**Corneal Nerve Fractal Dimension: A Novel Corneal Nerve Metric for the Diagnosis of Diabetic Sensorimotor Polyneuropathy**

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**OBJECTIVE.** Corneal confocal microscopy (CCM), an in vivo ophthalmic imaging modality, is a noninvasive and objective imaging biomarker for identifying small nerve fiber damage. We have evaluated the diagnostic performance of previously established CCM parameters to a novel automated measure of corneal nerve complexity called the corneal nerve fiber fractal dimension (ACNFrD).

**METHODS.** A total of 176 subjects (84 controls and 92 patients with type 1 diabetes) with and without diabetic sensorimotor polyneuropathy (DSPN) underwent CCM. Fractal dimension analysis was performed on CCM images using purpose-built corneal nerve analysis software, and compared with previously established manual and automated corneal nerve fiber measurements.

**RESULTS.** Manual and automated subbasal corneal nerve fiber density (CNFD) ($P < 0.0001$), length (CNFL) ($P < 0.0001$), branch density (CNBD) ($P < 0.05$), and ACNFrD ($P < 0.0001$) were significantly reduced in patients with DSPN compared to patients without DSPN. The areas under the receiver operating characteristic curves for identifying DSPN were comparable: 0.77 for automated CNFD, 0.74 for automated CNFL, 0.69 for automated CNBD, and 0.74 for automated ACNFrD.

**CONCLUSIONS.** ACNFrD shows comparable diagnostic efficiency to identify diabetic patients with and without DSPN.

**Keywords:** corneal confocal microscopy, diabetic neuropathy, image segmentation, nerve fiber quantification
means to quantify corneal nerve damage and repair in diabetic neuropathy.\textsuperscript{15} Since then multiple studies have shown that these parameters also identify nerve fiber degeneration and repair in a range of peripheral neuropathies including amyloid neuropathy,\textsuperscript{23} Charcot-Marie-Tooth disease type 1A,\textsuperscript{24} chronic inflammatory demyelinating polyneuropathy,\textsuperscript{25,26} and human immunodeficiency virus (HIV)-induced sensory neuropathy,\textsuperscript{27} as well as central neurodegenerative conditions including motor neuron disease,\textsuperscript{28} Parkinson’s disease,\textsuperscript{29} and multiple sclerosis.\textsuperscript{30,31} However, while corneal nerve fiber density, branch density, and length can quantify nerve fiber damage and repair, they cannot differentiate specific neurodegenerative conditions. We propose a novel metric of corneal nerve morphology, the fractal dimension (FD),\textsuperscript{32} to measure the spatial loss of nerve fibers, which may help to identify specific neurodegenerative conditions and augment the diagnosis of DSPN.

**METHODS**

**Study Subjects**

This research adhered to the tenets of the Declaration of Helsinki and was approved by the North Manchester Research Ethics Committee. Age between 14 and 85 years and a history of type 1 diabetes were used as the inclusion criteria. Exclusion criteria were a positive history of malignancy, connective tissue or infectious disease, deficiency of vitamin B\textsubscript{12} or folate, liver failure, chronic renal failure, active diabetic foot ulceration, and family history of peripheral neuropathy, active ocular disease, and systemic disease known to affect the cornea other than diabetes or chronic corneal pathologies. Informed written consent was obtained from all participants. Ninety-two patients with type 1 diabetes mellitus and 84 age-matched controls were recruited for the study. All subjects were assessed for the presence and severity of DSPN based on the updated Toronto consensus criteria.\textsuperscript{33} All participants underwent assessment of body mass index, glycated hemoglobin (HbA\textsubscript{1c}), total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides, and systolic and diastolic blood pressure.

**Peripheral Neuropathy Assessment**

Neurologic deficits (Neuropathy Disability score)\textsuperscript{34} and neuropathic symptoms (Diabetic Neuropathy Symptom score)\textsuperscript{34} were evaluated. Vibration perception threshold (VPT) was tested using a neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK), and cold and warm thresholds were evaluated on the dorsolateral aspect of the left foot, using the TSA-II NeuroSensory Analyser (Medoc Ltd., Ramat-Yishai, Israel). Electrodiagnostic studies were performed using a Dantec Keypoint system (Dantec Dynamics Ltd, Bristol, UK) equipped with a Diasa temperature regulator to keep limb temperature constantly between 32°C and 35°C. Sural sensory nerve conduction velocity (SSNCV), sural sensory nerve amplitude (SSNamp), peroneal motor nerve conduction velocity (PMNCV), and peroneal motor nerve amplitude (PMNamp) were assessed by a consultant neurophysiologist.

