Patterns of Quantitative Sensation Testing of Hypoesthesia and Hyperalgesia Are Predictive of Diabetic Polyneuropathy

A study of three cohorts

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OBJECTIVE — To test quantitative sensation testing (QST) patterns of hypoesthesia and hyperalgesia as indicators of diabetic polyneuropathy (DPN) and its severity.

RESEARCH DESIGN AND METHODS — We used Computer-Assisted Sensory Examination IV; characterized the QST results of the foot of each patient in three diabetic cohorts (~1,500 patients) as hyperesthetic (≤2.5th percentile), low-normal (2.5th–50th percentiles), high-normal (50th–97.5th percentiles), or hypoesthetic (≥97.5th percentile); and tested associations with symptoms, impairments, and test abnormalities.

RESULTS — Overall neuropathic impairment was most severe in the pancreas-renal transplant and nerve growth factor cohorts, but it was much less severe in the population-based Rochester Diabetic Neuropathy Study (RDNS) cohort. The frequency distribution of sensory abnormalities mirrored this difference. When the QST spectra of diabetic cohorts were compared with those of the control subject cohort for vibration and cooling sensations, the only abnormality observed was hypoesthesia, which was expressed as an increased number of subjects with values at or above the 97.5th percentile or by an increased percentage of cases with high-normal values. Symptoms and impairments of DPN were significantly more frequent in the subjects with values at or above the 97.5th percentile than in the subjects whose values were between the 50th and 97.5th percentiles. For heat pain (HP) sensation thresholds (intermediate pain severity [HP:5], pain threshold [HP:0.5], and pain-stimulus response slope [HP:5–0.5]), an increased frequency of both hypoalgesia and hyperalgesia was observed (especially in the RDNS cohort). Steeper pain-stimulus response slopes were significantly associated with sensory symptoms, including severity of pain.

CONCLUSIONS — *1*) Decreased vibratory sensation (hypoesthesia) appears to be characteristic of mild DPN, whereas panmodality hypoesthesia is characteristic of severe DPN. *2*) A shift of vibratory and cold detection thresholds (and also of attributes of nerve conduction and a measure of autonomic dysfunction) from low-normal (2.5th–50th percentiles) to high-normal (50th–97.5th percentiles) appears to precede overt expression of DPN and to thereby provide evidence of subclinical abnormality. *3*) Heat stimulus–induced hyperesthesia (low thresholds) occurs especially in mild DPN, and, because it correlates with DPN symptoms and impairments, it must be attributed to hyperalgesia rather than to supersensitivity. Therefore, hypoalgesia or hyperalgesia may be an indicator of early DPN.

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Quantitative sensation testing (QST) has been used to assess the effects of maturation, aging, anatomical location, anthropometric characteristics, and disease on thresholds of different modalities of cutaneous sensation (1,2). These techniques have been used to also study the physiological characteristics of sensory receptors and such dysfunctions as stimulus-induced hyperalgesia and pain (3–8). These approaches were used to show also that vibratory sensation persisted for a longer time after application of a tourniquet in diabetic subjects than in control subjects (3). This resistance to ischemic conduction block is now usually attributed to the greater availability of energy substrate in diabetic nerves (9). Increasingly, QST approaches are being used in epidemiological studies and controlled clinical trials (10–12), and they have been introduced into medical practice (13). In particular, QST has been used to detect and characterize sensation alteration in various human polyneuropathies, including diabetic polyneuropathy (DPN) (14–16), and this information has recently been reviewed (17).

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Abbreviations: CASE IV, Computer-Assisted Sensory Examination IV; CDT, cooling detection threshold; DPN, diabetic polyneuropathy; HBDB, heart beat response to deep breathing; HP, heat pain; NC, nerve conduction; NIS, Neuropathy Impairment Score; NIS(LL), NIS for impairment of lower limb; NIS(LL) + 4, NIS(LL) subscore with four nerve tests; NIS(S), NIS for sensation; NIS(W), NIS for weakness; NSC, Neuropathy Symptoms and Change; NSC[A], NSC subscore for autonomic dysfunction; NSC[LF], NSC subscore for large-diameter fiber sensation; NSC[P], NSC subscore for pain threshold; NSC[SF], NSC subscore for small-diameter fiber sensation; NSC[W], NSC subscore for weakness; PRT, pancreas-renal transplant; QST, quantitative sensation testing; RDNS, Rochester Diabetic Neuropathy Study; rhNGF, recombinant human nerve growth factor; VDT, vibratory detection threshold.

