ from a healthy cohort. Values are significantly different from those of healthy subjects, if their 95% confidence interval does not cross the zero line. Insets show numeric pain ratings for dynamic mechanical allodynia (DMA) on a logarithmic scale (0-100) and frequency of paradoxical heat sensation (PHS) (0-3). Blue symbols: cluster 1 "sensory loss" (42% in A and 53% in B). Red symbols: cluster 2 "thermal hyperalgesia" (33% in A and B). Yellow symbols: cluster 3 "mechanical hyperalgesia" (24% in A and 14% in B). CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NRS, Numerical Rating Scale; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

exclude solutions that are likely to be artificial. Silhouette widths that range between −1 and +1 for each patient in the analysis may indicate that clusters overlap by a small degree of negative values.[52] A high count of negative silhouettes or a cluster with a mean silhouette width below zero indicates a cluster solution that is highly fragmented. Thus, we excluded all solutions with at least 1 cluster with a negative mean silhouette width, or over 10% negative silhouette widths.

(2) To validate a solution that is not dependent on the clustering method, the remaining k-means solutions were compared with a robust hierarchical agglomerative clustering method (maximum linkage) and an expectation maximization (EM) algorithm.[15] We compared both solutions with the initial k-means clustering through the adjusted rand index (ARI) and the adjusted variation of information (AVI). Although the ARI measures similarity on a scale from 0 to 1 (*high* values are preferable), the AVI measures *dissimilarity* on the same scale (*low* values are preferable[49]).

(3) The final criterion for the decision between otherwise equally good k-means solutions with different numbers of clusters was the Bayesian information criterion (BIC), which captures the gain of information by an increased number of clusters. The higher number of clusters is preferable if the difference between the BICs of both solutions (delta-BIC) is >10.[53]

2.6. Validation data set

For external validation, patients with PNP, PNI, and PHN who were collected either within the DFNS after the database closure in 2010 (n = 143) or within the Europain consortia for treatment studies with oxcarbazepine and lidocaine (n = 90)[13,14] (not included in the flowchart, **Fig. 1**). Inclusion and exclusion criteria for the patients collected within the DFNS were identical to the criteria for the test data set. Inclusion and exclusion criteria for the patients collected within Europain were identical except that patients did not fill out questionnaires on pain qualities, depression, and pain course over the last 4 weeks. Test and validation data sets were equal in age, sex, pain duration, and current pain intensity. After transforming the individual QST values into z scores, a separate cluster analysis was performed within this data set.

3. Results

3.1. Patients

In total, 1848 data sets were included into the combined DFNS/Europain/Neuropain database. After applying the inclusion/exclusion criteria, we could assess 902 patients with peripheral neuropathic pain of different etiologies in the test cohort (**Fig. 1**). The validation cohort consisted of 233 patients. Demographic data of the entire patient cohort are shown in **Table 1**. Most of the patients had long-lasting chronic pain between 1 and 5 years. Pain intensity generally was moderate to severe with average current pain ratings close to 6 on a 0-to-10 Likert scale without relevant differences between the cohorts. Distributions of etiologies differed between the 2 cohorts because of the absence of patients with RAD in the validation cohort. Questionnaires were available from 724 of the 902 patients in the test cohort, but not from the validation cohort.

3.2. Cluster analysis

We used a distributive cluster analysis technique (k-means) that separates data sets for maximal similarity within clusters and dissimilarity between clusters in a multidimensional space (here: 13 dimensions) for a predetermined number of clusters. Therefore, the first step was to identify the optimal number of clusters in a data-driven manner (**Table 2**). We compared k-means cluster solutions for 2 to 10 clusters. According to the frequency of negative silhouette widths, we excluded the solutions with 4 to 10 clusters because they each presented at least 1 cluster with

Table 2

Determination of the number of clusters.

n (clusters)	2	3	4	5	6	7	8	9	10
*Mean silhouette width	0.29	0.25	0.23	0.20	0.15	0.17	0.19	0.19	0.21
†Minimum mean silhouette width per cluster	0.28	0.13	−0.24	−0.28	−0.10	−0.07	−0.003	−0.02	−0.06
‡Negative silhouettes, %	0.7	4.8	14.5	16.4	22.6	21.2	16.3	16.0	14.7
§Comparison with hierarchical: ARI	0.30	0.30							
‖Comparison with hierarchical: AVI	0.67	0.56							
§Comparison with EM: ARI	0.01	0.22							
‖Comparison with EM: AVI	0.95	0.69							
¶Comparison with EM: delta-BIC	0	708							

