Corneal Confocal Microscopy Predicts 4-Year Incident Peripheral Neuropathy in Type 1 Diabetes

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OBJECTIVE

This study determined if deficits in corneal nerve fiber length (CNFL) assessed using corneal confocal microscopy (CCM) can predict future onset of diabetic peripheral neuropathy (DPN).

RESEARCH DESIGN AND METHODS

CNFL and a range of other baseline measures were compared between 90 non-neuropathic patients with type 1 diabetes who did or did not develop DPN after 4 years. The receiver operator characteristic (ROC) curve was used to determine the capability of single and combined measures of neuropathy to predict DPN.

RESULTS

DPN developed in 16 participants (18%) after 4 years. Factors predictive of 4-year incident DPN were lower CNFL ($P = 0.041$); longer duration of diabetes ($P = 0.002$); higher triglycerides ($P = 0.023$); retinopathy (higher on the Early Treatment of Diabetic Retinopathy Study scale) ($P = 0.008$); nephropathy (higher albumin-to-creatinine ratio) ($P = 0.001$); higher neuropathy disability score ($P = 0.037$); lower cold sensation ($P = 0.001$) and cold pain ($P = 0.027$) thresholds; higher warm sensation ($P = 0.008$), warm pain ($P = 0.024$), and vibration ($P = 0.003$) thresholds; impaired monofilament response ($P = 0.003$); and slower peroneal ($P = 0.013$) and sural ($P = 0.002$) nerve conduction velocity. CCM could predict the 4-year incident DPN with 63% sensitivity and 74% specificity for a CNFL threshold cutoff of 14.1 mm/mm^2 (area under ROC curve = 0.66, $P = 0.041$). Combining neuropathy measures did not improve predictive capability.

CONCLUSIONS

DPN can be predicted by various demographic, metabolic, and conventional neuropathy measures. The ability of CCM to predict DPN broadens the already impressive diagnostic capabilities of this novel ophthalmic marker.

Diabetic peripheral neuropathy (DPN) can result in pain, foot ulceration, and lower extremity amputation (1). Unmyelinated nerve fibers can now be examined at approximately original magnification ×500 using a laser scanning corneal confocal microscope (CCM) to image the subbasal nerve plexus of the human cornea in vivo (2). This approach has been validated as a viable alternative for assessing DPN (3–5). Increased severity of DPN is associated with reduced corneal nerve fiber length (CNFL) (4,5) and corneal sensitivity (6,7), assessed using CCM and noncontact corneal esthesiometry (NCCE), respectively.

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A large body of literature has accrued during the past decade highlighting the potential of CCM for assessing DPN. This technique is considered to be a simple, rapid, noninvasive, reiterative, and cost-effective approach for quantifying small-nerve fiber loss (8). CCM has been shown to be capable of detecting (4) and stratifying the severity (5) of DPN and demonstrating good diagnostic utility (85% sensitivity, 84% specificity) (4) and repeatability (9). CCM is able to track the recovery of DPN after interventions such as improving risk factors for DPN (10), simultaneous pancreas and kidney transplantation (11), and continuous subcutaneous insulin infusion (12). Nerve parameters assessed using CCM correlate significantly with structural (skin punch biopsy) (13) and functional (14) measures of small-nerve fiber injury, and abnormalities in the corneal nerve plexus have been shown to precede neurophysiological abnormalities in patients with type 1 diabetes (15). However, the capacity for CCM to predict future onset of DPN is unknown. Here we evaluated the ability of CCM, NCCE, and a range of conventional tests of DPN to predict the development of this condition over 4 years in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Patients with type 1 diabetes without DPN (age range, 14–80 years) were recruited from local hospitals and clinics in Brisbane. The following "Toronto criteria" (16) were adopted for diagnosing DPN: *a*) abnormal nerve conduction velocity relative to age-referenced normative data derived in our laboratory, and *b*) a symptom or sign of neuropathy, defined as one or more of the following: *i*) diabetic neuropathy symptom score of $\geq$1 of 4 (17), or *ii*) neuropathy disability score of $\geq$3 of 10 (18). The gold standard Toronto criteria were derived by an expert consensus group (16) to provide a clear "yes/no" decision tree for diagnosing DPN and as such cannot be used to stage the severity of the condition.