The names of the investigators of the NGF Study Group will be listed in a scientific article that is being prepared for publication. Schwartz S, et al.: A phase III multicenter double-blind placebo-controlled study of the efficacy and safety of recombinant human nerve growth factor in subjects with diabetic polyneuropathy.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

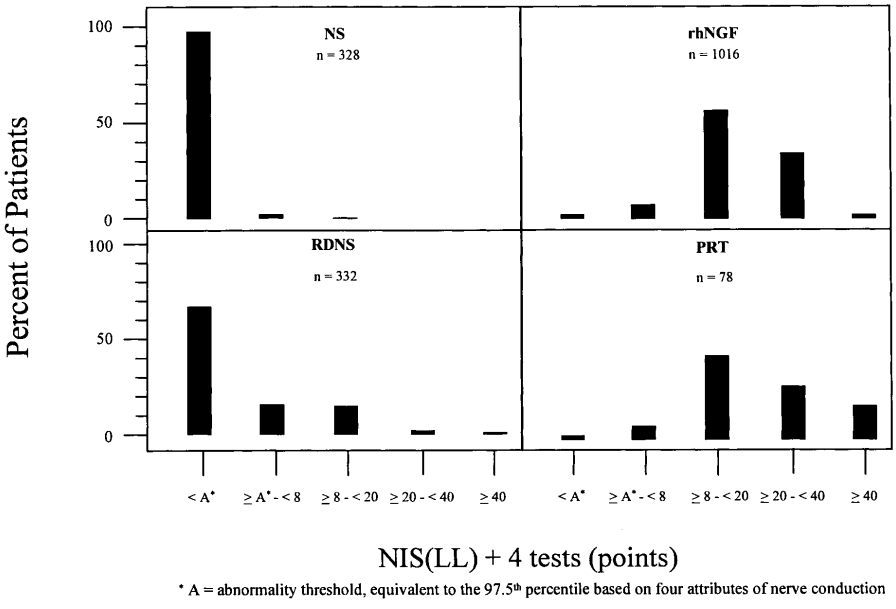

Figure 1—*Frequency distribution of severity of DPN as measured by NIS(LL)+4 tests for the three diabetic cohorts and the control cohort. The PRT and rhNGF cohorts tend to have more severe neuropathy than the RDNS cohort. The QST abnormalities mirrored the severity of neuropathy shown in the figure. NS, normal control subjects.*

We introduced interactive programmed computer-controlled systems to evaluate touch pressure, vibration, cooling, and heat pain (HP) thresholds by use of defined, standardized, and validated stimuli and preprogrammed algorithms of testing and finding thresholds (18,19). We estimated reference values that were specific for modality, site, age, sex, and other anthropometric characteristics so that the results could be expressed as a percentile value or as a standard deviate (see RESEARCHDESIGN ANDMETHODS) value (1,2). The use of such specific percentile values permitted a valid comparison of results for an individual at different ages, because age and anthropometric characteristics are considered when percentile values are calculated. By using across-test comparisons, we found that sensation loss was correlated with clinical, nerve conduction (NC), and morphometric measures of biopsied nerve (15,20). Vibratory sensation of the toe, using the Computer-Assisted Sensation Examination IV (CASE IV) and the 4-, 2-, and 1-stepping with null stimuli algorithm, performed favorably when compared with other nerve tests in assessing the changes over time in the severity of DPN (19).

In the present study, we compare QST results in three diabetic cohorts with quite different severities of polyneuropathy. Comparisons of the spectra of severity of polyneuropathy (as measured by the percent having defined levels of abnormality) and spectra of QST abnormalities permitted an assessment of how well QST results reflect overall neuropathy severity. Comparisons of the frequency of impairments or symptoms between those groups with and without hypoesthesia (decreased or absent sensitivity) or hyperesthesia (increased sensitivity) allowed inferences to be made about the value of QST in detecting and characterizing DPN.

RESEARCH DESIGN AND METHODS

Cohorts studied

We evaluated QST results and neuropathic impairments, symptoms, and test results (at first evaluation) in three diabetic cohorts: the Rochester Diabetic Neuropathy Study (RDNS) cohort, the recombinant human nerve growth factor (rhNGF) cohort, and the pancreas-renal transplant (PRT) cohort. The RDNS cohort consisted of all of the consenting diabetic patients from the city of Rochester, Minnesota, who did not have a confounding neurological disease and who were participating in a cross-sectional and longitudinal study of diabetic complications (21). For patients <70-years-old, comorbidity (as evaluated from coded discharge diagnoses) was not significantly different between participating and nonparticipating patients. The cohort was thought to be representative of northern U.S. cities of predominantly Caucasian extraction. Approximately one-half of the patients in this population had DPN, but such cases were usually mild, as described below.

The rhNGF cohort was recruited for trials of rhNGF therapy (Genentech, San Francisco, CA) as treatment for DPN. Patients <65 years old with stable diabetes, DPN (asymptomatic or symptomatic), and an abnormal (≥90th percentile) cold detection or HP:5 thresholds (albeit, one of the two thresholds had to be obtained) were recruited. This cohort consisted of 1,019 diabetic patients from 84 medical centers.

The diabetic PRT cohort consisted of 78 patients with type 1 diabetes who were <40-years-old, had end-stage diabetic nephropathy (and other diabetic complications), and had undergone pancreas and renal transplantation. Patients with symptomatic coronary and peripheral vascular disease were excluded.