Mean silhouette width per cluster below zero indicates clusters that do not separate from other clusters (4-10 cluster solutions).
* Measure of discriminatory power (0-1). 0: no discrimination, 1: perfectly separated clusters (high values are preferred).
† Measure of fragmentation of solution (−1 to +1). −1: cluster that is solely a fragment, +1: a solution that is not fragmented (solutions with values below zero were discarded).
‡ Measure of fragmentation of solution (0%-100%). 0%: no fragmentation, 100% a completely fragmented solution (solutions with values above 10% were discarded).
§ ARI (adjusted rand index): measure of similarity (0-1). 0: only random identity, 1: perfect identity (high values are preferred).
‖ AVI (adjusted variation of information): measure of dissimilarity (0-1). 0: no dissimilarity, 1: strong dissimilarity (low values are preferred).
¶ Delta-BIC (Bayesian information criterion): measure of gain of information by increasing the cluster number. If delta-BIC >10, the higher cluster number is recommended.
EM, expectation maximization.

a negative mean silhouette width that indicated an artifact. Furthermore, in each of these solutions, negative silhouettes were frequent (15%-23%). The remaining 2 and 3 cluster solutions were compared with 2 mathematically different clustering algorithms for the same number of clusters. Compared with agglomerative hierarchical cluster analysis, both 2- and 3-cluster solutions were equal according to the ARI criterion, but the 3-cluster solution was better according to the AVI criterion. In comparison to the EM algorithm, the 2-cluster solution failed to show similarity between k-means and EM clustering (ARI almost zero, AVI almost 1). Because the delta-BIC also strongly preferred the 3-cluster solution (**Table 2**), the 3-cluster solution was used for further analysis as the optimal number of clusters. This array of techniques gave multiple lines of converging evidence that patients should be grouped in exactly 3 clusters.

3.3. Sensory profiles of the 3-cluster solution

Figure 2 shows the mean z-score sensory profiles for the test data set (**Fig. 2A**) and the replication data set, which was also subjected to a k-means cluster analysis with k = 3 (**Fig. 2B**). In both data sets, the clusters represented similar percentages of patients: cluster 1 was the largest (42% in A, 53% in B), followed by cluster 2 (33% in A and B), and cluster 3 (24% in A, 14% in B). Sensory profiles were also replicated excellently. For nonnociceptive temperature sensation (CDT, WDT, and TSL), clusters 1 and 3 exhibited pronounced deficits with mean z scores near −2, whereas temperature sensation was essentially normal in cluster 2. This offset was similar for thermal pain sensitivity (CPT and HPT), but here clusters 1 and 3 exhibited less of a deficit, whereas cluster 2 exhibited significant sensory gain. Cluster 2 was therefore given the label "thermal hyperalgesia." For mechanical pain perceptions (PPT, MPT, and MPS), the rank order between clusters was different and cluster 1 and 3 were separated: although there was again a deficit for cluster 1, cluster 3 exhibited significant sensory gain. Cluster 3 was therefore given the label "mechanical hyperalgesia." Wind-up did not differentiate between clusters. For nonnociceptive touch sensation (MDT and VDT), cluster 2 was again close to normal, cluster 3 had some deficit, and cluster 1 exhibited the most pronounced deficit. Cluster 1 was given the label "sensory loss," because it was characterized by negative mean z scores across all QST parameters. Dynamic mechanical allodynia was most pronounced in cluster 3, which also exhibits the most pronounced hyperalgesia to pinprick (MPT and MPS) and blunt pressure (PPT). Paradoxical heat sensations were most pronounced in cluster 1, associated with diminished cold detection (CDT) but not cold hyperalgesia (CPT).