Exclusion criteria were a history of ocular trauma or surgery, ocular or systemic disease affecting the cornea, systemic disease other than diabetes, or a history of neuropathy of a nondiabetic cause. Participants were assessed at baseline and after 4 years. Ethical clearance was granted by partner hospitals, universities, and other relevant research ethics committees. Written informed consent was obtained from all participants, and the study was conducted in accordance with the principles of the Declaration of Helsinki as revised in 2000.

All tests were on the side of hand dominance, apart from the neuropathy disability score (a bilateral test; see below). Images of the central corneal subbasal nerve plexus were captured using a CCM (Heidelberg Retinal Tomograph III with Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany), after anesthetizing the cornea. CNFL was determined by analyzing eight of the clearest images using semiautomated software (19). The corneal sensation threshold was measured using a custom-made NCCE (6).

Blood pressure was measured with the patient supine using a Spot Vital Signs 4200B-E6 sphygmomanometer (Welch Allyn, Skaneateles Falls, NY). Symptoms were assessed using the diabetic neuropathy symptom score (17). Neurological deficits were evaluated by determining the neuropathy disability score (18), which involved bilateral measurement of vibration, pin prick, temperature perception, and ankle reflexes. The monofilament test (20) was performed by applying a 10-gauge nylon filament to three predetermined points on the sole of the foot. Vibration thresholds were measured using a Medoc VSA-3000 Vibratory Sensory Analyzer (Medoc Advanced Medical Systems, Ramat-Yishai, Israel), and warm and cold sensation and pain thresholds were determined using the Medoc TSA-II NeuroSensory Analyzer on the dorsolateral aspect of the foot. Peroneal and sural nerve conduction velocities were recorded by a certified neurophysiologist using a Neuropack S1 EMG/Evoked Potential Measuring System (Nihon Kohden, Tokyo, Japan).

Retinal fundus images (three-field) were captured through a dilated pupil and graded by an ophthalmologist according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale (21). Demographic, lifestyle, blood chemistry, and metabolic factors were also measured (Table 1).

The means $\pm$ SD of all parameters measured at baseline were compared between those who did and did not develop 4-year incident DPN. Because some variables were not normally distributed, comparisons between groups were made using a nonparametric test for independent samples (the Mann-Whitney *U* test). Comparisons between groups for categorical variables (sex and ethnicity) were made using the χ^2 test.

Receiver operating characteristic (ROC) curves were generated to assess the capability of seven of the neuropathy tests investigated in this work to diagnose DPN, according to gold standard Toronto criteria (16) (three neuropathy tests—nerve conduction assessment, neuropathy disability score, and diabetic neuropathy symptom score—were excluded from this analysis because these tests are used to diagnose DPN according to the Toronto criteria [16]). Specifically, we determined the area under the ROC curve for each of the neuropathy tests. For those tests that displayed a statistically significant area under the ROC curve, the diagnostic threshold (determined using the Youden index method), sensitivity, and specificity were calculated. An ROC curve was not generated for the monofilament test because this test yields limited ordinal data (4-point scale), which violates the assumption of normality required for ROC parametric analysis; the other seven tests yield continuous data, which are amenable to ROC analysis.

Because multiple sensory modalities are likely to be simultaneously impaired in a given person with neuropathy, we sought to evaluate the potential for enhancing predictive capacity by grouping the results from various combinations of neuropathy measures. This was achieved using the predicted probabilities from a multiple logistic regression (22).

RESULTS

The study recruited 101 participants with type 1 diabetes without DPN during a 12-month period. Of these, 90 were reexamined after 47 $\pm$ 3 months (range, 35–52 months), 8 withdrew from the study, and 3 were lost to follow-up (11% attrition).

Four-year incident DPN, which could not be attributed to nondiabetic causes, developed in 16 participants (18%). Table 1 compares the baseline characteristics of those who did and did not

Table 1—Baseline characteristics of patients with type 1 diabetes in whom DPN was absent or present after 4 years

