Corneal Confocal Microscopy Predicts the Development of Diabetic Neuropathy: A Longitudinal Diagnostic Multinational Consortium Study

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PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

Corneal nerve fiber length (CNFL) has been shown in research studies to identify diabetic peripheral neuropathy (DPN). In this longitudinal diagnostic study, we assessed the ability of CNFL to predict the development of DPN.

RESEARCH DESIGN AND METHODS

From a multinational cohort of 998 participants with type 1 and type 2 diabetes, we studied the subset of 261 participants who were free of DPN at baseline and completed at least 4 years of follow-up for incident DPN. The predictive validity of CNFL for the development of DPN was determined using time-dependent receiver operating characteristic (ROC) curves.

RESULTS

A total of 203 participants had type 1 and 58 had type 2 diabetes. Mean follow-up time was 5.8 years (interquartile range 4.2–7.0). New-onset DPN occurred in 60 participants (23%; 4.29 events per 100 person-years). Participants who developed DPN were older and had a higher prevalence of type 2 diabetes, higher BMI, and longer duration of diabetes. The baseline electrophysiology and corneal confocal microscopy parameters were in the normal range but were all significantly lower in participants who developed DPN. The time-dependent area under the ROC curve for CNFL ranged between 0.61 and 0.69 for years 1–5 and was 0.80 at year 6. The optimal diagnostic threshold for a baseline CNFL of 14.1 mm/mm^2 was associated with 67% sensitivity, 71% specificity, and a hazard ratio of 2.95 (95% CI 1.70–5.11; $P < 0.001$) for new-onset DPN.

CONCLUSIONS

CNFL showed good predictive validity for identifying patients at higher risk of developing DPN ∼6 years in the future.

Diabetic peripheral neuropathy (DPN) is the most frequent long-term complication of diabetes (1). Current diagnostic criteria require the presence of clinical signs and symptoms and abnormal nerve conduction measurements, both of which are weighted toward abnormalities of the large fibers (2). However, these diagnostic tests do not reliably detect early damage to the small nerve fibers, which may

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predate the large fiber abnormalities (3) and potentially represent an early subclinical phase of DPN. While thermal threshold and sudomotor testing and intraepidermal nerve fiber density allow an assessment of small fiber dysfunction and damage, the former are not widely available and the latter requires skin biopsy, an invasive procedure.

Corneal confocal microscopy (CCM) is a rapid, noninvasive, ophthalmic imaging tool that is comparable to skin punch biopsy in the diagnosis of DPN (4) and correlates with other established measures of small fiber neuropathy (5). In a large multinational cohort study, we established the diagnostic validity for CCM in the diagnosis of DPN (6). We previously showed that a rapid decline in corneal nerve fiber length (CNFL) was associated with the development of foot ulceration and Charcot foot, and recently we have shown that patients with diabetes with a more rapid decline in CNFL are at increased risk for the development and progression of DPN (7). Moreover, in two small cohort studies of patients with type 1 diabetes, we have previously demonstrated as a proof of concept that CNFL may have diagnostic validity to identify future incident DPN (8,9). Thus, it was proposed, but not yet confirmed, that a baseline measurement of CNFL may have diagnostic usefulness for predicting the future onset of DPN. This longitudinal, diagnostic, multinational consortium study aimed to provide robust evidence for the predictive diagnostic validity of CCM for the future onset of DPN in people with type 1 and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Design

This was a planned longitudinal analysis by an international consortium of data from five separate cohorts pooled into a single prospective study (ClinicalTrials.gov identifier: NCT02423434). The study design and baseline characteristics of the study population have been previously reported (6). The diagnostic index test was CNFL quantification using CCM, and the reference standard for DPN was based on clinical symptoms and signs and electrophysiology as per the Toronto consensus definition (2). Both the index test and the reference standard were undertaken in participants at baseline and annual follow-up visits. The staff performing the reference standard were blinded to results of the index test (and vice versa). This article follows the 2015 Standards for Reporting of Diagnostic Accuracy statement (10).

Study Population

This analysis creates a "neuropathy incidence cohort" from a subset of the original cohort established by the consortium (6). In brief, 998 people with diabetes (432 adults and 84 adolescents with type 1 diabetes and 482 adults with type 2 diabetes) completed baseline evaluations between 2008 and 2011. Participants were recruited from local diabetes, endocrinology, and neurology clinics, had type 1 or type 2 diabetes (in accordance with American Diabetes Association guidelines), and had unknown DPN status at the time of initial contact. Exclusion criteria included neuropathy due to nondiabetic causes, current eye infection or other conditions that precluded CCM, and allergy to the ocular anesthetic used during the CCM examination. The protocol and consent procedures at all sites were approved by local research ethics boards, and written informed consent was provided by all study participants or their legal guardians.