The Toronto Diabetic Neuropathy Expert Group\textsuperscript{33} recommended a new clinical tool to identify clinically detectable DSPN: (1) abnormal nerve conduction—a PMNCV of <42 m/s; (2) a symptom or sign of neuropathy, defined as one of the following: diabetic neuropathy symptom score of 1 or more, or DSPN disability score, or DSPN symptom score, or DSPN symptom score of 1 or more.

**Manual and Automated Corneal Nerve Quantification**

CCM images were captured using the Heidelberg Retina Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany),\textsuperscript{21,36} as shown in Figure 1. Each image is 384 × 384 pixels with a pixel size of 1.0417 μm. Six images of the subbasal nerve plexus from the right and left eyes were selected for analysis using purpose-written, proprietary software. CNFD (number of main fibers per mm\textsuperscript{2}), CNFL (total length of main fibers and branches per mm\textsuperscript{2}), and CNBD (number of branches per mm\textsuperscript{2}) were measured using manual (CCMetrics; M.A. Dabbah, Imaging Science, University of Manchester) and automated (ACCMetrics) software.\textsuperscript{19} The FD measurement is fully automated and consists of a nerve fiber detection step based on a machine-learning method\textsuperscript{57} (Fig. 1). As shown in Figure 1, most of the nerve fibers can be robustly detected, even with a noisy background, and a previous study has validated the utility of this method.\textsuperscript{37} The nerve fiber FD measures the structure complexity as a ratio of the change in detail to the change in scale.\textsuperscript{32} For the implementation of FD in this study, it is calculated using a box-counting method based on the detected nerve fibers from the CCM images.\textsuperscript{38} Different-sized boxes (size of $1 \times 1, 2 \times 2, 4 \times 4, \ldots, 2^n \times 2^n$) are used to check each pixel location in the image. If any part of the detected nerve fiber is within a box, the number of boxes for this particular size box increases by 1. A set of points can be plotted based on the number of boxes against the corresponding box sizes. A line is then fitted to these points using the least square method, where the slope of the line is the FD value. Intuitively, the slope of the line is larger when a larger number of small boxes are counted, indicating a more complicated structure. A high FD value corresponds to an evenly distributed complex nerve fiber structure that likely belongs to a healthy subject. In contrast, fewer distorted nerve fibers results in a lower FD value that may reflect abnormality.

The automated corneal nerve fiber fractal dimension (ACNFrD) measurement is now included in our automated nerve fiber quantification software, which is freely available from a dedicated Web site via the University of Manchester portal.\textsuperscript{59} To assess the diagnostic ability of the standard corneal nerve metrics (CNFD, CNBD, CNFL) with ACNFrD for DSPN, we compared control subjects to diabetic subjects with and without DSPN.