Characteristic	4-year incident diabetic neuropathy		
	Absent (n = 74)	Present (n = 16)	P
Demographic and lifestyle measures			
Sex (n)			0.608*
Female	37	8	
Male	37	8	
Ethnicity† (n)			0.678*
White	71	16	
Nonwhite	3	0	
Age at baseline visit (years)	42 ± 16 (14–73)	51 ± 14 (29–77)	0.083
Diabetes duration (years)	15 ± 12 (1–43)	29 ± 16 (1–55)	0.002‡
Height (m)	171 ± 10 (150–194)	172 ± 9 (156–187)	0.635
Weight (kg)	76 ± 14 (48–112)	82 ± 19 (51–111)	0.239
BMI (kg/m^2)	26 ± 4 (19–38)	28 ± 6 (19–41)	0.378
Waist circumference (cm)	88 ± 12 (69–129)	92 ± 17 (69–123)	0.463
Smoking (cigarettes/day)	4 ± 8 (0–30)	4 ± 7 (0–20)	0.852
Alcohol (units/week)	6 ± 8 (0–60)	5 ± 5 (0–15)	0.974
Systolic blood pressure (mmHg)	118 ± 14 (90–168)	118 ± 3 (88–141)	0.937
Diastolic blood pressure (mmHg)	73 ± 7 (56–87)	68 ± 9 (48–80)	0.067
Retinopathy status (ETDRS)	16 ± 9 (10–35)	26 ± 16 (10–61)	0.008‡
Blood biochemistry and metabolic measures			
HbA_{1c} (%)	7.9 ± 1.2 (5.8–11.6)	8.0 ± 1.1 (6.2–9.7)	0.711
HbA_{1c} (mmol/mol)	63 ± 13 (40–103)	64 ± 13 (44–83)	
Albumin-to-creatinine ratio (mg/mmol)	1.1 ± 3.2 (0.1–26.0)	3.1 ± 4.5 (0.2–18.2)	0.001‡
eGFR (mL/min)	84 ± 10 (46–99)	80 ± 14 (54–90)	0.511
Total cholesterol (mmol/L)	4.7 ± 0.8 (2.9–6.8)	4.6 ± 1.2 (3.6–8.6)	0.312
HDL (mmol/L)	1.5 ± 0.4 (0.9–2.7)	1.4 ± 0.4 (0.8–2.9)	0.163
LDL (mmol/L)	2.7 ± 0.7 (1.1–4.4)	2.7 ± 1.1 (1.1–6.3)	0.258
Triglycerides (mmol/L)	0.9 ± 0.4 (0.4–2.4)	1.3 ± 0.7 (0.5–2.5)	0.023‡
Vitamin B_{12} (ng/L)	462 ± 198 (99–1,178)	550 ± 254 (175–1,261)	0.135
Neuropathy measures			
DNSS (score/4)	0.1 ± 0.4 (0–2)	0.3 ± 0.6 (0–2)	0.373
NDS (score/10)	0.5 ± 0.8 (0–4)	0.9 ± 0.9 (0–3)	0.037‡
Cold sensation threshold (°C)	28.1 ± 4.6 (9.5–31.5)	23.9 ± 6.8 (6.0–30.3)	0.001‡
Warm sensation threshold (°C)	36.8 ± 3.5 (33.0–46.4)	39.6 ± 4.1 (33.7–46.2)	0.008‡
Cold pain threshold (°C)	12.9 ± 9.8 (0.0–29.0)	7.7 ± 9.8 (0.0–25.3)	0.027‡
Warm pain threshold (°C)	46.3 ± 3.5 (36.9–50.0)	48.2 ± 2.4 (42.2–50.0)	0.024‡
Vibration threshold (Hz)	7.5 ± 9.3 (0.9–47.7)	21.5 ± 26.1 (2.2–104.4)	0.003‡
Monofilament (responses/3)	2.9 ± 0.5 (0–3)	2.7 ± 0.5 (2–3)	0.003‡
Peroneal NCV§ (m/s)	46.7 ± 6.2 (13.4–57.4)	39.6 ± 12.3 (13.4–52.8)	0.013‡
Sural NCV§ (m/s)	41.1 ± 5.3 (30.4–51.9)	35.6 ± 6.1 (30.4–49.1)	0.002‡
CNFL (mm/mm^2)	16.2 ± 3.5 (6.3–24.2)	14.0 ± 4.1 (7.4–21.0)	0.041‡
Corneal sensation threshold (mbars)	0.6 ± 0.6 (0.3–4.3)	0.5 ± 0.2 (0.3–0.8)	0.824

Data are presented as mean ± SD (range) for ordinal data; n for categorical data (sex, ethnicity). DNSS, diabetic neuropathy symptom score; eGFR, estimated glomerular filtration rate; NCV, nerve conduction velocity; NDS, neuropathy disability score. *χ^2 Test (all other paired data were analyzed using the Mann-Whitney U test). †Whites were of European descent; nonwhites were of Asian, South East Asian, Middle Eastern, or other ethnic origin. ‡Significant findings ($P < 0.05$). §No reproducible response substituted with lowest value recorded in our laboratory for peroneal and sural nerve conduction velocity (13.0 and 30.4 m/s, respectively).

develop DPN after 4 years. There was no difference between the two groups with respect to sex or ethnicity or between the clinical characteristics of the 90 participants who did and 11 participants who did not present for reexamination.