Figure 3 illustrates the distinction of the 3 clusters in a 2-D scatter plot of those 2 QST parameters that exhibited the best separation of clusters: WDT and MPS. Patients in cluster 1 had loss of pinprick sensitivity, whereas those in cluster 3 had pinprick

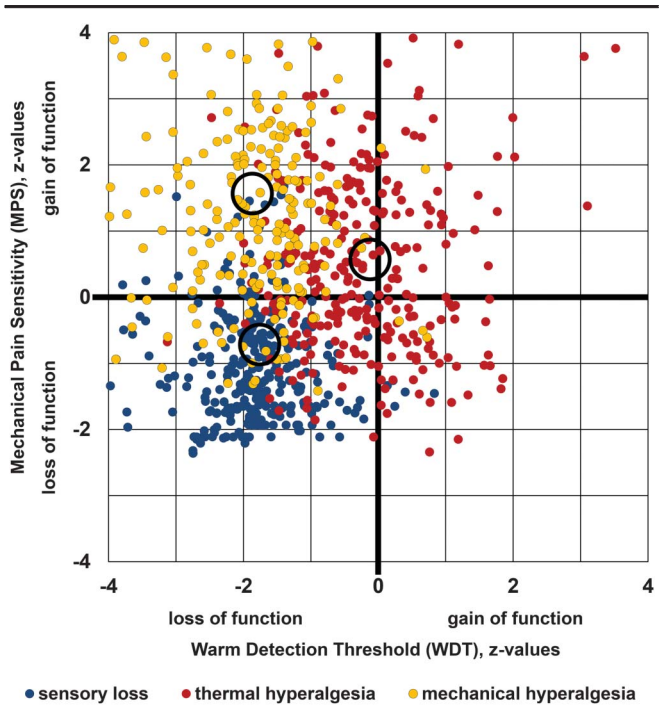

Figure 3. Cluster separation projected onto 2-dimensional space. Scatter plot of the 2 quantitative sensory testing (QST)-parameters that gave the best cluster separation: mechanical pain sensitivity (MPS) plotted against warm detection threshold (WDT). Blue dots: cluster 1 "sensory loss" (n = 381 patients); red dots: cluster 2 "thermal hyperalgesia" (n = 302 patients); yellow dots: cluster 3 "mechanical hyperalgesia" (n = 219 patients). Circles indicate centroids of each cluster.

hyperalgesia. Most patients in cluster 2 had WDT within the normal range of ±1.96 z values, whereas many of clusters 1 and 3 had hypoaesthesia to warmth (z values below −1.96). Although the k-means cluster separation was calculated in 13-dimensional space, this 2-D projection illustrates some of the main characteristics how the 3 clusters differ between each other. Partial overlap between clusters may also be due to 2 mechanisms present in the same patient.

3.4. Patient characteristics of the 3 clusters

The patients' sex and mean age did not differ between the 3 clusters (**Table 3**). The pain intensity also did not differ between the 3 groups. Depressive symptoms occurred significantly more frequently in the "sensory loss" cluster. Spontaneous pain described by the patients as "stabbing" was comparable across the clusters, but "burning" pain was significantly more frequent in the "mechanical hyperalgesia" cluster and hence cannot be taken as evidence for heat hyperalgesia. Information on current medication of the patients is available only from Europain and Neuropain (**Table 3**). Patients in the group "sensory loss" most frequently took tricyclic antidepressants who also presented an increased frequency of depressive symptoms. Anticonvulsants were most frequently taken in the "thermal hyperalgesia" group at least partly matching to the finding that Na-channel anticonvulsants are more effective in a very similar subgroup ("irritable nociceptor," see 4.4.[14]). Importantly, no specific drug was present in more than half of the patients in any group that shows that the sensory patterns do not result from drug effects. Furthermore, when cluster analyses were applied in the 2 largest groups of medication (tricyclic antidepressants, anticonvulsants), 3 clusters with similar pattern emerge (data not shown).

According to the published DFNS reference data, each QST parameter in each patient can be individually rated as within or outside the 95% CI of variability in healthy age- and sex-matched subjects. This analysis is presented in **Figure 4**. Of patients in cluster 1 ("sensory loss"), more than 50% had significant nonnociceptive sensory loss on an individual basis. Paradoxical

Table 3

Cluster characteristics and medication.