**Statistical Analysis**

Statistical analysis and the receiver operating characteristic (ROC) curves were performed and generated using MATLAB (version R2012a, The MathWorks, Inc., Natick, MA, USA). One-way ANOVA (nonparametric Kruskal-Wallis) was used to evaluate within- and between-group differences (control versus no DSPN versus DSPN). A $P < 0.05$ was considered significant. Area under the ROC curve (AUC) values, 95% confidence intervals were calculated using Stata (version 14.1, StataCorp, College Station, TX) and SPSS (version 23.0, IBM, Armonk, NY).
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TABLE 1. Clinical Demographic Results and Neuropathy Assessment in Control Subjects and Type 1 Diabetic Patients Without (DSPN [−]) and With (DSPN [+] ) Neuropathy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control, n = 84</th>
<th>DSPN(−), n = 63</th>
<th>DSPN(+), n = 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46 ± 15</td>
<td>44 ± 15</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>Neuropathy disability score*</td>
<td>0.4 ± 1.2</td>
<td>1.5 ± 2.0</td>
<td>6.7 ± 2.3</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>N/A</td>
<td>20.0 ± 11.1</td>
<td>19.9 ± 11.7</td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>5.6 ± 0.3</td>
<td>8.3 ± 1.4</td>
<td>8.6 ± 1.5</td>
</tr>
<tr>
<td>HbA1c, %/mmol/mol</td>
<td>37.4 ± 5.5</td>
<td>63.9 ± 21.2</td>
<td>70.4 ± 16.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.2 ± 4.9</td>
<td>26.4 ± 4.8</td>
<td>27.0 ± 3.6</td>
</tr>
<tr>
<td>Total cholesterol, mM*</td>
<td>5.0 ± 0.9</td>
<td>4.3 ± 0.9</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>Blood pressure systolic/diastolic, mm Hg</td>
<td>126.0 ± 17.8/71.4 ± 9.7</td>
<td>130.5 ± 18.6/70.3 ± 9.1</td>
<td>145.2 ± 24.2/73.7 ± 10.0</td>
</tr>
<tr>
<td>Vibration perception threshold, V‡</td>
<td>5.6 ± 4.6</td>
<td>8.5 ± 6.5</td>
<td>28.8 ± 12.7</td>
</tr>
<tr>
<td>Warm sensation threshold, °C†</td>
<td>36.6 ± 2.8</td>
<td>38.5 ± 4.0</td>
<td>43.3 ± 4.7</td>
</tr>
<tr>
<td>Cold sensation threshold, °C‡</td>
<td>28.5 ± 1.9</td>
<td>26.8 ± 2.9</td>
<td>19.0 ± 9.8</td>
</tr>
<tr>
<td>Peroneal motor nerve conduction velocity, m/s‡</td>
<td>49.3 ± 3.3</td>
<td>43.7 ± 3.1</td>
<td>31.5 ± 8.8</td>
</tr>
<tr>
<td>Sural sensory nerve conduction velocity, m/s‡</td>
<td>51.1 ± 4.1</td>
<td>45.1 ± 4.7</td>
<td>36.0 ± 6.9</td>
</tr>
<tr>
<td>Peroneal motor nerve amplitude, mV‡</td>
<td>5.4 ± 2.1</td>
<td>5.3 ± 7.0</td>
<td>1.2 ± 1.4</td>
</tr>
<tr>
<td>Sural sensory nerve amplitude, µV‡</td>
<td>21.4 ± 9.7</td>
<td>11.8 ± 6.8</td>
<td>3.3 ± 3.2</td>
</tr>
<tr>
<td>Manual corneal nerve fiber density, no./mm²‡</td>
<td>36.17 ± 6.2</td>
<td>27.7 ± 7.9</td>
<td>17.4 ± 9.9</td>
</tr>
<tr>
<td>Manual corneal nerve branch density, no./mm²‡</td>
<td>87.1 ± 36.7</td>
<td>57.4 ± 31.3</td>
<td>45.6 ± 31.7</td>
</tr>
<tr>
<td>Manual corneal nerve fiber length, mm.mm²‡</td>
<td>25.6 ± 5.3</td>
<td>20.1 ± 5.5</td>
<td>14.6 ± 8.2</td>
</tr>
<tr>
<td>Automated corneal nerve fiber density, no./mm²‡</td>
<td>28.9 ± 6.9</td>
<td>21.9 ± 7.6</td>
<td>13.5 ± 8.7</td>
</tr>
<tr>
<td>Automated corneal nerve branch density, no./mm²‡</td>
<td>38.7 ± 17.7</td>
<td>25.9 ± 17.5</td>
<td>16.0 ± 15.5</td>
</tr>
<tr>
<td>Automated corneal nerve fiber length, mm.mm²‡</td>
<td>16.9 ± 3.4</td>
<td>13.3 ± 3.7</td>
<td>8.7 ± 4.7</td>
</tr>
<tr>
<td>Automated corneal nerve fractal dimension‡</td>
<td>1.50 ± 0.02</td>
<td>1.45 ± 0.05</td>
<td>1.40 ± 0.07</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD, statistically significant differences using ANOVA/Kruskal-Wallis. N/A, not applicable for this group.
* P < 0.05.
† P < 0.001.
‡ P < 0.0001 post hoc results for DSPN(+) significantly different from control subjects and DSPN(−).
§ Control subjects.
|| DSPN(−).

