Does the Prevailing Hypothesis That Small-Fiber Dysfunction Precedes Large-Fiber Dysfunction Apply to Type 1 Diabetic Patients?

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OBJECTIVE

The prevailing hypothesis that early subclinical small-fiber injury precedes large-fiber damage in diabetic sensorimotor polyneuropathy (DSP) is based on lower intraepithelial nerve fiber density in type 2 prediabetic patients despite normal nerve conduction studies (NCSs). We aimed to confirm the same hypothesis in type 1 diabetic patients by examining whether: *1*) subjects without DSP include a spectrum with both normal and abnormal small-fiber measures and *2*) subjects with DSP have concurrent evidence of abnormal small-fiber measures.

RESEARCH DESIGN AND METHODS

A healthy control population (*n* = 53) was used to generate threshold values for four small-fiber tests: cooling detection thresholds (CDTs), laser Doppler imaging of heat-evoked flare (LDI_{flare}), heart rate variability (HRV), and corneal confocal microscopy. Based on NCS results, type 1 diabetic patients (*n* = 131) were dichotomized according to the presence or absence of DSP.

RESULTS

Threshold values derived from healthy control subjects were 26.5°C, 1.4 cm^2, 13%, and 12.9 mm/mm^2 for CDT, LDI_{flare}, HRV, and corneal nerve fiber length, respectively. Among type 1 diabetic patients, 57 of 131 had evidence of DSP, and 74 of 133 did not. Using abnormality of any small-fiber test to define small-fiber dysfunction, 55 of 57 (96.5%) DSP patients and 39 of 74 (52.7%) control subjects without DSP had concurrent small-fiber damage. The severity of small-fiber abnormalities worsened with an increasing number of NCS abnormalities (ANOVA, $P < 0.01$).

CONCLUSIONS

Our findings in type 1 diabetes support the prevailing hypothesis that small-fiber dysfunction occurs early in DSP. However, further research is required to determine which combination of small-fiber tests is most suitable as a surrogate marker in clinical trials.

Diabetic sensorimotor polyneuropathy (DSP) is a common complication of diabetes, affecting 28–55% of patients (1). A prospective Finnish study found evidence of probable or definite neuropathy in 8.3% of diabetic patients at the time of diagnosis, 16.7% after 5 years, and 41.9% after 10 years (2). Diabetes-related peripheral neuropathy results in serious morbidity, including chronic neuropathic pain, leg

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weakness and falls, sensory loss and foot ulceration, and amputation (3). Health care costs associated with diabetic neuropathy were estimated at $10.9 billion in the U.S. in 2003 (4). However, despite the high prevalence of diabetes and DSP, and the important public health implications, there is a lack of serum- or tissue-based biomarkers to diagnose and follow patients with DSP longitudinally. Moreover, numerous attempts at treatment have yielded negative results. However, with future therapeutic options in mind, current research has focused on the earliest identification of DSP in order to allow for intervention at the most mild stage, possibly when most amenable to treatment.

DSP is known to cause injury to both large-diameter, myelinated (Aα and Aβ) fibers and small-diameter, unmyelinated nerve (Aδ and C) fibers; however, the sequence of nerve fiber damage remains uncertain. While earlier reports seemed to indicate simultaneous loss of small- and large-diameter nerve fibers, with preserved small/large ratios (5), more recent studies have suggested the presence of early involvement of small-diameter Aδ and C fibers (6–11). Some suggest a temporal relationship of small-fiber impairment preceding that of large fibers. For example, impairment in the density of the small intraepidermal nerve fibers in symptomatic patients with impaired glucose tolerance (prediabetes) have been observed in the face of normal large-fiber function, as assessed by nerve conduction studies (NCSs) (9,10). In addition, surveys of patients with DSP have demonstrated an overwhelming predominance of sensory and autonomic symptoms, as compared with motor weakness. Again, this has been interpreted as indicative of preferential small-fiber dysfunction (12). Though longitudinal studies are limited, such studies have lead to the current prevailing hypothesis for the natural history of DSP that measures of small-fiber morphology and function decline prior to those of large fibers. One implication of this hypothesis is that small-fiber testing could serve as an earlier, subclinical primary end point in clinical trials investigating interventions for DSP (13).