	Sensory loss	Thermal hyperalgesia	Mechanical hyperalgesia
Original data set*	381 (42)	302 (33)	219 (24)
Age, y	59 ± 14	56 ± 14	59 ± 15
Female*	169 (39)	152 (35)	108 (25)
Depression*	104 (47)‡	69 (31)	49 (22)
Pain intensity†	6.1 ± 3.1	5.8 ± 3.2	6.1 ± 3.0
Burning pain†	4.5 ± 3.4	4.3 ± 3.3	5.1 ± 3.2‡
Stabbing pain†	4.7 ± 3.2	4.3 ± 3.2	5.0 ± 3.0
Medication§	126 (86)‡	64 (71)	62 (78)
NSAID	28 (19)	18 (20)	13 (16)
SNRI	16 (11)	6 (7)	12 (15)
TCA	60 (41)‡	20 (22)	21 (26)
Anticonvulsant	41 (28)	34 (38)‡	20 (25)
Opioid	36 (25)	20 (22)	20 (25)
Validation data set*	124 (53)	77 (33)	32 (14)

Importantly, no specific drug was present in more than half of the patients in any cluster, which shows that the sensory patterns do not result from drug effects.

* n (%).

† Rated on a 0-to-10 Numerical Rating Scale scale.

‡ $P < 0.05$.

§ This information is available for n = 316 patients.

NSAID, nonsteroidal anti-inflammatory drug; SNRI, serotonin-norepinephrine-reuptake-inhibitor; TCA, tricyclic antidepressant.