Intervals, and sensitivity/specificity both at the equal error rate point and at the threshold of 2 standard deviations below the mean of the control group were calculated and compared.40

RESULTS

Demographic, Metabolic, and Anthropometric Assessment

Demographic, anthropometric, and metabolic measurements in patients with diabetes and control subjects are summarized in Table 1. Age was comparable, but HbA1c was significantly higher in diabetic patients compared with control subjects, with no difference between patients with and without DSPN. Body mass index was significantly higher in diabetic patients with DSPN compared to controls. Total cholesterol was significantly lower in diabetic patients with and without DSPN, while HDL and triglycerides did not differ between groups. Systolic blood pressure was significantly higher in diabetic patients with and without DSPN, compared to control subjects.

Neurologic Assessment

The neuropathy disability score was significantly higher in patients with DSPN compared to control subjects.

Quantitative Sensory Testing

The VPT was significantly higher in patients with DSPN compared to patients without DSPN and control subjects. Warm sensation threshold was significantly higher while cold sensation threshold was lower in patients with and without DSPN compared to control subjects.

Electrophysiology

PMNCV, SSNC, and SSNamp were significantly reduced in diabetic patients with and without DSPN compared to controls; PMNCV, SSMCV, PMNamp, and SSNamp were significantly reduced in diabetic patients with and without DSPN compared to controls and in diabetic patients with DSPN compared to patients without DSPN.

Manual CCM

MCNFD was significantly reduced in diabetic patients with (P < 0.0001) and without (P < 0.0001) DSPN compared to control subjects and was reduced in patients with DSPN compared to patients without DSPN (P < 0.0001) (Table 1). MCNBD was significantly reduced in diabetic patients with and without neuropathy (P < 0.0001) compared to control subjects. MCNFL was significantly reduced in diabetic patients with (P < 0.0001) and without (P < 0.0001) DSPN compared to control subjects and was reduced in diabetic patients with DSPN compared to patients without DSPN (P = 0.001).

Automated CCM

Automated corneal nerve fiber density (ACNFD), automated corneal nerve fiber length (ACNFL), and ACNFD were all significantly reduced in diabetic patients with (P < 0.0001) and without (P < 0.0001) DSPN compared to control subjects and were further reduced in diabetic patients with DSPN compared...
Differences between the ROC curves comparing ACNFrD to ACNFL (P = 0.30), ACNBD (P = 0.21), and ACNFL (P = 0.50) for discriminating control from DSPN(−) (Fig. 2c). There were no statistically significant differences between the ROC curves for ACNFrD compared to ACNFL (P = 0.50), ACNBD (P = 0.40), and ACNFL (P = 0.35) for discriminating DSPN(−) from DSPN(+).

**DISCUSSION**

Objective surrogate endpoints of diabetic neuropathy that accurately detect early disease and quantify disease progression and measure therapeutic response are required. Small fiber neuropathy is implicated in a number of clinically relevant outcomes including neuropathic pain, erectile dysfunction, altered gait, and foot ulceration. Although IENFD has been proposed as a valid measure of diabetic neuropathy, the invasive nature of skin biopsy limits its practical use as a diagnostic test.

CCM is a rapid, noninvasive opthalmic imaging technique that can quantify small nerve fiber degeneration and regeneration in patients with diabetic neuropathy, and that has been related to the severity of diabetic neuropathy. Additionally, IENFD has been proposed as a valid measure of diabetic neuropathy, and has also shown that corneal nerve loss occurs in patients without diabetic retinopathy and significantly worsens in those with diabetic retinopathy. Moreover, we have also recently shown that a reduction in corneal nerve fiber length predicts worsening of diabetic retinopathy. CCM has also been deployed to assess nerve regeneration in clinical trials evaluating new therapies in sarcoid and diabetic neuropathy and after simultaneous pancreas and kidney transplantation.

This is the first study to assess the diagnostic utility of corneal nerve fractal dimension. We show that ACNFrD is comparable to CNFD, CNBD, and CNFL in diagnosing patients with and without diabetic neuropathy. However, we believe that the additional utility of this measure may arise by characterizing the structural complexity of the corneal nerves, to provide an additional means of differentiating patients with neuropathies of different etiologies including amyloid neuropathy, CMT1A, chronic inflammatory demyelinating polyneuropathy, and HIV. It may also help to identify patterns of subbasal corneal nerve loss associated with central neurodegenerative conditions, including motor neuron disease, Parkinson’s disease, multiple sclerosis, and stroke.