Four-year incident DPN was predicted by lower CNFL (P = 0.041) but not by corneal sensation threshold measurement (P = 0.824). Other factors predictive of 4-year incident DPN were longer duration of diabetes (P = 0.002); higher triglyceride levels (P = 0.023); retinopathy (higher ETDRS score) (P = 0.008); nephropathy (higher albumin-to-creatinine ratio) (P = 0.001); higher neuropathy disability score (P = 0.037); lower cold sensation (P = 0.001) and cold pain (P = 0.027) thresholds; higher warm sensation (P = 0.008), warm pain (P = 0.024), and vibration (P = 0.003) thresholds; impaired monofilament response (P = 0.003); and slower peroneal (P = 0.013) and sural (P = 0.002) nerve conduction velocity.

The ROC curves for 4-year incident DPN in 90 participants with type 1 diabetes are presented for seven neuropathy tests in Fig. 1. The area under the ROC curve of each of these tests is presented in Table 2. With the exception of corneal sensation threshold, the area under the curve was significantly different from the line of no discrimination for all neuropathy tests. The diagnostic capability of the six significant tests was similar, ranging from 0.66 (CNFL) to 0.77 (cold sensation threshold). CCM could predict 4-year incident DPN with 63% sensitivity and 74% specificity for a

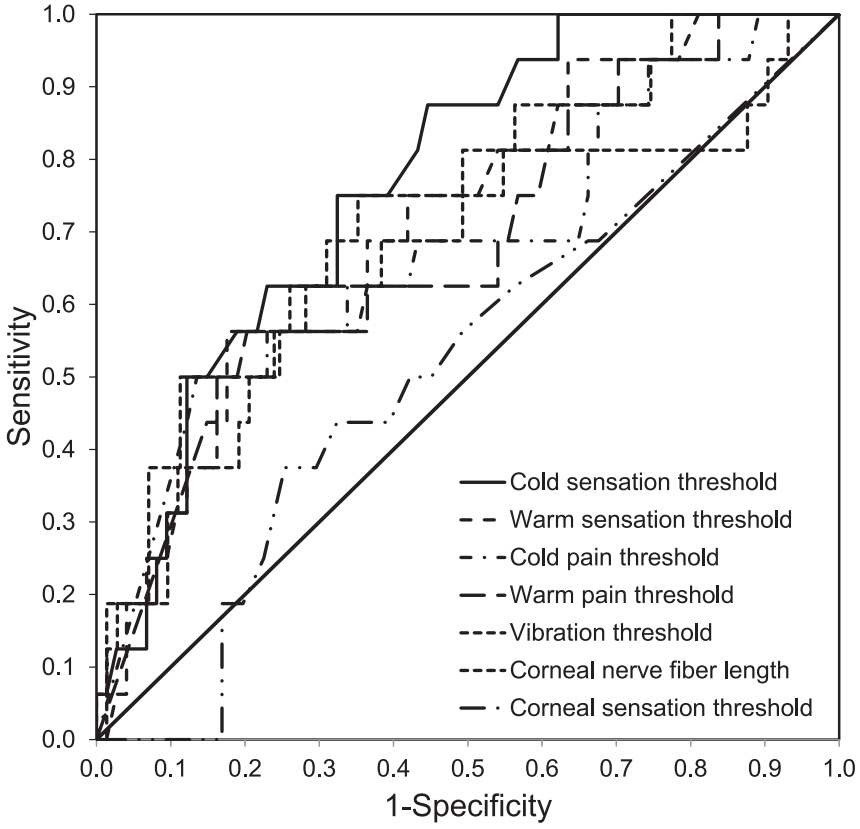

Figure 1—ROC curves for 4-year incident DPN in 90 participants with type 1 diabetes for seven neuropathy measures. The diagonal line of no discrimination is shown as $y = x$.