Test results were expressed as percentiles and standard deviates, based on a study of 330 randomly selected and neurologically examined subjects from Rochester, Minnesota, who showed no signs of diabetes, neurological disease, or diseases predisposing to neuropathy (control subject cohort) (1,2)

CASE IV system

The CASE IV system was used to study the control subjects, the RDNS cohort, and the PRT cohort. The system was used to obtain vibratory detection thresholds (VDTs), cooling detection thresholds (CDTs), and HP thresholds (intermediate pain [HP:5], pain threshold [HP:0.5], and pain-stimulus response slope [HP:5–0.5]). The system consists of a personal computer, controlling electronics, transducers, a cueing device, a keyboard, and ancillary devices, as previously described (18). The stimulus waveform, the null stimulus, the 4-, 2-, and 1-stepping with null stimuli (for VDTs and CDTs), and the nonrepeating ascending with null stimuli (for HP tests) algorithms have been previously described and validated (19). Patient instructions by use of cueing cards were standard. A commercial model, the Computer-Assisted Sensation Evaluator IV (WR Medical Electronics, Stillwater, MN), was used in the rhNGF trial. The design of the WR system was modeled after our system and uses the same stimuli,

algorithms of testing, and finding threshold and reference values. Calibration of the vertical displacement was calibrated for both systems with the vibrating tactator resting on a silicone tube (11/16" OD, 1/2" ID, shore A 50) by use of laser triangulation (Keyence, Osaka, Japan). The thermode was calibrated for absolute temperature by using a high-purity platinum resistor (Goodrich, Minneapolis, MN) interposed between the thermode and a Styrofoam block at controlling temperatures of 10 and 40°C for periods of 15 min at each temperature. Rapid fluxes (e.g., the 4°C/s rise from baseline and return to baseline) were monitored with bead thermistor, thermocouple, or infrared thermometer. Variation over the surface of the thermoelectric unit was monitored with cholesterol ester mixtures. The vibratory tactator rested on the skin with a constant baseline load. The baseline temperature for the CDT was 30°C, and the baseline temperature for the HP threshold was 34°C. For the VDT, CDT, and the HP threshold, 25 exponentially increasing steps of stimulus magnitude are standard. QST results were monitored in a central reading laboratory to ensure that *1*) the correct site, algorithm, and conditions of testing were used; *2*) practice and algorithm thresholds were within two steps of each other (except for HP, for which a practice sequence was not used); *3*) the stimulus response pattern did not show inattention or drowsiness; *4*) sufficient turn-arounds to estimate threshold had been attained; *5*) the order of testing by modality was correct; and *6*) sufficient trials had been used to find threshold. If the test did not pass quality assurance criteria, then it was repeated.

For VDTs or CDTs, the quantity estimated was the stimulus step that was felt 50% of the time. For HP thresholds, we estimated threshold (HP:0.5), an intermediate severity of HP (HP:5), and the pain-stimulus response slope (HP:5–0.5). QST results are expressed as noticeable difference step of stimulus magnitude (1 to 25), the corresponding physical measurement (μm of displacement or the change from 0°C), and the corresponding percentile or standard deviate to make appropriate corrections for modality, anatomical site, age, sex, and applicable physical characteristics.