CCM Examination (Index Test)

Participants underwent examination of the subbasal nerve plexus of the cornea using the Heidelberg Tomograph Rostock Cornea Module III (Heidelberg Engineering GmbH, Heidelberg, Germany, and Heidelberg Engineering, Smithfield, RI) according to published methods. The device is a laser scanning in vivo confocal microscope that uses a visible 670-nm red wavelength diode laser source to highlight the area of the cornea being scanned for the examiner and to illuminate its structures. In brief, after application of topical anesthetic eye drops, a viscous gel medium was applied, permitting a visual gel bridge between the cornea and the sterile single-use cap on the microscope's objective lens. Subjects fixed their gaze on a target positioned behind the CCM device, and the examiner used a side view digital video camera to ensure that the apex—or the central area—of the cornea was scanned. The examiner manually focused the CCM lens on the subbasal nerve plexus adjacent to the Bowman layer of the cornea and captured the first in-focus high-contrast image. Images were taken through the subbasal layer over a depth of ~60 μm using methods that had minor procedural variation between centers (11). Six to eight images of the central subbasal nerve plexus were selected by site staff according to quality, position, and depth, and CCM parameters were determined using an automated protocol (ACCMetrics software) (12). Measured parameters were CNFL, expressed as the total length of nerves in mm/mm^2 of image area; corneal nerve branch density (CNBD), expressed as branches/mm^2; and corneal nerve fiber density (CNFD), expressed as fibers/mm^2. CCM operators were either trained in optometry or ophthalmology or were research assistants who underwent training by the microscope manufacturer. Published data have demonstrated similar cohort in vivo CCM characteristics, reproducibility, and concurrent validity, regardless of study site (4,6,11,13–19). For sensitivity analysis, we examined corneal nerve fiber area (CNFA), and manual CNFL (CNFL$_{Manual}$) at the baseline visit.

Neuropathic Symptoms, Deficits, and Electrophysiology (Reference Standard)

All participants were free of DPN at baseline, and incident DPN was defined at the first follow-up visit using the following criteria based on the Toronto consensus: the presence of one or more neuropathic symptoms and/or the presence of two or more signs of neuropathy corroborated by the presence of electrophysiological abnormality in the lower limbs (2,20). For determination of neuropathic symptoms, the Queensland site used the Diabetic Neuropathy Symptom (DNS) scoring system, the Calgary site used the Neuropathy Symptom Score (NSS) system, the Manchester site used the Neuropathy Symptom Profile (NSP), and the Toronto site used the Toronto Clinical Neuropathy Score (TCNS) symptom subscale (6). For neuropathic signs, comprehensive neurological examination was operationalized at the Toronto site using the TCNS sign subscale system; all other sites used the Neuropathy Disability Score (NDS) system (6). An algorithm was applied to the patient-level data to determine DPN status (both at baseline and during follow-

up). Additional details of the methods used to define DPN can be found in our consortium's baseline article and its supplementary materials (6).

Statistical Analysis

Between-group comparisons of clinical and DPN characteristics were made using ANOVA, the Kruskal-Wallis test, or the χ^2 test (depending on distribution). For each participant, the change in clinical and neuropathic variables over follow-up was calculated as the difference between the baseline and final follow-up observation. To account for censoring and varying length of follow-up, the predictive diagnostic validity of CCM was determined using time-dependent receiver operating characteristic (ROC) curves. Time-dependent ROC curves are constructed using methods that extend standard cross-sectional ROC curves into the longitudinal setting using survival analysis techniques. The incident cases/dynamic controls method of Heagerty and colleagues (21,22) was used to construct the time-dependent ROC curves. In this setting, the ROC curve at time *t* compares CCM parameters of incident cases with new-onset DPN at time *t* to all control subjects who remained DPN free through time *t*. The corresponding area under the ROC curve at time *t* [AUC(t)] can then be interpreted as the probability that a random incident case subject who experienced the event at time *t* had a lower CCM parameter value than a random control subject who remained event free through time *t* (assuming that both are event free up to time *t*). As an estimate of overall concordance between the index test and reference standard, Harrell C-statistic was calculated. Baseline CCM measurements were used to determine AUC(t) and the C-statistic. The crude area under the curve (AUC) using ROC curves ignoring time was also calculated for comparison with AUC(t). Optimal diagnostic threshold values were determined by finding the point on the ROC curves closest to the upper-left-hand corner of the plot.