CNFL threshold cutoff of 14.1 mm/mm^2 (area under ROC curve = 0.66, $P = 0.041$). The test with the highest predictive capability for diagnosing DPN was cold sensation threshold, with a sensitivity of 88% and specificity of 55% for a threshold cutoff of 29.2°C.

Analysis of the diagnostic utility of five different combinations of between two and six neuropathy measures yielded values of area under the ROC curve ranging from 0.69 to 0.79 (Table 2). These findings do not represent an appreciable improvement in predictive capability compared with those achieved using single measures.

CONCLUSIONS

Our finding of a 4-year incident neuropathy of 18% among patients with type 1 diabetes is lower than the 29% reported by Perkins et al. (20). This difference may be partly because the current study used more stringent recruitment criteria that limited the numbers of high-risk subjects; indeed, BMI, blood pressure, and HbA_{1c} were lower among patients in the current study compared with Perkins et al. (20) Also, Perkins et al. (20) examined predominantly type 2 diabetic patients who were older than the type 1 diabetic patients examined in the current study.

The novel finding of this study is the demonstrated ability of CCM to predict the development of DPN with 63% sensitivity and 74% specificity, for a CNFL threshold cutoff of 14.1 mm/mm^2. This finding therefore extends the diagnostic utility of CCM for evaluating neuropathic changes in patients with type 1 diabetes. Although assessment of corneal sensitivity using NCCE has good concurrent validity for diagnosing DPN (6,7), the current study failed to demonstrate predictive validity for this technique.

Contrary to the report of Perkins et al. (20), our findings demonstrate that the presence of early signs of retinopathy (higher ETDRS score) and nephropathy (higher albumin-to-creatinine ratio) are predictive of 4-year incident neuropathy.

Table 2—ROC of single and combined neuropathy measures for the diagnosis of 4-year incident DPN in type 1 diabetes

Neuropathy measures	Area under the ROC curve	95% CI	*P**	Threshold	Sensitivity (%)	Specificity (%)
Single measures						
Cold sensation threshold (CST)	0.77	0.66, 0.88	0.001	29.2°C	88	55
Warm sensation threshold (WST)	0.71	0.58, 0.85	0.008	39.1°C	56	82
Cold pain threshold (CPT)	0.68	0.52, 0.83	0.027	0.2°C	50	86
Warm pain threshold (WPT)	0.68	0.53, 0.83	0.025	49.5°C	56	80
Vibration threshold (VT)	0.74	0.60, 0.87	0.003	6.0 Hz	75	65
CNFL	0.66	0.50, 0.83	0.041	14.1 mm/mm^2	63	74
Corneal sensation threshold	0.52	0.36, 0.67	0.827	—	—	—
Combined measures						
CST+WST	0.74	0.62, 0.87	0.002	—	63	81
CPT+WPT	0.69	0.54, 0.84	0.019	—	44	89
CST+WST+CPT+WPT	0.74	0.61, 0.88	0.002	—	75	76
CST+WST+CPT+WPT+VT	0.79	0.67, 0.90	<0.001	—	81	72
CST+WST+CPT+WPT+VT+CNFL	0.79	0.67, 0.92	<0.001	—	75	83

*Significance of difference between ROC curve and line of no discrimination.

Whereas Perkins et al. (20) found the superficial pain score was predictive of 4-year incident neuropathy, we found no such association using the diabetic neuropathy symptom score. These discrepancies may be attributed to differences in the nature of the symptom tests used and characteristics of the patient cohorts in these two studies, as described above.

The capacity for impaired vibration and monofilament sensitivity to predict the future onset of DPN as reported here is consistent with the findings of Perkins et al. (20). These are long established measures used in the routine clinical assessment of DPN and would be expected to predict neuropathy.

With the exception of corneal sensitivity and the diabetic neuropathy symptom score (both discussed above), deficits in all of the other neuropathy measures investigated here—CNFL, neuropathy disability score, cold and warm sensation and pain thresholds, vibration threshold, monofilament response, and peroneal and sural nerve conduction velocity—were demonstrated to be predictive of 4-year incident neuropathy. Although the Toronto criteria (16) remain the gold standard, clinicians may wish to use some of the alternative tests investigated here for predicting the onset of DPN; however, combining test data does not appreciably enhance predictive validity.

These findings confirm an expanded role of CCM in the assessment of DPN as a supplement to the wide array of neurological tests currently in use.

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