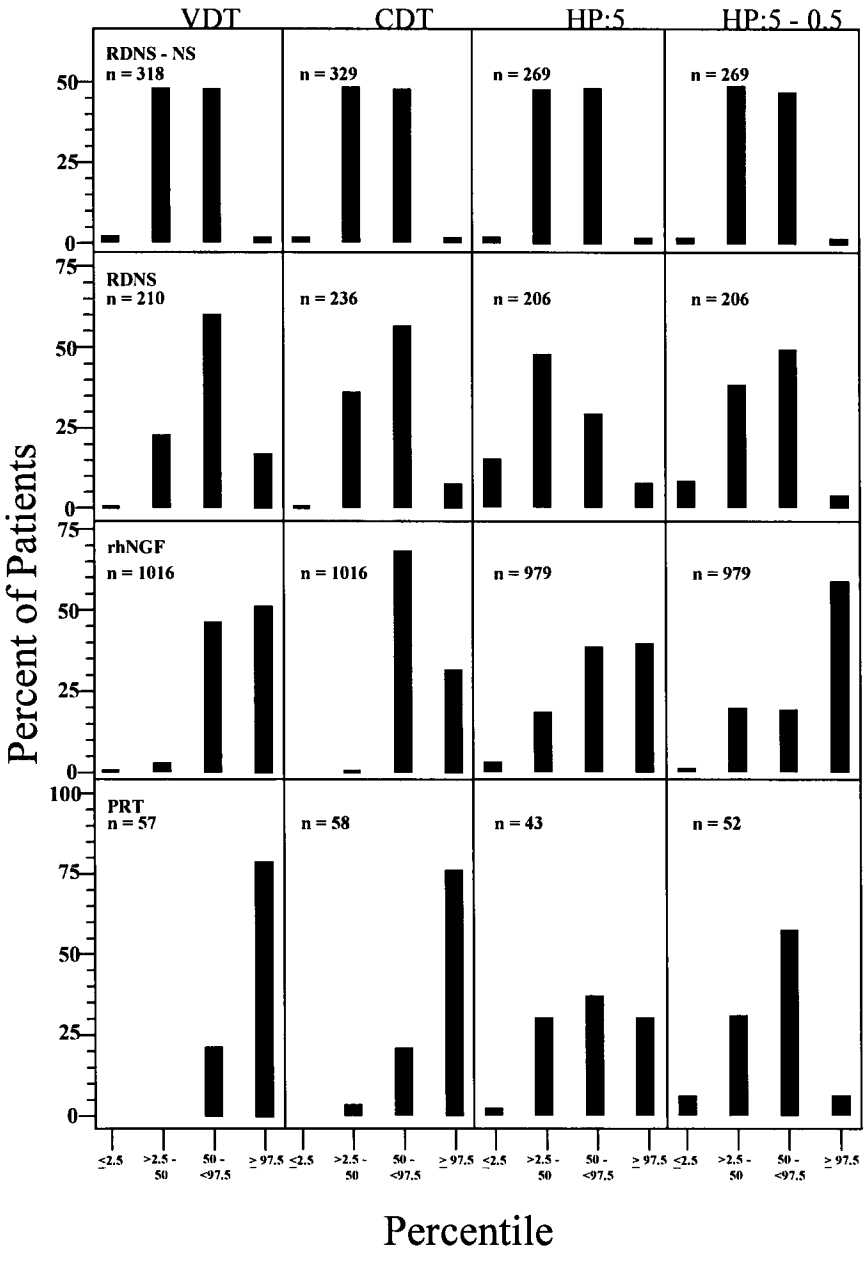

Figure 2—*Each patient's QST threshold and each of the three modalities tested were expressed as a percentile specific for test, site, age, sex, and anthropometric characteristics. The frequency distributions by categories of percentile abnormality are shown for each of the studied cohorts. The characteristics, associations, and inferences that were derived from these data are described in text. NS, normal control subjects.*

Other neuropathy tests

Standardized measurements of neuropathic impairment (Neuropathy Impairment Score [NIS]), neuropathic symptoms (Neuropathy Symptoms and Change [NSC]), attributes of NC, and the heartbeat response to deep breathing (HBDB) were compared with the QST results of each patient. In NIS muscle weakness, reflex diminution or loss and sensation diminution or loss were summated based on a standard evaluation of a predetermined group of muscles, muscle stretch reflexes, and sensation modalities of index fingers and great toes with abnormality expressed as a composite score (points). The examiner graded abnormality of each item of the NIS specific for age, sex, physical fitness, and other anthropometric characteristics. NIS subscores for impairment of lower limb (NIS[LL]), weakness (NIS[W]), and sensation (NIS[S]) were derived. We also used a composite score that included the results of the NIS(LL) and four nerve tests (NIS[LL] + 4), which

Table 1—*Average composite severity of DPN of patients in the RDNS, rhNGF, and PRT cohorts*

	VDT					CDT				
	50th to <97.5th		≥97.5th			50th to <97.5th		≥97.5th		
	n	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*
RDNS										
NIS(LL) + 4 tests	126	3.36	36	12.46	<0.001	133	4.44	16	17.09	<0.001
NIS(LL)	126	1.49	36	8.21	<0.001	133	2.26	17	11.53	<0.001
Lower-limb NIS(W)	126	0.15	36	2.10	<0.001	133	0.22	17	3.76	<0.001
Lower-limb NIS(S)	126	0.48	36	3.08	<0.001	133	0.89	17	4.00	<0.001
rhNGF										
NIS(LL) + 4 tests	466	15.73	514	20.06	<0.001	691	16.88	319	19.85	<0.001
NIS(LL)	467	12.01	516	14.99	<0.001	694	12.88	319	14.62	<0.001
Lower-limb NIS(W)	467	1.52	516	2.01	0.008	694	1.64	319	2.00	0.026
Lower-limb NIS(S)	467	7.44	516	8.86	<0.001	694	8.02	319	8.34	0.310
PRT										
NIS(LL) + 4 tests	12	9.92	43	22.28	<0.001	11	13.45	41	22.73	0.012
NIS(LL)	12	4.50	43	13.66	0.001	11	6.73	41	14.43	0.012
Lower-limb NIS(W)	12	1.67	43	5.45	0.034	11	3.18	41	5.77	0.190
Lower-limb NIS(S)	12	0.17	43	4.02	<0.001	11	0.91	41	4.15	0.007