A priori, the recruitment goal called for 70% of the baseline cohort to be followed for 4–8 years; this planned sample size would be sufficient to detect a crude AUC of 0.70 (representing good predictive validity). At study closeout, 261 participants without DPN at baseline had at least 4 years of follow-up (62% of planned sample size). The planned analysis called for stratification by diabetes type. We included two sensitivity analyses. First, as an alternative to restricting the analysis to the baseline CCM parameters only, time-updated CCM values (taken during follow-up) were used to calculate AUC(t). Second, we included a pooled type 1 and type 2 diabetes analysis. An α-level of 0.05 was used for tests of statistical significance. Time-dependent ROC curve analysis was performed using the R software environment ("meanrankROC" package) (22). All other statistical analyses were performed using SAS version 9.4.

RESULTS

A study flow diagram is presented in Supplementary Fig. 1. Of the 998 participants with a valid index test and reference standard data included in the baseline concurrent validity study, 415 had DPN at baseline while 583 did not. There were 387 of 583 (66%) participants without DPN who had at least one follow-up visit with valid reference standard data; 261 of 387 had at least 4 years of follow-up and were eligible for analysis.

Baseline characteristics of the 261 participants included in the primary analysis are shown in Table 1. There were 203 (78%) participants with type 1 diabetes and 58 (22%) with type 2 diabetes. These two groups differed in their demographic and clinical disposition, and the type 1 diabetes subcohort had lower mean age (36 ± 19 vs. 60 ± 7 years; $P < 0.001$) and higher HbA_{1c} (8.2 ± 1.5 vs. 7.3 ± 1.0%; $P < 0.001$). Although no participants met the reference standard definition for neuropathy at baseline, participants with type 2 diabetes had a higher prevalence of DPN signs and/or symptoms, lower sural nerve amplitude and conduction velocity, and lower peroneal nerve F-wave latency. Baseline CNFL was significantly lower in the type 2 diabetes subcohort compared with the type 1 diabetes subcohort (13.6 ± 3.6 vs. 15.3 ± 3.6 mm/mm^2; $P = 0.003$).

In the primary analysis set, mean ± SD follow-up time was 5.8 ± 1.6 years (median 6.0 years [interquartile range 4.2–7.0]) over a median of five visits (interquartile range 3–5). New-onset DPN was present in 60 participants (cumulative incidence rate 23%; incidence rate 4.29 events per 100 person-years). Clinical characteristics at baseline and their change over the follow-up period are shown for participants without DPN and case subjects with new-onset DPN in Table 2. Participants who developed DPN were older and had a higher BMI, higher prevalence of type 2 diabetes, and longer duration of diabetes. The baseline electrophysiology results were mainly in the normal ranges, but participants who developed DPN had significantly more impaired values compared with controls. Baseline CCM parameters were all significantly lower in participants who developed DPN. The mean values for CCM parameters were relatively stable over follow-up in both groups.

Details of the predictive diagnostic validity analysis—performed using time-dependent ROC curves—are shown in Table 3, which provides the estimates of AUC(t) at years 2, 3, 4, 5, and 6; the estimate of crude AUC; and the C-statistic for each of the index tests. We highlight the following observations: First, in the type 1 and type 2 diabetes subcohorts and pooled data set, CNFL numerically had the highest AUC(t) and crude AUC among the CCM parameters. Second, AUC(t) values for CNFL tended to be higher in type 1 diabetes compared with type 2 diabetes, and AUC(t) values were highest at year 5 or 6. Third, the overall C-statistic for CNFL was 0.63 in the type 1 and type 2 diabetes subcohorts and in the pooled data set; the 95% CI did not include the value 0.50 in the three groups, indicating moderate, but statistically significant overall predictive diagnostic validity. Fourth, as part of the sensitivity analysis, the time-varying CCM parameters had similar or lower AUC(t) and C-statistic values compared with the baseline parameters.