The hypothesis described above has been investigated exclusively in type 2 diabetic or prediabetic patients. Through the study of a cohort of healthy volunteers and type 1 diabetic subjects (who were accrued to determine the performance characteristics of corneal confocal microscopy [CCM]), we had the opportunity to evaluate in cross-sectional analysis the relationship between measures of large-fiber function and small-fiber structure and function. Under the hypothesis that small-fiber abnormalities precede large-fiber dysfunction in the natural history of DSP, we sought to determine if: *1*) the majority of subjects who meet criteria for large-fiber dysfunction have concurrent evidence of small-fiber dysfunction and *2*) the subset of patients without DSP includes a spectrum with normal small-fiber tests (indicating lack of initiation of nerve injury) as well as abnormal small-fiber tests (indicating incipient DSP).

RESEARCH DESIGN AND METHODS

The study protocol was approved by the research ethics board of the University Health Network (Toronto, Ontario, Canada), and informed consent was obtained from all participants. Study subjects (n = 131) and control subjects (n = 53) were accrued between November 2008 and December 2012 from the Diabetes and Endocrinology Clinic and the Diabetic Neuropathy Clinic at Toronto General Hospital as part of a longitudinal cohort study funded by the Juvenile Diabetes Research Foundation (operating grant 17-2008-715). All experimental subjects were ≥18 years of age and had a confirmed diagnosis of type 1 diabetes mellitus. Patients were excluded if they had an alternative etiology for peripheral neuropathy, including genetic neuropathy, B12 deficiency, monoclonal gammopathy, uremia, active thyroid dysfunction, alcoholism, malignancy, chemotherapy, and toxic drug exposure, among others. Control subjects were ≥18 years of age and had no history of diabetes or symptoms suggestive of peripheral neuropathy. They were recruited as part of the same cohort study mentioned above and often included family members of type 1 diabetic patients. The control population consisted mainly of healthy patients (n = 53); however, there was a smaller group (n = 18) with serological evidence of prediabetes [defined as HbA_{1c} 5.7–6.4 (14)] who were excluded from the analysis. As part of the initial cohort study, each participant underwent comprehensive medical and neurologic evaluation for the assessment of neuropathy-related symptoms and comorbidities, physical examination, serological testing (HbA_{1c}), NCSs, and noninvasive small-diameter nerve fiber testing. The current study involved the extraction of demographic, clinical, laboratory, and electrophysiological data from the research database.

Large-Diameter Nerve Fiber Testing: Electrophysiologic Studies

All subjects underwent NCSs, as per our laboratory protocol. Limb temperature measurements were performed, and warming techniques were used to ensure a limb temperature of ≥32°C in the hands and ≥31°C in the feet. Studies were performed using Cadwell Sierra Wave EMG equipment (Cadwell Laboratories, Inc., Kennewick, WA). Routine investigation included unilateral peroneal motor conduction study, peroneal F-wave response, and unilateral sural sensory conduction study. All tracings were reviewed manually, and all action potentials were marked to demonstrate the onset latencies and the deviation from baseline to maximal negative peak. This allowed for determination of the peroneal nerve distal motor latency, compound motor action potential amplitude, conduction velocity, minimal F-wave latency, and the sural sensory nerve action potential (SNAP) amplitude and conduction velocity. Normative values were height- and age-dependent, as per the protocols of the Toronto General Hospital EMG laboratory. Nerve conduction testing was performed by certified technicians and overseen by neurologists, according to the standards of the Canadian Society of Clinical Neurophysiologists and the American Academy of Neuromuscular and Electrodiagnostic Medicine (14,15). Diabetes-related peripheral neuropathy was defined according to the England et al. (16) criteria, which requires the presence of neuropathy symptoms or signs, one abnormal peroneal nerve parameter (amplitude, velocity, or minimal F-wave latency), and one abnormal sural nerve parameter (amplitude or velocity). In addition, sensitivity analysis was performed using the following alternative definitions of DSP: *1*) presence of symptoms or signs and abnormal sural SNAP amplitude or conduction velocity;