	HP:5					HP:5–0.5				
	50th to <97.5th		≥97.5th			50th to <97.5th		≥97.5th		
	n	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*
RDNS										
NIS(LL) + 4 tests	61	5.61	16	14.31	0.004	102	5.52	8	4.50	0.659
NIS(LL)	61	2.80	16	10.56	0.002	102	3.05	8	2.50	0.415
Lower-limb NIS(W)	61	0.43	16	3.69	0.027	102	0.63	8	0	0.582
Lower-limb NIS(S)	61	0.92	16	4.25	<0.001	102	1.13	8	1.00	0.487
rhNGF										
NIS(LL) + 4 tests	378	16.13	389	20.21	<0.001	576	17.55	190	19.30	0.041
NIS(LL)	379	12.09	390	15.40	<0.001	578	13.30	191	14.62	0.094
Lower-limb NIS(W)	379	1.18	390	2.34	<0.001	578	1.65	191	2.44	0.432
Lower-limb NIS(S)	379	7.54	390	9.14	<0.001	578	8.13	191	8.34	0.541
PRT										
NIS(LL) + 4 tests	15	21.00	11	25.27	0.311	23	22.35	0	—	—
NIS(LL)	15	12.73	11	16.59	0.274	26	14.97	0	—	—
Lower-limb NIS(W)	15	5.33	11	5.68	0.507	26	5.86	0	—	—
Lower-limb NIS(S)	15	2.67	11	7.00	0.009	26	4.81	0	—	—

Data were attained by use of Wilcoxon's rank-sum test. The data for HP:0.5 (not shown) were similar to the data for HP:5.

were conducted by procedures previously outlined (20). The four tests consisted of the peroneal nerve motor nerve conduction velocity, the compound muscle action potential, the motor nerve distal latency, and the tibial distal latency tests. The NSC score is obtained through a standardized physician-completed questionnaire that is used to judge the number, severity, and change of symptoms. Subscores on the severity of the NSC were also obtained to measure weakness (NSC[W]), sensation (NSC[S]), autonomic dysfunction (NSC[A]), large-diameter fiber sensation (NSC[LF], small-diameter fiber sensation (NSC[SF]), and pain (NSC[P]). In all cases, only symptoms occurring in the lower limbs were used.

QST and DPN severity spectra

Each patient's QST results, by modality of sensation, were categorized as being at or below the 2.5th percentile (hyperesthetic), between the 2.5th and 50th percentiles (low-normal), between the 50th and 97.5th percentiles (high normal), and at or above the 97.5th percentile (hypoesthetic). This categorization was decided on a priori without assessing other limits. Small percentiles represent small-magnitude stimuli, and large percentiles represent large-magnitude stimuli. Overall severity of DPN was represented by the points of the NIS(LL) + 4 tests (20).

Analysis

To compare different test results among different modalities of sensation and attributes of NC, we used percentile values derived by study of the control cohort (2), as previously described (1). Thus, it was possible to express each of the test results as a specific percentile value that considered test, site, age, sex, and applicable anthropometric characteristics. A standard deviate value relates each percentile value to a normal distribution. For example, for the 50th percentile, the corresponding standard deviate is 0, because 50% of the values in a normal distribution fall below 0. The standard deviate corresponding to the 95th percentile is 1.96, because 95% of the values in a normal distribution fall below 1.96. The mean neuropathic impairments (as measured by NIS, NIS[LL], NIS[LL] + 4, NIS[LL][W], or NIS[LL][S]) was compared among patients with different categories of sensation abnormality by use of two-sided Wilcoxon's rank-sum tests.

RESULTS

The severity of DPN among the cohorts studied

There was a striking difference in the severity spectra of DPN, as measured by NIS(LL) + 4 tests, among the three disease cohorts studied, as compared with the control cohort (Fig. 1). In the RDNS cohort, only 35% of the subjects had DPN and only a few patients had NIS(LL) + 4 test values ≥20 points, which indicates severe neuropathy. By contrast, virtually all of the patients in the rhNGF and PRT cohorts had DPN, and ~ 40% of each cohort had NIS(LL) + 4 test values ≥20 points. DPN in the PRT cohort was somewhat more severe than that in the rhNGF cohort.

The severity spectrum of QST abnormalities

The severity spectrum of QST results for VDTs, CDTs, and HP thresholds of the foot are shown in Fig. 2 for the control subjects and the three disease cohorts. Results for HP:0.5 are not shown. VDTs and CDTs were shifted from left (≤50th percentile) to right (>50th percentile), resulting in more patients in the 50th–97.5th percentile category than in the 2.5th–50th percentile category, with an increase in patients with unequivocal

hypersensitivity or insensitivity (≥97.5th percentile). In the RDNS, the VDTs were much more frequently abnormal (≥97.5th percentile) than they were for the CDTs or HP:5 thresholds. By comparison, for the PRT cohort, the frequency of VDT and CDT abnormality (≥97.5th percentile) were approximately equal for the rhNGF cohort. The pattern was approximately intermediate between the RDNS and PRT cohorts. For the HP threshold, the pattern of abnormality was strikingly different than that described for the VDT and CDT (Fig. 2). Notably, in the RDNS cohort, and to a lesser degree in the PRT cohort, abnormality (i.e., an increased frequency of subjects with hypoalgesia and hyperalgesia) at both ends of the spectrum was seen. In the RDNS cohort, there were more patients who had HP:5 in the low-normal than in the high-normal range. By contrast, in the other two disease cohorts, there were more patients whose HP:5 were in the high-normal than in the low-normal range.