In the type 1 diabetes derivation set, the optimal threshold of CNFL for identifying new-onset DPN was 13.9 mm/mm^2 at 2 years and 14.1 mm/mm^2 at years 3–6. The optimal threshold for the crude ROC curve was 14.1 mm/mm^2. These values were confirmed in the type 2 diabetes validation set, with values of 14.2 mm/mm^2 at years 2–5, 14.9 mm/mm^2 at year 6, and a crude estimate of 14.1 mm/mm^2. In the pooled data set, the optimal threshold value

Table 1—Baseline characteristics of the study participants without DPN at baseline for the total cohort and the type 1 and type 2 diabetes subcohorts.

Characteristic	Total (N = 261)	Type 1 diabetes (n = 203)	Type 2 diabetes (n = 58)	P value
Clinical and demographic variables				
Age, years	41 ± 19	36 ± 19	60 ± 7	<0.001
Age <18 years, n (%)	59 (23)	59 (29)	0 (0)	—
Female sex, n (%)	128 (49)	100 (49)	28 (48)	0.89
Diabetes duration, years	15 ± 12	16 ± 12	12 ± 7	0.073
BMI, kg/m^2	26.0 ± 5.3	24.9 ± 4.7	30.3 ± 5.3	<0.001
HbA_{1c}, %	8.0 ± 1.5	8.2 ± 1.5	7.3 ± 1.0	<0.001
HbA_{1c}, mmol/mol	64 ± 16	66 ± 16	56 ± 11	<0.001
LDL cholesterol, mmol/L	2.39 ± 0.79	2.48 ± 0.80	2.06 ± 0.70	<0.001
eGFR, mL/min/1.73 m^2*	78 ± 14	78 ± 11	79 ± 20	0.73
Neuropathy measurements				
Signs and/or symptoms present, n (%)	92 (35)	50 (25)	42 (72)	<0.001
Sural nerve amplitude, μV	12.7 ± 8.7	13.5 ± 9.0	9.7 ± 6.9	0.002
Sural nerve conduction velocity, m/s	42.5 ± 7.3	42.0 ± 7.0	44.2 ± 8.0	0.017
Peroneal nerve amplitude, mV	4.9 ± 2.4	5.0 ± 2.4	4.5 ± 2.1	0.16
Peroneal nerve conduction velocity, m/s	46.0 ± 5.7	45.9 ± 5.7	46.1 ± 6.1	0.69
Peroneal nerve F-wave latency, ms	54.5 ± 10.2	55.8 ± 10.8	51.1 ± 7.4	0.007
Automated CCM measures				
CNFL, mm/mm^2†	14.9 ± 3.7	15.3 ± 3.6	13.6 ± 3.6	0.003
CNBD, branches/mm^2	26.3 ± 16.3	26.3 ± 16.4	26.6 ± 16.0	0.91
CNFD, fibers/mm^2	21.2 ± 8.0	21.2 ± 7.6	21.1 ± 9.5	0.98

Data are mean ± SD unless otherwise indicated. P values are for comparison between type 1 and type 2 diabetes subcohorts. eGFR, estimated glomerular filtration rate. *At baseline and follow-up, no participants had kidney failure (renal replacement therapy or eGFR <15 mL/min/1.73 m^2). eGFR <60 mL/min/1.73 m^2 occurred in 30 (11%) participants over the course of follow-up. This represented 12 participants with eGFR <60 mL/min/1.73 m^2 at baseline, no participants with eGFR <30 mL/min/1.73 m^2 at baseline, and 1 participant with 15 < eGFR < 30 mL/min/1.73 m^2 over the course of follow-up. †On the basis of our previous work on concurrent validity in individuals with diabetes (6), we identified a CNFL of 8.6 mm/mm^2 as abnormal. In the current study, we found that 16 of 261 (6%) individuals had a CNFL ≤8.6 mm/mm^2. The onset of large fiber damage was defined by the development of DPN and ranged from 3 to 5 years. Indeed, the group with baseline CNFL <8.6 mm/mm^2 had a higher incidence of DPN (7 of 16, 44%) and a shorter time to DPN onset than those with a CNFL >8.6 mm/mm^2 (3 vs. 5 years).

was 14.1 mm/mm^2 at all time points; values below this threshold had a hazard ratio for developing new-onset DPN of 2.95 (95% CI 1.70–5.11; $P < 0.001$) compared with those above this threshold. The Kaplan-Meier curves illustrating this hazard ratio are shown in Fig. 1. The optimal threshold value corresponded to an overall sensitivity of 67%, specificity of 71%, positive diagnostic likelihood ratio of 2.26, and negative diagnostic likelihood ratio of 0.46.