2) presence of symptoms or signs and abnormal sural SNAP amplitude; and *3*) presence of symptoms or signs alone.

Small-Diameter Nerve Fiber Testing

All subjects underwent testing of small-diameter nerve fiber function using four separate techniques: cooling detection thresholds (CDTs), laser Doppler imaging of heat-evoked flare (LDI_{flare}), CCM, and heart rate variability (HRV).

CDTs were measured using the TSA-II NeuroSensory Analyzer (Medoc Advanced Medical Systems, Ramat Yishai, Israel). Testing was performed using the method of limits (17), in which the stimulus temperature is set at 32°C and progressively lowered until the patient detected a difference from the previous stimulus. Three trials were averaged and a single false trial was performed to ensure specificity.

LDI_{flare} was used to measure the axon reflex-mediated neurogenic vasodilation in response to cutaneous heating on the moorLDI2 instrument (Moor Instruments Ltd, Axminster, U.K.). A skin-heating probe comprised of a 0.64-cm^2 metallic disc was placed on the dorsum of the foot, between the first and second metatarsal heads. It was used to heat the skin to 44°C over 20 min. After probe removal, the cutaneous flare response was measured by LDI_{flare} over a surface area of 36 cm^2.

HRV was measured during deep breathing, as previously described (18), using the Dantec Keypoint Workstation (Natus Medical, San Carlos, CA). Participants were instructed to avoid alcohol, smoking, caffeinated beverages, and large meals for 2 h prior to testing. They were maintained in a resting state, in the supine position, for 30 min prior to testing. Two surface electrodes were used to record an electrocardiogram tracing for 1 min. During the recording, patients were instructed to breathe deeply at a rate of 6 breaths/min. The software generated a plot of *R*-R intervals versus time, which was used to calculate the *R*-R interval variation, as measured by the following formula: $RR_{var} = ([RR_{max} - RR_{min}]/RR_{mean}) \times 100\%$.

CCM was used to visualize the small nerve fibers of the subbasal nerve layer of the Bowman layer of the cornea. Corneal examination was performed as previously described (19) using a 0.3-mm^2 field-of-view lens on the Rostock Cornea Module of the Heidelberg Tomograph II (Heidelberg Engineering, Smithfield, RI) to produce a 0.3 × 0.3-mm image. Subsequently, 40 contiguous 0.3 × 0.3-mm images were acquired by the method of volume scanning, and the procedure was repeated twice. The technician performing the scan selected optimal images, based on image quality, and maximal density of nerve fibers. The procedure was repeated in each eye, and the mean corneal nerve fiber length (CNFL) was calculated (20).

Patient Categorization and Statistical Analysis

All study participants were categorized according to the number of NCS parameter abnormalities (0–6) and the presence or absence of DSP according to the England et al. (16) definition, as described above. Patients were also categorized as having normal or abnormal noninvasive small-fiber test results based on cutoffs established using the 5th percentile value from the healthy control population (see Table 1 footnote). Subsequently, patients were dichotomized based on the presence or absence of structural or functional small-fiber abnormalities (SFA_{bn}). Unfortunately, there is no consensus regarding which combination of small-fiber abnormalities is sufficient to diagnose small-fiber neuropathy (SFN). In addition, the individual tests assess a variety of small-caliber nerves, including the trigeminal, vagal, and spinal nerve branches (21). Therefore, we conducted a sensitivity analysis,to explore the frequency of small-fiber dysfunction based on varying definitions of SFA_{bn}. Analogous to the England et al. (16) criteria for NCS abnormalities in DSP, which allows for abnormalities of any sural or peroneal nerve conduction parameter, we used a broad definition of SFA_{bn} based on abnormality of any one or two tests. Finally, additional sensitivity analysis was performed to confirm that our results were robust, despite modifications to the definition of DSP. We considered alternative definitions of DSP including: the presence of symptoms/signs plus abnormal sural SNAP amplitude or conduction velocity; presence of symptoms/signs plus abnormal sural SNAP amplitude; and presence