The association of QST hypoesthesia with NIS

As shown in Table 1, mean severity of DPN, as measured by the NIS(LL) + 4 tests, the NIS(LL), the lower-limb NIS(W), or the lower-limb NIS(S) subscores, was consistently greater in patients whose QST thresholds were at or above the 97.5th percentile than in those patients whose thresholds were between the 50th and 97.5th percentiles for VDT, CDT, and HP:5 in all three cohorts. Usually, the differences were statistically significant. With only a few exceptions, mean symptom scores were larger when QST results were at or above the 97.5th percentile than when they were between the 50th and 97.5th percentiles (Table 2). In 20 of 63 comparisons, symptoms were significantly more severe in the groups with thresholds at or above the 97.5th percentile vs. those groups with thresholds between the 50th and 97.5th percentiles. A statistically significant association was observed most frequently with the following symptoms: NSC(SF) (n = 5), NSC severity (n = 5), NSC(LF) (n = 4), NSC(S) (n = 3), NSC(W) (n = 2), and NSC(P) (n = 1). For autonomic symptoms, there were no significant associations. Of the 63 comparisons, none showed symptoms significantly greater in the high-normal group, as compared with the hypoesthetic group.

Table 2—*Average NSC scores of severity of patients in the RDNS, rhNGF, and PRT cohorts*

	VDT					CDT				
	50th to <97.5th		≥97.5th			50th to <97.5th		≥97.5th		
	n	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*
RDNS										
NSC severity	126	0.48	36	1.56	0.068	133	0.51	17	1.71	0.116
NSC(W)	126	0.02	36	0.28	0.010	133	0.07	17	0.24	0.384
NSC(S)	126	0.22	36	0.94	<0.001	133	0.32	17	0.88	0.091
NSC(A)	126	0.23	36	0.33	0.847	133	0.13	17	0.59	0.070
NSC(LF)	126	0.13	36	0.50	0.003	133	0.17	17	0.41	0.159
NSC(SF)	126	0.05	36	0.28	0.094	133	0.06	17	0.47	0.079
NSC(P)	126	0.05	36	0.17	0.098	133	0.09	17	0	0.378
rhNGF										
NSC severity	467	8.81	516	10.53	<0.001	694	9.24	319	10.56	0.001
NSC(W)	467	0.20	516	0.25	0.990	694	0.22	319	0.27	0.894
NSC(S)	467	6.64	516	8.41	<0.001	694	7.11	319	8.39	<0.001
NSC(A)	467	1.96	516	1.87	0.250	694	1.91	319	1.90	0.802
NSC(LF)	467	1.60	516	2.16	<0.001	694	1.82	319	2.01	0.210
NSC(SF)	467	1.21	516	2.00	<0.001	694	1.43	319	2.01	<0.001
NSC(P)	467	3.82	516	4.22	0.182	694	3.84	319	4.34	0.070
PRT										
NSC severity	12	1.67	45	4.09	0.083	12	0.58	44	3.82	0.042
NSC(W)	12	0.33	45	1.04	0.407	12	0.08	44	0.75	0.232
NSC(S)	12	1.00	45	2.53	0.056	12	0.25	44	2.48	0.032
NSC(A)	12	0.33	45	0.51	0.680	12	0.25	44	0.59	0.578
NSC(LF)	12	0.17	45	0.76	0.216	12	0	44	0.77	0.059
NSC(SF)	12	0	45	0.87	0.030	12	0.25	44	0.89	0.259
NSC(P)	12	0.75	45	1.27	0.186	12	0.08	44	1.30	0.115

	HP:5					HP:5–0.5				
	50th to <97.5th		≥97.5th			50th to <97.5th		≥97.5th		
	n	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*
RDNS										
NSC severity	61	0.82	16	1.81	0.6716	102	0.75	8	0.63	0.680
NSC(W)	61	0.11	16	0.31	0.5743	102	0.04	8	0	0.806
NSC(S)	61	0.26	16	1.25	0.2701	102	0.43	8	0.63	0.952
NSC(A)	61	0.44	16	0.25	0.8550	102	0.28	8	0	0.362
NSC(LF)	61	0.11	16	0.63	0.1099	102	0.25	8	0.25	0.907
NSC(SF)	61	0.13	16	0.31	0.1509	102	0.10	8	0	0.639
NSC(P)	61	0.02	16	0.31	0.0451	102	0.08	8	0.38	0.251
rhNGF										
NSC severity	379	9.12	390	10.74	<0.001	578	9.47	191	10.38	0.166
NSC(W)	379	0.16	390	0.32	0.005	578	0.24	191	0.27	0.900
NSC(S)	379	7.01	390	8.41	<0.001	578	7.31	191	8.31	0.050
NSC(A)	379	1.94	390	2.01	0.766	578	1.91	191	1.80	0.610
NSC(LF)	379	1.64	390	2.22	<0.001	578	1.86	191	1.96	0.283
NSC(SF)	379	1.30	390	2.10	<0.001	578	1.85	191	1.85	0.028
NSC(P)	379	4.05	390	4.07	0.590	578	3.84	191	4.50	0.100
PRT										
NSC severity	16	5.50	13	6.31	0.564	30	5.07	3	11.33	0.261
NSC(W)	16	1.25	13	1.08	0.844	30	0.97	3	3.33	0.145
NSC(S)	16	3.25	13	4.31	0.188	30	3.20	3	8.00	0.234
NSC(A)	16	1.00	13	0.92	0.953	30	0.90	3	0	0.482
NSC(LF)	16	1.00	13	1.15	0.446	30	1.03	3	3.00	0.135
NSC(SF)	16	0.69	13	2.15	0.003	30	1.13	3	2.67	0.249
NSC(P)	16	1.94	13	2.54	0.338	30	2.17	3	11.33	0.118