CONCLUSIONS

This large, multinational, longitudinal diagnostic study has shown that CCM has significant predictive diagnostic validity for identifying patients with type 1 and type 2 diabetes at higher risk of new-onset DPN ∼6 years in the future. Participants who developed DPN had a higher prevalence of symptoms and signs, more abnormal sural and peroneal nerve electrophysiology, and lower CNFD, CNBD, CNFL, and CNFA at baseline. Furthermore, the predictive diagnostic validity of CNFL was relatively stable over the follow-up period and was associated with a nearly threefold risk of developing new-onset DPN.

CCM has been used to identify a subclinical reduction in corneal nerve fibers with a comparable utility to quantitative sensory testing and electrophysiology in diagnosing patients with DPN (4,23,24). In a large, multinational cohort of patients with type 1 and type 2 diabetes, we also established the diagnostic validity and thresholds for CNFL in the diagnosis of DPN (6). In relation to concurrent validity, a CNFL value <8.6 mm/mm^2 was associated with a specificity of 88% and a positive likelihood ratio of ∼3.0 for DPN, while a CNFL value >15.3 mm/mm^2 was associated with a sensitivity of 88% and negative likelihood ratio of ∼0.3. Values between 8.6 and 15.3 mm/mm^2 represented future risk of DPN. Our current study of predictive validity demonstrates that values <14.1 mm/mm^2 represent the greatest risk for future-onset DPN. Though not confirmed by independent studies, these numbers arose from the largest neuropathy cohort for CCM, and they propose practical thresholds to define the presence, the absence, and the future risk for DPN for use in future clinical diagnostic research studies.

One may argue that an AUC of ∼70% represents modest performance; however, the reference standard for identifying DPN was for more advanced large fiber damage rather than early subclinical DPN associated with small fiber damage. Furthermore, the relative risk of developing DPN varies according to risk factors and ongoing treatment, which may well impact on the predictive validity of any test. In the current study the development of DPN was associated with older age, type 2 diabetes, a longer duration of diabetes, and higher BMI. Indeed, the development of DPN may be determined by multiple

Table 2—Baseline and change in clinical variables and neuropathy and CCM measures in participants who did and did not develop DPN

Characteristic	Did not develop DPN (n = 201)	Developed DPN (n = 60)	P value
Baseline clinical variables†			
Age, years	43 ± 20	59 ± 15	<0.001
Type 1 diabetes, n (%)	166 (83)	37 (62)	<0.001
Type 2 diabetes, n (%)	35 (17)	23 (38)	
Female sex, n (%)	103 (51)	25 (42)	0.19
Diabetes duration, years	19 ± 11	25 ± 14	0.001
BMI, kg/m^2	26.1 ± 4.3	28.4 ± 5.6	0.006
HbA_{1c}, %	7.9 ± 1.6	7.7 ± 1.2	0.33
HbA_{1c}, mmol/mol	63 ± 18	61 ± 13	0.33
LDL cholesterol, mmol/L	2.34 ± 0.79	2.20 ± 0.74	0.36
Change in clinical variables†			
BMI, kg/m^2	0.8 ± 2.6	−0.2 ± 2.4	0.013
HbA_{1c}, %	−0.1 ± 1.4	0.0 ± 1.0	0.50
HbA_{1c}, mmol/mol	−1 ± 15	0 ± 11	0.50
LDL cholesterol, mmol/L	−0.01 ± 0.77	−0.40 ± 0.87	0.014
Baseline neuropathy measurements			
Signs and/or symptoms present, n (%)	58 (29)	34 (57)	<0.001
Sural nerve amplitude, μV	12.0 ± 7.7	7.0 ± 6.4	<0.001
Sural nerve conduction velocity, m/s	45.0 ± 7.1	38.7 ± 6.6	<0.001
Peroneal nerve amplitude, mV	5.3 ± 2.2	3.5 ± 2.2	<0.001
Peroneal nerve conduction velocity, m/s	45.7 ± 3.9	38.6 ± 4.2	<0.001
Peroneal nerve F-wave latency, ms	52.5 ± 9.3	61.3 ± 10.4	<0.001
Change in neuropathy measurements†			
Increase in number of signs and/or symptoms, n (%)	16 (8)	26 (43)	<0.001
Sural nerve amplitude, μV	−2.0 ± 8.5	−0.8 ± 5.8	0.24
Sural nerve conduction velocity, m/s	1.5 ± 7.1	−0.2 ± 5.9	0.095
Peroneal nerve amplitude, mV	0.2 ± 2.2	−0.4 ± 2.1	0.056
Peroneal nerve conduction velocity, m/s	−1.6 ± 5.0	−2.8 ± 5.7	0.11
Peroneal nerve F-wave latency, ms	1.2 ± 11.1	3.7 ± 9.2	0.14
Baseline CCM measurements			
CNFL, mm/mm^2	15.5 ± 3.4	13.2 ± 4.0	<0.001
CNBD, branches/mm^2	27.5 ± 15.8	22.7 ± 17.6	0.049
CNFD, fibers/mm^2	22.2 ± 7.9	17.8 ± 7.6	<0.001
$CNFL_{Manual}$, mm/mm^2	19.8 ± 5.9	17.6 ± 5.6	0.012
CNFA, μm/mm^2	22,197 ± 8,132	18,558 ± 7,565	0.002
Change in CCM measurements†			
CNFL, mm/mm^2	0.0 ± 3.1	−0.3 ± 3.9	0.53
CNBD, branches/mm^2	1.8 ± 16.5	−1.6 ± 14.6	0.16
CNFD, fibers/mm^2	0.1 ± 7.7	0.0 ± 8.8	0.94