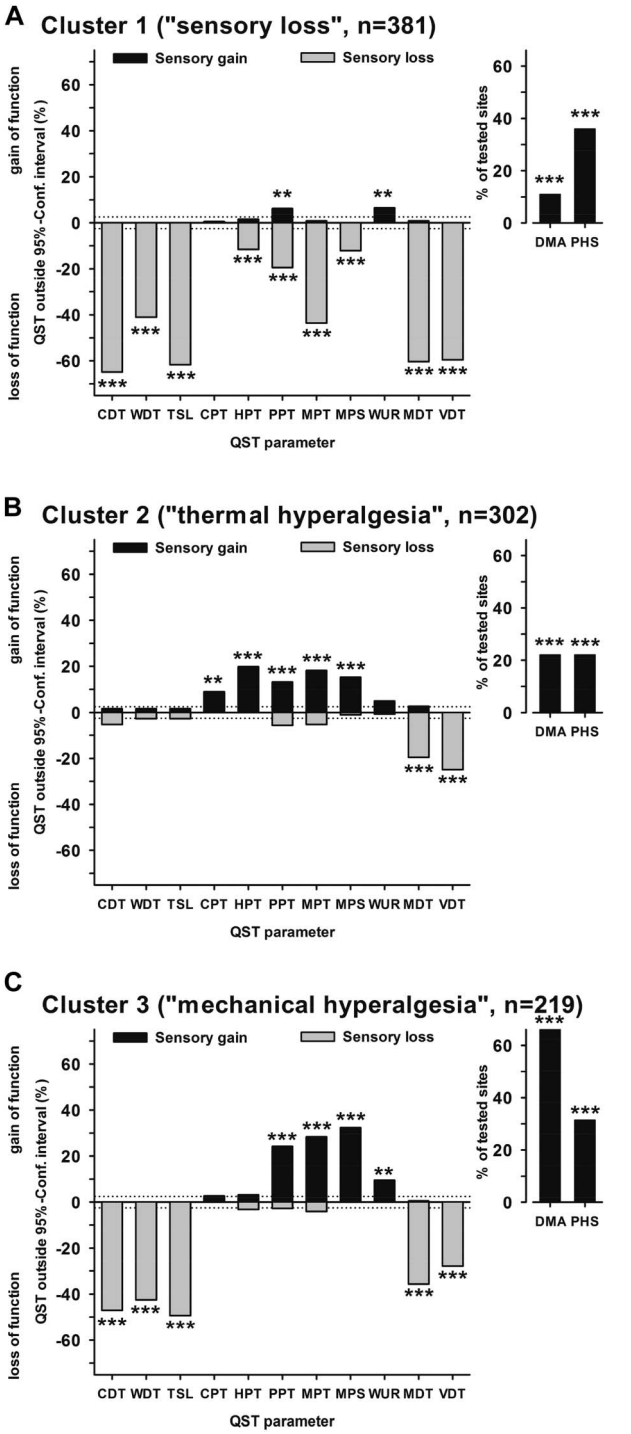

Figure 4. Frequencies of abnormal quantitative sensory testing (QST) findings for the test data set (n = 902). Each column gives the percentage of patients with abnormal findings for that particular QST parameter (outside the 95% CI of healthy subjects). Positive values indicate positive sensory signs (hyperalgesia), whereas negative values indicate negative sensory signs (hypoaesthesia and hypoalgesia). Dashed lines: Expected value for healthy subjects (±2.5%). A: cluster 1 "sensory loss" (n = 381 patients), B: cluster 2 "thermal hyperalgesia" (n = 302 patients), C: cluster 3 "mechanical hyperalgesia" (n = 219 patients). Significant compared with the expected value (2.5%) on *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NRS, Numerical Rating Scale; PHS, paradoxical heat sensation; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

heat sensation occurred in 40% and sensory loss for pain sensitivity was also prevalent, although at less than 50%. Patients of cluster 2, in contrast, exhibited hardly any sensory loss (except for touch in about 20% of patients), but significant proportions of patients presented with hyperalgesia to various stimuli. Cold and heat hyperalgesia were only significant for this cluster, but—probably at least partly due to the substantial variability of CPT and HPT in healthy subjects—all percentages were clearly below 50%. Patients of cluster 3 were characterized by a combination of loss of detection of nonnociceptive stimuli and hyperalgesia no noxious stimuli. However, in contrast to cluster 1, the sensory loss was more pronounced for small fiber function, ie, diminished temperature perception but relatively preserved tactile perception, and hyperalgesia was present only for mechanical stimuli. Dynamic mechanical allodynia was present in the majority of these patients. Because each individual sensory sign was present in less than 100% of patients per cluster, future analysis on assignment of individual patients to these cluster prototypes will thus also have to take subclinical sensory abnormalities into account.

3.5. Distribution of clusters across etiologies of peripheral neuropathic pain

Figure 5 illustrates that in principle, all 3 clusters were distributed across all 4 etiologies, which demonstrates that the sensory signs of neuropathic pain that are produced by these etiologies overlap considerably. Each of the different etiologies, however, showed a characteristic pattern of sensory profiles. In PNI, patients with "thermal hyperalgesia" were significantly more frequent (40.1%) than patients with other sensory profiles. "Thermal hyperalgesia" was the least frequent in patients with PNP. Patients with diabetic PNP only very rarely show this sensory profile (20%, cf. Ref. 57) indicating a predominant progressive dying-back axonal degeneration in this etiology. Therefore, "sensory loss" was the most frequent profile in PNP (51.8%) and RAD (42.7%). Patients with PHN were concentrated in the "mechanical hyperalgesia" cluster (46.6%).

4. Discussion

We had hypothesized that patients with peripheral neuropathic pain can be grouped into subtypes based on sensory profiles and that these profiles may reflect neurobiological mechanisms. According to the concept that damaged and surviving nociceptors are the key players in the pathophysiology of neuropathic pain,[10] one might have expected 2 clusters. Cluster analyses suggested that 3 subgroups best describe patients with peripheral neuropathic pain. All subgroups occurred in relevant numbers across etiologies, but frequencies differed between the entities. This 3-cluster solution and the structure of the sensory profiles could be reproduced in the validation cohort. It quite nicely matches the 3 subgroups described in smaller studies in patients with PHN almost 20 years ago.[7,20,31,61]

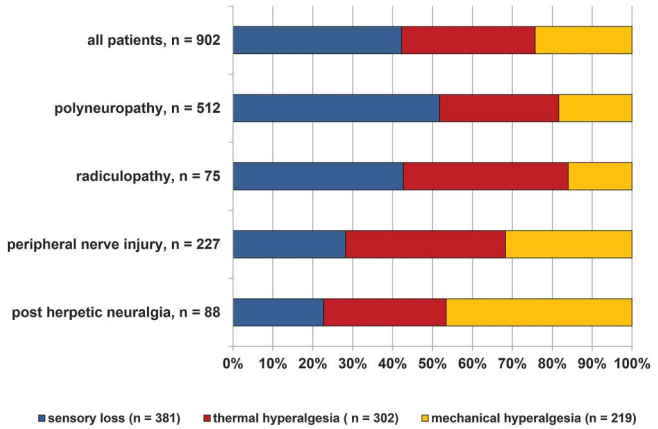

Figure 5. Distribution of the 3 clusters within each neuropathic pain etiology. Blue bars: cluster 1 "sensory loss" (n = 381 patients), red bars: cluster 2 "thermal hyperalgesia" (n = 302 patients), yellow bars: cluster 3 "mechanical hyperalgesia" (n = 219 patients). Cluster 1 was most frequent in polyneuropathy, cluster 2 in peripheral nerve injury and radiculopathy, and cluster 3 in postherpetic neuralgia.