Table 1—Baseline characteristics of the 53 healthy volunteers and 131 type 1 diabetic subjects

Characteristics	Healthy volunteers (*n* = 53)	Type 1 diabetic subjects (*n* = 131)	*P* value
Age (years)	35 ± 14	42 ± 16	0.007
Female sex [*n* (%)]	27 (51)	70 (53)	0.76
Diabetes duration (years)	—	23 ± 14	—
DSP [*n* (%)]	—	57 (44)	—
Weight (kg)	73 ± 17	77 ± 16	0.88
Height (m)	1.71 ± 0.09	1.72 ± 0.11	0.67
BMI (kg/m^2)	24.9 ± 5.0	25.9 ± 4.4	0.30
Systolic blood pressure (mmHg)	123 ± 14	129 ± 16	0.02
Diastolic blood pressure (mmHg)	76 ± 9	71 ± 9	0.004
HbA_{1c} (%)	5.4 ± 0.2	8.0 ± 1.7	<0.0001
(mmol/mol)	64 ± 2.2	36 ± 18.6	
TCNS	0 (0–2)	5 (2–9)	<0.0001
Large-fiber tests			
Sural nerve amplitude potential (μV)	19.0 ± 8.2	7.8 ± 5.9	<0.0001
Sural nerve conduction velocity (m/s)	51.5 ± 4.3	43.5 ± 5.2	<0.0001
Peroneal nerve amplitude potential (mV)	6.5 ± 2.2	4.4 ± 2.7	<0.0001
Peroneal nerve conduction velocity (m/s)	48.3 ± 3.2	40.4 ± 5.6	<0.0001
Peroneal nerve F-wave latency (ms)	47.2 ± 6.7	58.4 ± 11.4	<0.0001
Small-fiber tests			
CNFL (mm/mm^2)	19.2 ± 4.3	15.2 ± 4.6	<0.0001
CDT (°C)	29.6 (28.5–30.0)	26.8 (20.0–29.3)	<0.0001
LDI_{flare} area (cm^2)	3.44 ± 1.81	1.95 ± 1.17	<0.0001
HRV (%)	38 ± 21	34 ± 22	0.32

Data presented as mean ± SD, median (interquartile range), or *n* (%). Cutoff (5th percentile) values: CNFL, 12.9 mm/mm^2; CDT (average of two sides), 26.5°C; LDI_{flare}, 1.4 cm^2; and HRV, 13%.

of clinical symptoms/signs alone (Toronto Clinical Neuropathy Score [TCNS] ≥5).

All statistical analysis was performed using SAS version 9.3 (SAS Institute, Cary, NC). Baseline characteristics were compared using the *t* test (for normally distributed continuous variables), the Mann–Whitney rank-sum test for nonparametric continuous variables, or the χ^2 test for categorical variables. *P* values of <0.05 were considered to be statistically significant. ANOVA and linear regression were used to examine the relationship between the number of nerve conduction parameter abnormalities and mean small-fiber test results. We used qualitative analysis of the 2 × 2 contingency tables to explore the frequency of SFA_{bn} in patients with evidence of large-fiber dysfunction (i.e., abnormal NCS results consistent with DSP).