Data were attained by use of Wilcoxon's rank-sum test. The data for HP:0.5 (not shown) were similar to the data for HP:5.

Table 3—*Average composite severity of impairments and symptoms of DPN of patients in the RDNS cohort by HP thresholds*

	HP:5					HP:05					HP:5–0.5				
	≤2.5th		>2.5th–50th			≤2.5th		>2.5th–50th			≤2.5th		>2.5th–50th		
	n	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*	*n*	Mean	*n*	Mean	*P*
Impairments															
NIS(LL) + 4 tests	31	2.90	98	2.63	0.899	10	2.10	141	2.89	0.909	17	4.41	79	3.10	0.356
NIS(LL)	31	1.16	98	1.29	0.256	10	1.00	141	1.33	0.818	17	3.06	79	1.50	0.230
Lower-limb NIS(W)	31	0.29	98	0	0.012	10	0	141	0.06	0.720	17	0.76	79	0.21	0.033
Lower-limb NIS(S)	31	0.42	98	0.38	0.963	10	0.20	141	0.45	0.770	17	1.35	79	0.35	0.014
Symptoms															
NSC severity	31	0.39	98	0.47	0.568	10	1.00	141	0.48	0.287	17	1.35	79	0.41	0.122
NSC(W)	31	0.03	98	0	0.078	10	0	141	0.01	0.811	17	0.35	79	0.04	0.026
NSC(S)	31	0.29	98	0.31	0.929	10	0.50	141	0.27	0.983	17	0.65	79	0.19	0.131
NSC(A)	31	0.06	98	0.16	0.352	10	0.50	141	0.20	0.181	17	0.35	79	0.18	0.203
NSC(LF)	31	0.03	98	0.20	0.264	10	0.20	141	0.16	0.805	17	0.29	79	0.06	0.182
NSC(SF)	31	0	98	0.03	0.432	10	0	141	0.02	0.720	17	0.12	79	0.05	0.484
NSC(P)	31	0.26	98	0.07	0.391	10	0.30	141	0.09	0.304	17	0.24	79	0.08	0.028

Data were attained by use of Wilcoxon's rank-sum test.

The associations of QST hyperesthesia with NIS

These associations were tested only for the RDNS cohort because hyperesthesia was too infrequent in the rhNGF and PRT cohorts to allow meaningful testing. Because hyperesthesia was found only for HP and not for VDT or CDT, only associations of impairments and symptoms with HP were compared (Table 3). Generally, in the RDNS cohort, impairments or symptoms were not consistently larger or smaller in patients with HP:5 or HP:0.5 in patients whose thresholds were at or below the 2.5th percentile, as compared with those patients whose thresholds were between the 2.5th and 50th percentiles. All four neuropathic impairments and all seven composite symptom scores were greater in patients whose HP:5–0.5 were at or below the 2.5th percentile than in patients whose thresholds were between the 2.5th and 50th percentiles. The differences were significant for lower-limb NIS(W) (P = 0.033), for NSC(W) (P = 0.026), and for NSC(P) (P = 0.028).

CONCLUSIONS

The unique studies characterizing vibration, cooling, and HP thresholds of the foot and their association with severity of DPN, as reported here, were made possible because of the following reasons: *1*) data from three diabetic cohorts that consisted of ~1,500 patients; *2*) all patients of each cohort had severity of DPN quantitated using a comprehensive score of severity and different symptom scores; and *3*) vibration, cooling, and HP thresholds of the foot were assessed using graded, quantitated, and invariant stimuli by use of predetermined algorithms and the CASE IV. QST results were expressed as a percentile value or as a normal deviate, which allowed individual thresholds to be characterized as abnormally low (hyperesthetic, low-normal, or high-normal) or as abnormally high (hypoesthetic). Studies of this kind had not previously been possible, because large cohorts had not previously been characterized by these approaches.