4.1. Cluster 1 (sensory loss)

Cluster 1 (42%) was characterized by a loss of small and large fiber function and the presence of PHSs (**Table 4**). These patients did not suffer from sensory gain except a mild DMA in few patients. About 52% of patients with polyneuropathies fell into this category indicating dying-back degeneration of nearly all fiber classes. Interestingly, 43% of patients with painful RAD demonstrated this sensory pattern, suggesting severe degeneration of sensory fibers within the affected nerve root. Paradoxical heat sensation was most frequent, which suggests that it is induced by a loss of afferent input although at face value, it is a positive sensory sign possibly related to a central disinhibition process.[29,69]

The sensory profile is similar to that of a compression nerve block.[7,24,70] It likely represents the "deafferentation" or "painful hypoesthesia" subgroups described by others.[7,20,31,61] The spontaneous pain was likely due to ectopic action potentials generated in proximal sites of injured nociceptors,[10] eg, in the dorsal root ganglion or in deafferented central nociceptive neurons.[16,46,54] Laboratory tests for neuropathic pain assessment are likely to show denervation and loss of function (**Table 4**).[28]

4.2. Cluster 2 (thermal hyperalgesia)

Cluster 2 was characterized by relatively preserved large and small fiber sensory functions in combination with heat and cold hyperalgesia and only low-intensity DMA. This pattern occurred in 33% of all patients with peripheral neuropathic pain regardless of etiology. The fact that in one third of all patients the cutaneous sensory function was relatively well preserved despite documented nerve damage indicates that peripheral neuropathic pain may be associated with effective cutaneous regeneration and sensitized nociceptors.

The sensory profile is similar to that of a UV-B burn lesion[27] and is likely due to peripheral sensitization.[59] It represents the "irritable nociceptor" subgroup described by others.[13,14,20,45] Sensitized nociceptors are associated with overexpression of channels and receptors leading to pathological spontaneous discharges and a lowered activation threshold for thermal (heat and cold) and mechanical stimuli. Ongoing hyperactivity in surviving nociceptors may be responsible for ongoing pain[10] and may lead to some central sensitization in the spinal cord dorsal horn, so that tactile stimuli conveyed in A-fibers become capable of activating central nociceptive neurons. As a result, mechanical stimuli induce enhanced pain percepts, ie, pinprick hyperalgesia and DMA.[64] Because these types of mechanical hyperalgesia were only present in about 20% of the patients, peripheral nociceptor drive obviously does not always induce central sensitization.[60] Structural laboratory tests for

Table 4

Cluster characteristics, hypotheses on underlying pathophysiology, and rational pharmaceutical treatment.

	Sensory loss	Thermal hyperalgesia	Mechanical hyperalgesia
Original data set, n (%)	381 (42)	302 (33)	219 (24)
Validation data set, n (%)	124 (53)	77 (33)	32 (14)
Sensory profile			
Sensory loss	Touch, thermal, pain	None	Mostly thermal
Hyperalgesia	None	Mostly cold and heat	Mostly pressure and pin
DMA	Little	Little	Much
PHS	Much	Little	Little
Pathophysiology			
Sensory loss	Small and large fibres	—	Mostly small fibres
Hyperalgesia	—	Mostly peripheral sensitization	Mostly central sensitization
Ongoing pain	Ectopic activity in damaged nociceptors or in CNS neurons	Spontaneous activity in surviving nociceptors	(Ectopic?) activity in nociceptors
Predicted findings			
IENFD	Loss	None	Mild loss
CCM	Loss	None	Mild loss
Peripheral MRI	Damage	None	Mild damage
LEP	Reduction	Normal or gain	Mild reduction
RIII	Reduction	Normal or gain	Gain
μENG	Denervation	Sensitization	Little denervation
Predicted efficacy			
NSAIDS	—	(+)	—
Botox		+	
Topical capsaicin		+	
NMDA-antagonist			+
Antidepressant	++	+	+
Gabapentinoid	+	+	++
Na-channel blocker	+	++	++
Opioid	++	+	+