RESULTS

Baseline demographic data for type 1 diabetic patients and healthy control subjects (with HbA_{1c} <5.7%) are displayed in Table 1. Patients with type 1 diabetes demonstrated statistically significant differences in mean age (42 ± 16 vs. 35 ± 14 years), systolic (129 ± 16 vs. 123 ± 14 mmHg) and diastolic blood pressure (71 ± 9 vs. 76 ± 9 mmHg), HbA_{1c} (8.0 ± 1.7 vs. 5.4 ± 0.2% [64 ± 18.6 vs. 36 ± 2.2 mmol/mol]), and TCNS (5 [2–9] vs. 0 [0–2]) as compared with control subjects. In addition, the type 1 diabetic group exhibited lower mean sural and peroneal nerve amplitudes and conduction velocities and lower CNFL, CDTs, and LDI_{flare} results as compared with healthy control subjects ($P < 0.05$). There were no significant differences in sex, height, weight, BMI, or HRV. The healthy control population was used to generate threshold values for abnormality in small-fiber testing based on the 5th percentile values. Cutoffs for the presence of SFA_{bn} were: 12.9 mm/mm^2 for CNFL, 26.5°C for CDT (average of two sides), 1.4 cm^2 for LDI_{flare}, and 13% for HRV (see Table 1 footnote).

We sought to determine if a relationship exists between worsening DSP severity, as reflected by a greater number of abnormal parameters on NCS, and worsening small-fiber testing. The resulting box-and-whisker plots are displayed in Figs. 1 and 2. Analysis of variance demonstrated lack of equality among the different categories. Linear regression analysis demonstrated a weak-moderate relationship between worsening DSP and worsening mean CNFL (r^2 = 0.34), CDT (r^2 = 0.41), LDI_{flare} (r^2 = 0.27), and HRV (r^2 = 0.17), with all results reaching statistical significance ($P < 0.0001$).