Data are mean ± SD unless otherwise indicated. †Expressed as difference from baseline to final follow-up visit (or first visit where new onset was apparent).

factors, including hyperglycemia-driven abnormalities of the polyol pathway, advanced glycation end products, and dyslipidemia (25). Furthermore, high BMI, hypertension, and cholesterol and triglyceride levels are associated with incident DPN in type 1 diabetes (26), and age, BMI, waist circumference, LDL cholesterol, and HDL cholesterol are associated with incident DPN in type 2 diabetes (27). Treatment with fibrates and statin therapy is associated with a reduced incidence of DPN (28), and increased triglycerides are associated with incident DPN (29) and amputation (30). There are also differences in risk factors for corneal nerve loss between patients with type 1 and type 2 diabetes (31–33). Thus, a longer duration of diabetes has been associated with reduced CNFD and CNBD in patients with type 1 diabetes (34), while higher LDL and total cholesterol was related to lower CNFD and CNFL in patients with type 2 diabetes (27). More recently, we have shown a significant association of reduced CNFL with age, HbA_{1c}, and weight in patients with type 2 diabetes and with duration of diabetes, LDL cholesterol, and triglycerides in patients with type 1 diabetes (35). Indeed, normalization of blood glucose following simultaneous pancreas and kidney transplantation (36), improvement in HbA_{1c} with basal bolus insulin or glucagon-like peptide 1 therapy (37), and bariatric surgery are associated with a significant improvement in corneal nerve morphology (38).

We acknowledge several limitations to our study. First, the sample size did not permit independent validation sets

Table 3—Area under the ROC curve values at selected time points and overall concordance statistic for each CCM parameter