CCM, confocal corneal microscopy; CNS, central nervous system; DMA, dynamical mechanical allodynia; IENFD, intraepidermal nerve fiber density; LEP, laser evoked potential; NMDA, *N*-methyl-D-aspartate; PHS, paradoxical heat sensation; RIII, flexor reflex; μENG, microneurography.
MRI, magnetic resonance imaging.

neuropathic pain assessment are likely to be normal, whereas functional tests may show gain of function (**Table 4**).[28]

4.3. Cluster 3 (mechanical hyperalgesia)

Cluster 3 (24%) was characterized by a predominant loss of cold- and heat-sensitive small fiber function in combination with blunt pressure hyperalgesia, pinprick hyperalgesia, and marked and more frequent DMA. Burning pain quality in this cluster was more prominent than in the other groups, consistent with findings in Guillain–Barré syndrome in which burning pain was associated with small fiber deficits[43] and with the concept of synthetic heat[12] rather than peripheral sensitization to heat. The profile was most commonly present in patients with PHN (47%). It is similar to the one induced by high-frequency electrical stimulation of the skin that is capable of inducing spinal long-term potentiation[37,50] and likely equivalent to "neurogenic hyperalgesia" or "central sensitization" subgroups described by others.[7,20] Central sensitization is prominent for mechanical stimuli[6,55,59] but not thermal stimuli. The dissociation of thermal and mechanical hyperalgesias may be explained by differences in neural signalling of thermal and mechanical pain that starts with peripheral encoding in distinct subsets of nociceptors.[11,32] Ongoing pain in this subgroup indicates spontaneous activity in the nociceptive system, which may originate in the peripheral and/or central nervous system. Laboratory tests for neuropathic pain assessment are likely to reflect mild loss of function; few tests are sensitive to reflect central sensitization (**Table 4**).[28]

4.4. Subgrouping identifies responders

Several trials in neuropathic pain have used baseline QST profiling to identify predictors of treatment response[8] that can be tentatively assigned to the 3 clusters:

Patients with a baseline QST profile similar to our cluster 2 ("heat hyperalgesia") exhibited a higher efficacy in a prospective randomized placebo-controlled trial with oxcarbazepine,[14] in a preplanned analysis of a placebo-controlled trial with botulinum toxin,[1] and in a retrospective analysis of a study using topical capsaicin patches without a placebo arm.[41] A retrospective analysis of a placebo-controlled trial with topical lidocaine demonstrated lower efficacy.[65]

Patients with a baseline QST profile similar to our cluster 1 ("sensory loss") exhibited a higher efficacy in a retrospective analysis of a placebo-controlled trial with oral opioids.[17] A prospective randomized placebo-controlled trial with oxcarbazepine demonstrated lower efficacy.[14]

Patients with a baseline QST profile similar to our cluster 3 ("mechanical hyperalgesia") exhibited a higher efficacy in retrospective analyses of placebo-controlled trials with oral pregabalin,[56] topical lidocaine,[65] lamotrigine,[23] or intravenous lidocaine.[3]

The different pharmacological profiles support the clinical relevance of our clusters. Our predictions for differential efficacy of major neuropathic pain medications across clusters are summarized in **Table 4**. The size of the difference in treatment response between clusters remains to be proven in future prospective trials.

4.5. Limitations

Because the inclusion criteria slightly differed between the 3 consortia, there is no perfect homogeneity of patients within etiologies. Furthermore, in contrast to short-term stability of QST,[26] long-term stability over weeks has not been studied, and hence it is possible that patients can shift from one cluster into another. It should be noted that implementation of the DFNS QST protocol requires formal training, which has been undergone by about 70 centers around the world so far.