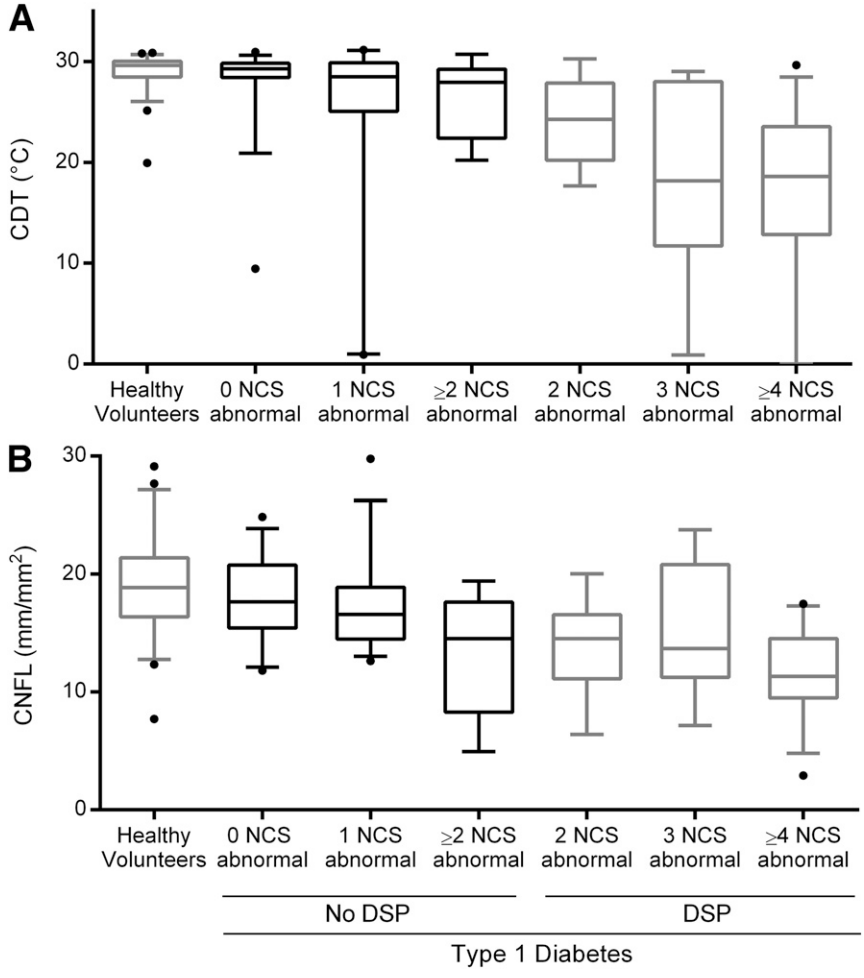

Figure 1—Mean CDTs (*A*) and CNFL (*B*) in patients with increasing number of nerve conduction parameter abnormalities. R^2 values for *A* and *B* are 0.41 and 0.34, respectively. All ANOVA *P* values are ≤0.0001. Box is median and interquartile range, whiskers are 5th–95th percentiles, and dots are outliers.

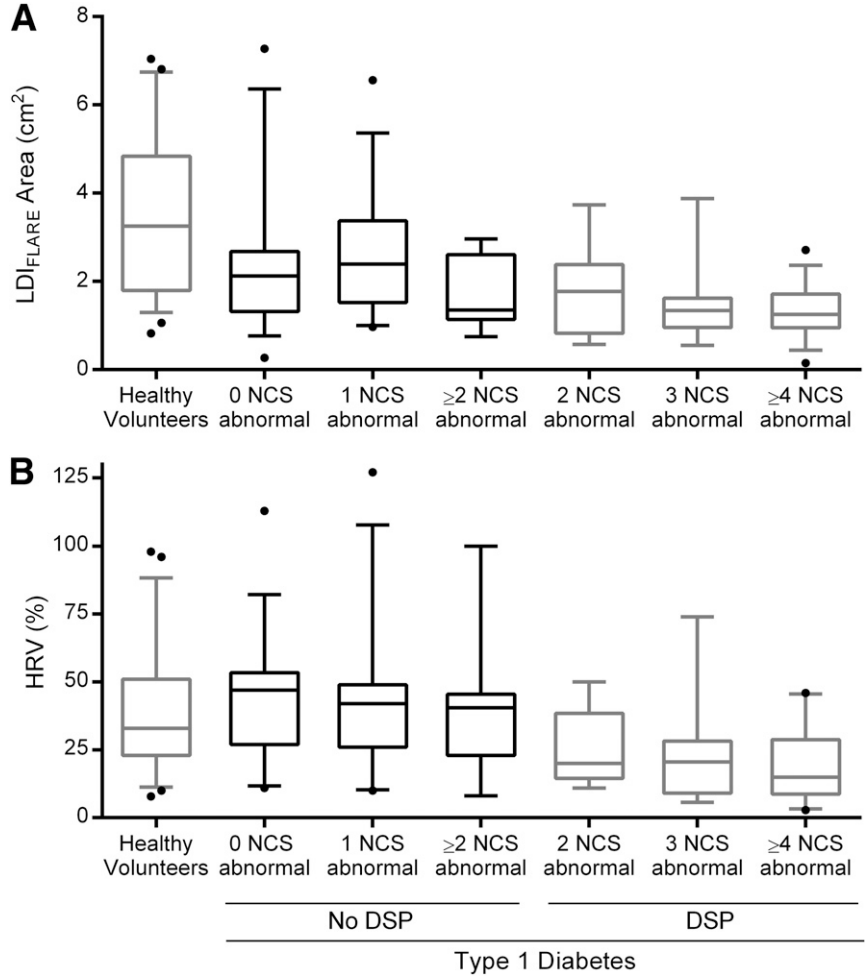

Figure 2—Mean LDI_{flare} (*A*) and HRV (*B*) in patients with increasing number of nerve conduction parameter abnormalities. R^2 values for *A* and *B* are 0.27, and 0.17, respectively. All ANOVA *P* values are ≤0.0001. Box is median and interquartile range, whiskers are 5th–95th percentiles, and dots are outliers.