A limitation of the present study is the relatively small number of patients with established neuropathy. However, we do not have DSPN(+). Automated corneal nerve branch density (ACNBD) was significantly reduced in diabetic patients with (P < 0.0001) and without (P < 0.0001) DSPN compared to control subjects and was reduced in diabetic patients with DSPN compared to patients without DSPN (P = 0.002). The box plots shown in Figure 2a indicate that the fractal dimension values decreasing significantly from the control group to the DSPN(−) group (P < 0.0001) and from the DSPN(−) group to the DSPN(+) group (P < 0.0001).

**ROC Analysis**

Diabetic patients were divided into two groups: no DSPN(−) (n = 63) and DSPN(+) (n = 29). Table 2 shows the AUC values, 95% confidence intervals, and sensitivity/specificity at the equal error rate point on the ROC curve for both manual and automated CCM features. The highest AUC values among the manual and automated CCM measures were obtained for ACNFL, with AUC values of 0.79 and 0.77, respectively. The areas under the ROC curve for ACNFL were 0.76 and 0.74, respectively (Figs. 2b, 2c). There was no significant difference between the ROC curves comparing ACNFL to ACNFL (P = 0.30), ACNBD (P = 0.21), and ACNFL (P = 0.50) for discriminating control from DSPN(−) (Fig. 2c). There were no statistically significant differences between the ROC curves for ACNFL compared to ACNFL (P = 0.50), ACNBD (P = 0.40), and ACNFL (P = 0.35) for discriminating DSPN(−) from DSPN(+).

**Table 2.** AUC, 95% Confidence Interval Values, and Sensitivity/Specificity for Manual and Automated CCM for the Diagnosis of DSPN(+) From DSPN(−)

<table>
<thead>
<tr>
<th>CCM</th>
<th>AUC</th>
<th>95% Confidence Interval</th>
<th>Sensitivity/Specificity at Equal Error Rate</th>
<th>Sensitivity/Specificity at Mean ± 2 SD (Threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual corneal nerve fiber density</td>
<td>0.7890</td>
<td>[0.68 0.89]</td>
<td>0.72</td>
<td>0.79/0.71 (23.8)</td>
</tr>
<tr>
<td>Manual corneal nerve fiber length</td>
<td>0.7137</td>
<td>[0.59 0.83]</td>
<td>0.65</td>
<td>0.55/0.86 (14.9)</td>
</tr>
<tr>
<td>Manual corneal nerve branch density</td>
<td>0.6136</td>
<td>[0.49 0.74]</td>
<td>0.59</td>
<td>0.17/0.96 (15.8)</td>
</tr>
<tr>
<td>Automated corneal nerve fiber density</td>
<td>0.7720</td>
<td>[0.65 0.87]</td>
<td>0.65</td>
<td>0.65/0.79 (15.1)</td>
</tr>
<tr>
<td>Automated corneal nerve fiber length</td>
<td>0.7435</td>
<td>[0.63 0.86]</td>
<td>0.62</td>
<td>0.62/0.83 (10.2)</td>
</tr>
<tr>
<td>Automated corneal nerve branch density</td>
<td>0.6901</td>
<td>[0.56 0.80]</td>
<td>0.58</td>
<td>0.24/0.98 (5.3)</td>
</tr>
<tr>
<td>Automated corneal nerve fractal dimension</td>
<td>0.7378</td>
<td>[0.62 0.85]</td>
<td>0.65</td>
<td>0.61/0.78 (1.45)</td>
</tr>
</tbody>
</table>

This is the first study to assess the diagnostic utility of corneal nerve fractal dimension. We show that ACNFL is comparable to CNFD, CNBD, and CNFL in diagnosing patients with and without diabetic neuropathy. However, we believe that the additional utility of this measure may arise by characterizing the structural complexity of the corneal nerves, to provide an additional means of differentiating patients with neuropathies of different etiologies including amyloid neuropathy, CMT1A, chronic inflammatory demyelinating polyneuropathy, and HIV. It may also help to identify patterns of subbasal corneal nerve loss associated with central neurodegenerative conditions, including motor neuron disease, Parkinson’s disease, multiple sclerosis, and stroke.
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have introduced and evaluated the clinical utility of ACNFrD and shown that it is comparable to established CCM parameters in identifying patients with and without diabetic neuropathy. Further work is required to confirm the utility of ACNFrD in differentiating other peripheral and central neurodegenerative conditions.

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References


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