Dynamic QST, ie, assessment of a change of a QST parameter to an external stimulus,[4] is not the focus of our testing protocol. The only dynamic marker used, WUR, did not distinguish between subgroups. Another option of dynamic QST, the conditioned pain modulation, has demonstrated a potential in response prediction. This paradigm uses the fact that pain sensitivity is physiologically modulated by monoaminergic descending pathways originating in the brainstem and projecting to the spinal nociceptive transmission centers.[67] Individuals with diabetic painful neuropathy with a malfunctioning pain modulation benefit more from duloxetine treatment than do patients with a normal modulation pattern.[68]

4.6. Summary and conclusions

Using an unbiased hypothesis-free data segmentation approach on a broad range of peripheral neuropathic pain diagnoses, we identified 3 clusters that are consistent with previous smaller studies in the field, are pathophysiologically plausible, and can be tentatively related to pharmacological sensitivity. An important challenge will be to develop an algorithm that assigns individual patients to one of the clusters described in this study. We propose a Bayes network that provides probabilities for a patient to belong to each cluster. Based on this algorithm, future clinical trials should classify all included patients according to the 3 clusters and test for differential drug efficacy across clusters as a planned secondary analysis. In case a consistent pattern emerges, further trials could then use the clusters for stratification or as an inclusion criterion. The resulting label for a medication licensed this way is likely to be restricted to the respective cluster profile, but any disadvantages of this restricted label should be offset by a higher responder rate. As a result of the presented data, the European Medicines Agency (EMA) has recently acknowledged in a "CHMP qualification advice" that sensory profiling and subgrouping as proposed in this study is an adequate stratification tool for determining specific sensory phenotypes of patients in exploratory trials on neuropathic pain.[19]

Conflict of interest statement

R. Baron has received grants/research support from Pfizer, Genzyme, Grünenthal and Mundipharma. EU Project No 633491 DOLORisk. German Federal Ministry of Education and Research (BMBF): ERA-NET NEURON, IM-PAIN Project. German Research Network on Neuropathic Pain, NoPain system biology. German Research Foundation (DFG). He has received speaking fees from Pfizer, Genzyme, Grünenthal, Mundipharma, Sanofi Pasteur, Medtronic, Eisai, Lilly, Boehringer Ingelheim, Astellas, Desitin, Teva Pharma, Bayer-Schering, MSD, and Seqirus. He has been a consultant for Pfizer, Genzyme, Grünenthal, Mundipharma, Allergan, Sanofi Pasteur, Medtronic, Eisai, Lilly, Boehringer Ingelheim, Astellas, Novartis, Bristol-Myers-Squibb, Biogenidec, AstraZeneca, Merck, AbbVie, Daiichi Sankyo, Glenmark Pharmaceuticals, Seqirus, Teva Pharma, Genentech, and Galapagos.

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G. Cruccu has received honoraria for lectures or advisory boards from Astellas, Biogen-Convergence, Sigma Tau, Angelini, and Teva.

N. B. Finnerup has received honoraria for consulting or travel support from Grünenthal, Teva Pharmaceuticals, Novartis Pharma and Astellas Pharma.

M. Haanpää has received lecturing fees from Astellas, Allergan, MSD, Orion, Pfizer, and Sanofi Pasteur. She has been advisory board member of AbbVie, Astellas, and Pfizer.

She has received congress travel costs from Astellas and Pfizer.

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P. Hüllemann has received speaking fees and travel expenses from Pfizer, Grünenthal, and Genzyme.

T. S. Jensen undertakes consultancy for Pfizer, Grünenthal, and Orion. He has received a grant from Astellas.

R. Freynhagen declared research support, consulting, or lecture fees in the past 2 years from Astellas, Develco, Galapagos NV, Grünenthal GmbH, Eli Lilly & Company, Merck Sharp & Dohme, Mitsubishi Tanabe Pharma, and Pfizer Inc.

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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, Toray, Galapagos, Merck, Medivir, Mitsubishi, Aquilas, Asahi Kasei, Relmada, Novartis, and Orion. He was the owner of share options in Spinifex Pharmaceuticals from which personal benefit accrued upon the acquisition of Spinifex by Novartis in July 2015 and from which future milestone payments may occur. Research grant to Imperial College from Astellas as part of a European Commission and European Federation of Pharmaceutical Industries and Associations (EFPIA), Innovative Medicines Initiative Grant (EUROPAIN).

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Supplemental media

Video content associated with this article can be found online at http://links.lww.com/PAIN/A363

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