Contingency-table analysis for agreement between abnormality according to large fiber function and abnormality according to small fiber measures is illustrated in Table 2. Overall, 57 of 131 (43.5%) type 1 diabetic patients met DSP criteria, and 74 of 131 (56.5%) did not meet DSP criteria. Abnormality of CCM was present in 30 of 57 (52.6%) DSP patients and 6 of 74 (8.1%) type 1 diabetic patients without DSP. Abnormality of CDT was present in 47 of 56 (83.9%) DSP patients and 17 of 73 (23.3%) without DSP. Abnormality of LDI_{flare} was present in 30 of 57 (52.6%) DSP patients and 20 of 72 (27.8%) without DSP. Abnormality of HRV was present in 18 of 45 (40.0%) DSP patients and 6 of 70 (8.6%) without DSP. Based on these results, a definition of SFA_{bn} based on individual small-fiber tests was not sufficient to illustrate the prevailing hypothesis. However, sensitivity analysis for alternative SFA_{bn} definitions revealed that abnormality of any one of the four small-fiber measures was present in 55 of 57 (96.5%) DSP patients (Table 2, top) and 39 of 74 (52.7%) type 1 diabetic patients without DSP. Similarly, abnormality of any two of the four small-fiber measures was present in 43 of 57 (75.4%) DSP patients (Table 2, middle) and 9 of 74 (12.2%) without DSP. Finally, abnormality of either CDT or CCM (with these two tests selected based on their high reliability) was noted in 53 of 57 (93.0%) DSP patients

Table 2—Illustration of 2 × 2 contingency table analysis of SFA_{bn} versus DSP for different definitions of SFA_{bn}

	SFA_{bn} (−)	SFA_{bn} (+)
SFA_{bn} = abnormality on one of four tests (CDT, CCM, LDI_{flare}, and HRV)		
DSP (−)	35	39
DSP (+)	2	55
SFA_{bn} = abnormality on two of four tests (CDT, CCM, LDI_{flare}, and HRV)		
DSP (−)	65	9
DSP (+)	14	43
SFA_{bn} = abnormality of either CCM or CDT		
DSP (−)	53	21
DSP (+)	4	53

and 21 of 74 (28.4%) patients without DSP (Table 2, bottom).

Contingency-table analysis was repeated using alterative definitions of DSP for the purposes of sensitivity analysis. When DSP was defined based on symptoms and signs plus abnormal sural SNAP amplitude *or* conduction velocity, there were 68 of 131 patients who met DSP criteria and 63 of 131 who did not. Abnormality of any one of the four small-fiber measures was present in 63 of 68 (92.6%) DSP patients and 31 of 63 (49.2%) type 1 diabetic patients without DSP. When DSP was defined based on symptoms and signs plus abnormal sural SNAP amplitude, there were 64 of 131 patients who met DSP criteria and 67 of 131 who did not. Abnormality of any one of the four small-fiber measures was present in 59 of 64 (92.2%) DSP patients and 35 of 67 (52.2%) type 1 diabetic patients without DSP. Finally, if DSP was defined based on clinical symptoms and signs alone, with TCNS $\geq$5, there were 68 of 131 patients who met DSP criteria and 63 of 131 who did not. Abnormality of any one of the four small-fiber measures was present in 62 of 68 (91.2%) DSP patients and 32 of 63 (50.8%) type 1 diabetic patients without DSP.