Set and parameter	Area under the ROC curve						C-statistic (95% CI)
	Crude estimate	2 years	3 years	4 years	5 years	6 years	
Pooled							
Baseline							
CNFL	0.69	0.67	0.61	0.62	0.69	0.80	0.63 (0.54–0.72)
CNBD	0.62	0.52	0.47	0.52	0.56	0.66	0.57 (0.47–0.67)
CNFD	0.67	0.62	0.59	0.56	0.46	0.51	0.63 (0.54–0.72)
$CNFL_{Manual}$	0.61	0.49	0.50	0.49	0.52	0.59	0.56 (0.47–0.65)
CNFA	0.64	0.55	0.46	0.46	0.59	0.76	0.59 (0.49–0.68)
Time varying							
CNFL	—	0.70	0.60	0.60	0.66	0.75	0.63 (0.54–0.72)
CNBD	—	0.60	0.53	0.53	0.56	0.65	0.59 (0.504–0.68)
CNFD	—	0.62	0.57	0.58	0.43	0.46	0.61 (0.52–0.70)
Type 1 diabetes							
Baseline							
CNFL	0.67	0.67	0.54	0.56	0.91	0.85	0.63 (0.51–0.75)
CNBD	0.61	0.42	0.45	0.50	0.76	0.76	0.55 (0.42–0.67)
CNFD	0.65	0.65	0.50	0.54	0.51	0.58	0.63 (0.51–0.74)
$CNFL_{Manual}$	0.60	0.41	0.58	0.55	0.47	0.49	0.54 (0.43–0.65)
CNFA	0.63	0.57	0.43	0.44	0.61	0.73	0.56 (0.44–0.68)
Time varying							
CNFL	—	0.72	0.50	0.51	0.86	0.73	0.63 (0.52–0.74)
CNBD	—	0.61	0.49	0.49	0.79	0.72	0.59 (0.48–0.69)
CNFD	—	0.72	0.54	0.54	0.50	0.49	0.63 (0.52–0.73)
Type 2 diabetes							
Baseline							
CNFL	0.71	0.60	0.63	0.65	0.62	0.75	0.63 (0.503–0.76)
CNBD	0.69	0.66	0.57	0.61	0.56	0.62	0.62 (0.47–0.77)
CNFD	0.72	0.71	0.58	0.56	0.53	0.48	0.61 (0.45–0.76)
$CNFL_{Manual}$	0.66	0.56	0.64	0.68	0.65	0.69	0.59 (0.45–0.74)
CNFA	0.65	0.54	0.58	0.63	0.64	0.78	0.64 (0.51–0.78)
Time varying							
CNFL	—	0.55	0.59	0.61	0.61	0.75	0.63 (0.49–0.77)
CNBD	—	0.59	0.51	0.55	0.50	0.61	0.59 (0.45–0.73)
CNFD	—	0.65	0.52	0.50	0.46	0.48	0.57 (0.43–0.71)

for type 1 and type 2 diabetes as it did for our prior evaluation of concurrent validity (6). While validation in separate cohorts is important, the similar diagnostic thresholds regardless of diabetes type and overall AUC in this cohort and the baseline cohort assure us that our estimates are stable. Second, we acknowledge the possible presence of selection bias as participants most likely to volunteer for this study may have had a greater likelihood of early neuropathic symptoms despite not meeting diagnostic criteria for neuropathy and, thus, were more likely to have new-onset neuropathy at follow-up. Finally, there were small variations in the CCM image acquisition protocols, though image selection for analysis was undertaken by the same investigator (M.F.) on the basis of our established criteria (39).

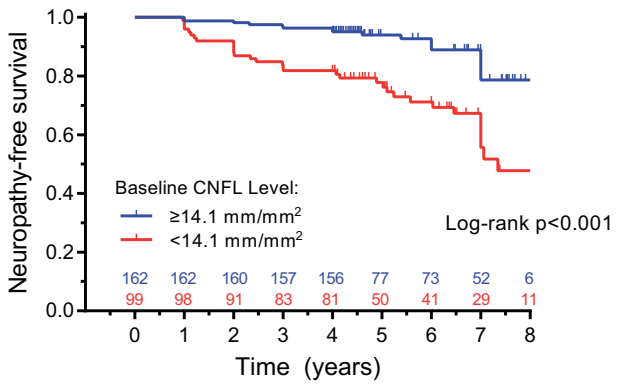

Figure 1—Kaplan-Meier curves for neuropathy-free survival according to baseline CNFL level. The blue line represents higher CNFL levels at baseline, and the red line represents lower. The vertical hash marks represent censored observations. Number at risk is given at the bottom of the graph.

In conclusion, systematic results of a neuropathy incidence cohort demonstrate that CCM represents a rapid, noninvasive, small nerve fiber imaging technique to identify patients with type 1 or type 2 diabetes at higher future risk of developing DPN over 6 years of follow-up. This study provides further support for the utility of CCM as a means to identify populations at high risk of neuropathy onset for clinical

research and in clinical practice and supports its value as a surrogate marker for nerve injury in DPN.

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Author Contributions. B.A.P., L.E.L., E.J.H.L., and R.A.M. wrote the first draft of the manuscript. B.A.P., V.B., N.E., and R.A.M. designed the study. L.E.L. carried out the data analysis, including the statistical analyses. M.F., A.O., K.E., N.P., A.R., C.D., D.P., K.R., J.K.M., M.J., A.M., R.M.S., R.P.-B., S.I.L., M.T., A.J.M.B., N.E., and R.A.M. conducted the study. All authors reviewed the manuscript for scholarly content and accuracy, and all authors read and approved the final version of the manuscript. B.A.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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