Our studies have confirmed that these QST approaches are useful not only for the characterization of sensation alterations in diabetes, but also for the detection and characterization of DPN. The findings of our study are as follows: *1*) hypoesthesia is recognized by an increased frequency of patients with an unequivocal abnormality at or above the 97.5th percentile and by an increased frequency of patients with threshold values that fall between the 50th and 97.5th percentiles; *2*) among modalities of sensation, vibration threshold was most frequently abnormal when polyneuropathy was mild (as in the RDNS cohort), but all modalities tended to be abnormal when polyneuropathy was more severe (as in the rhNGF and PRT cohorts); *3*) patients with hypoesthesia of VDT or CDT have significantly more impairments and symptoms of DPN than do patients with high-normal thresholds; *4*) increased frequencies of vibration or cooling hyperesthesia were not encountered in any of the cohorts studied, but testing of cooling was not in the cold-painful range; and *5*) increased frequencies of patients with HP hyperesthesia (hyperalgesia) were observed most notably in the RDNS cohort, and, in this cohort, several neuropathic end points were significantly greater than those in patients whose thresholds fell in the low-normal range. The strong and consistent correlation between sensory loss (thresholds at or above the 97.5th percentile) and other markers of DPN and even of symptoms of polyneuropathy provides additional validation that QST hypoesthesia is an indicator of DPN (15).

The greater-than-expected frequency of patients with sensation thresholds between the 50th and 97.5th percentiles, as compared with that of patients with sensation thresholds between the 2.5th and 50th percentiles, provides evidence of an unequivocal subclinical functional abnormality. Such evidence was observed in all three cohorts. This shift in severity spectra of QST abnormality, which was not previously described, implies a subtle change in nerve function, presumably before the development of minimal criteria for the diagnosis of DPN. By assessing NCs and variations in HBDB, we found a similar subclinical shift to abnormality. At the last evaluation of the RDNS cohort, we found that peroneal motor nerve conduction velocity was more frequent in the patients between the 2.5th and 50th percentiles (~60%) than in the patients between the 50th and 97.5th percentiles (~10%). It should be noted, unlike hypoesthesia, abnormality of conduction velocity and HBDB is in the lower tail of the distribution. Likewise, 63% of HBDB responses fell into the 2.5th–50th percentile category as compared with 27% in the 50th–97.5th

percentile category. Early investigators did not categorize NC as a percentile abnormality, but they did demonstrate that the conduction velocities of diabetic patients without findings of DPN were significantly lower than those of control subjects (22). Both their demonstration and our demonstration of subtle QST, NC dysfunction, and autonomic dysfunction, before the development of overt DPN, undoubtedly have biological, disease, and treatment implications. Earlier detection of subclinical abnormality and more intensive treatment may later result in prevention of an adverse clinical outcome (23,24).

Our observation that abnormality of VDT is much more frequent than abnormality of CDT or HP:5 in the RDNS cohort, even though the frequency of these abnormalities is nearly identical in the rhNGF and PRT cohorts, requires an explanation. It may be that this phenomenon simply reflects a milder involvement of a length-dependent polyneuropathy in the RDNS cohort versus that in the other two cohorts. Thus, in the RDNS cohort, sensation abnormality would be found in the toe (the site of vibratory testing) but not on the dorsum of the foot (the site of cooling and HP testing), whereas in the rhNGF and PRT cohorts, sensation abnormality would occur at all sites. The alternative explanation is that the vibratory receptors and/or fibers are affected in mild neuropathy, whereas all sensory fibers are affected in more severe neuropathy. Regardless of the explanation, the difference in the pattern of QST abnormalities by modality provides a useful indicator of severity.

This study provides new information on hyperesthesia in DPN. First, hyperesthesia was found only for HP and not for vibration or cooling. Because we do not routinely test cooling to the cold-pain range by use of the CASE IV, we do not know whether cold-pain hyperalgesia would have been found if we had tested for it. Secondly, stimulus-induced HP hyperesthesia (hyperalgesia) was more frequent than expected, but only in the RDNS cohort. Conceptually, this hyperesthesia might be due to either improved but normal sensation (supersensitivity) or to abnormal sensation (hyperalgesia). Because patients with this HP hyperesthesia had significantly greater neuropathic impairment and symptoms, we infer that hyperesthesia is a manifestation of hyperalgesia. The best indicator of hyperalgesia was the HP:5–0.5 slope. Further, it is of interest that hyperalgesia could be demonstrated only in the RDNS cohort, the mildest spectrum of DPN. The mechanisms underlying HP hyperalgesia are not completely understood, but electrophysiological and chemical (pain peptides and cytokines) events in the periphery or in the dorsal horn of the spinal cord have been implicated (8,25,26). Irrespective of the site or mechanisms involved, it is now clear that neuropathic abnormality from QST must be inferred not only from hypoesthesia but also from hyperalgesia.

The demonstration that hyperalgesia (or, in our study, a steeper stimulus response slope) is a useful marker of injured human nerve has been emphasized by Lindblom and Ochoa (5), Verdugo and Ochao (6), and Ochoa (7). The present studies suggest that it may be worthwhile to assess for hyperalgesia also in patients with DPN, even when they do not report allodynia (pain from stimuli that are not usually painful) or hypersensitivity.

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