CONCLUSIONS

This study demonstrates a number of novel findings regarding the relationship between small- and large-caliber nerve measures in patients with type 1 diabetes mellitus. Qualitative analysis of contingency tables shows that the majority of patients with DSP have concurrent evidence of small-fiber dysfunction, and patients without DSP include a spectrum with normal small-fiber tests (indicating lack of initiation of nerve injury) as well as abnormal small-fiber tests. Evidence of isolated large-fiber injury was much less frequent, especially when definitions of SFA_{bn} based on combinations of small-fiber tests were used. These findings suggest that small-fiber damage may herald the onset of DSP in type 1 diabetes. In addition, the above findings remained true when alternative definitions of DSP were explored in a sensitivity analysis. The second important finding was the linear relationships noted between small-fiber structure and function tests (CDT, CNFL, LDI_{flare}, and HRV) (18,21–23) and the number of NCS abnormalities (a marker of large-fiber function). This might indicate that once the process of large-fiber nerve injury in DSP has begun, damage to large and small nerve fibers occurs simultaneously. Finally, our study has provided threshold values for noninvasive small-fiber tests, which are lacking at present, based on a single cohort of healthy, asymptomatic volunteers with euglycemia according to HbA_{1c} criteria.

Recent publications support the prevailing hypothesis that alterations of small-caliber nerve fibers represent the earliest effects of diabetes on the peripheral nervous system (6–8,10,11,24). This has been based on a number of observations, including: *1*) findings of altered thermal thresholds in patients with normal NCSs (7,8); *2*) reduced intraepithelial nerve fiber density (IENFD) at the ankle and IENFD ratios in diabetic patients without symptoms or signs of peripheral neuropathy and normal NCSs (8,11); and *3*) reduced IENFD in patients with impaired glucose tolerance (6,10,24). Based on these findings, some authors have recommended that small-fiber testing be used as a surrogate measure in future clinical trials (25), and, in particular, skin-punch biopsy measuring IENFD has been singled out for its high sensitivity in identifying SFN (26,27). However, this concept has been demonstrated exclusively in cohorts of type 2 diabetic patients. Our study adds to the current literature by demonstrating a similar natural history of neuropathy in a type 1 diabetic cohort, thus providing support for the prevailing hypothesis.

Our analysis has limitations. We did not perform skin-punch biopsy for measurement of IENFD, which is considered by many investigators as a reference standard for diagnosis of SFN (26,27). We instead used a panel of morphology and function tests as surrogate markers for the diagnosis of small-fiber abnormalities. Second, our study was carried out using a cross-sectional design rather than a prospective one. Third, there have not been reference-standard threshold values established for the small-fiber tests, which may result in misclassification bias. In order to circumvent this issue, we used 5th percentile values from the healthy volunteer cohort to propose cutoff values for the purpose of classification, but we have not validated the specific levels in a large, independent cohort. Fourth, there was a significant difference in the baseline age of patients and control subjects, which may be of importance given that nerve parameters vary with age. Fifth, our study does not identify which combinations of abnormal noninvasive small-fiber tests are sufficient to establish a conclusive diagnosis of SFN. Finally, we cannot generalize these findings to type 2 diabetes.

Apart from the limitations noted above, we feel that our study has made an important contribution to the literature. First, we have shown a linear association between measures of large- and small-fiber function. Second, and most importantly, our study demonstrates that the current prevailing hypothesis of early small-fiber dysfunction is generalizable to type 1 diabetic patients, which would suggest that early disease surveillance via small-fiber tests is applicable in this patient population. Early identification of neuropathy may be helpful in the future, once targeted therapies are available, to allow for intervention at the earliest possible stage of disease. In carrying out our analysis, we have not relied on published literature values for small-fiber test abnormalities, which have varied significantly from laboratory to laboratory. Instead, we derived threshold values for the evaluation of small-fiber morphology and function based on data from our own healthy volunteer group. Future research directions would include a prospective study of peripheral nerve dysfunction in diabetes, exploration of the relationship between early small-fiber abnormalities and eventual clinical phenotype (insensate versus painful neuropathy), as well as further clarification of which combination of noninvasive small-fiber tests may be the most accurate surrogate markers.

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