Corneal confocal microscopy compared with quantitative sensory testing and nerve conduction for diagnosing and stratifying the severity of diabetic peripheral neuropathy

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ABSTRACT

Introduction Diabetic neuropathy can be diagnosed and assessed using a number of techniques including corneal confocal microscopy (CCM).

Research design and methods We have undertaken quantitative sensory testing, nerve conduction studies and CCM in 143 patients with type 1 and type 2 diabetes without neuropathy (n=51), mild neuropathy (n=47) and moderate to severe neuropathy (n=45) and age-matched controls (n=30).

Results Vibration perception threshold (p<0.0001), warm perception threshold (WPT) (p<0.001), sural nerve conduction velocity (SNCV) (p<0.001), corneal nerve fiber density (CNFD) (p<0.0001), corneal nerve branch density (CNBD) (p<0.0001), corneal nerve fiber length (CNFL) (p=0.002), inferior whorl length (IWL) (p=0.0001) and average nerve fiber length (ANFL) (p=0.0001) showed a progressive abnormality with increasing severity of diabetic neuropathy. Receiver operating characteristic curve analysis for the diagnosis of diabetic neuropathy showed comparable performance in relation to the area under the curve (AUC) but differing sensitivities and specificities for vibration perception threshold (AUC 0.79, sensitivity 55%, specificity 90%), WPT (AUC 0.67, sensitivity 50%, specificity 76%), cold perception threshold (AUC 0.64, sensitivity 80%, specificity 47%), SNCV (AUC 0.70, sensitivity 76%, specificity 54%), CNFD (AUC 0.71, sensitivity 58%, specificity 83%), CNBD (AUC 0.70, sensitivity 69%, specificity 65%), CNFL (AUC 0.68, sensitivity 64%, specificity 67%), IWL (AUC 0.72, sensitivity 70%, specificity 65%) and ANFL (AUC 0.72, sensitivity 71%, specificity 66%).

Conclusion This study shows that CCM identifies early and progressive corneal nerve loss at the inferior whorl and central cornea and has comparable utility with quantitative sensory testing and nerve conduction in the diagnosis of diabetic neuropathy.

How might these results change the focus of research or clinical practice?

► Corneal confocal microscopy may be used to diagnose and stratify the severity of diabetic neuropathy.

To date, most studies have undertaken central corneal nerve assessment. However, recent animal and small human studies suggest that quantification of corneal nerve morphology at the inferior whorl may detect earlier nerve fiber damage.11–14 Given that DPN is a length-dependent distal axonopathy, nerves at the inferior whorl are expected to be affected before central corneal nerves.15 Indeed, we have previously shown greater corneal nerve loss at the inferior whorl compared with the patients with diabetic neuropathy.1,3 It is a rapid, non-invasive nerve imaging technique with high reproducibility and moderate to high sensitivity and specificity in the diagnosis of diabetic peripheral neuropathy (DPN).36–40

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INTRODUCTION

Corneal confocal microscopy (CCM) has been used to identify nerve fiber degeneration in
central cornea, even though central corneal nerve fiber length (CNFL) is considered to be optimal for diagnosing DPN. While Pritchard et al. reported a comparable diagnostic utility for central and inferior whorl corneal nerve length, Petropoulos et al. showed that inferior whorl length (IWL) increased the sensitivity in diagnosing DPN. More recently, Kalteniece et al. combined CNFL and IWL and showed that it was more reliable than either alone, in identifying patients with diabetic neuropathy. Corneal nerve loss in larger images incorporating nerves from the center and inferior whorl has been demonstrated in patients with recently diagnosed type 2 diabetes. Furthermore, in our recent longitudinal study, a reduction in IWL occurred more rapidly than CNFL in patients with diabetic neuropathy.

We have compared the utility of quantifying corneal nerve loss at the inferior whorl and central cornea with quantitative sensory testing and nerve conduction in the diagnosis and assessment of the severity of DPN.

RESEARCH DESIGN AND METHODS

Study participants
One hundred and forty-three patients with diabetes and 30 control participants underwent a comprehensive assessment of peripheral neuropathy and CCM. Each participant provided informed consent prior to participation in the study.

Patients were excluded if they had a history of connective tissue or infectious disease, malignancy, deficiency in B12 or folate, chronic renal and liver failure, current or active diabetic foot ulceration, previous ocular trauma, systemic disease other than diabetes that could cause neuropathy or affect the cornea, corneal surgery, and a history of or current contact lens wear.

Clinical and peripheral neuropathy assessment
Each participant underwent assessment of body mass index (BMI), blood pressure, glycated hemoglobin (HbA1c) and lipid profile. The simplified Neuropathy Disability Score (NDS), which assesses vibration, pinprick, temperature perception, and presence or absence of ankle reflexes, was used to stratify patients into three groups: no (NDS=0–2), mild (NDS=3–5) and moderate to severe (NDS=6–10) neuropathy.

Vibration perception threshold (VPT) was assessed using a neurothesiometer (Scientific Laboratory Supplies, Wilford, Nottingham, UK), and cold perception threshold (CPT) and warm perception threshold (WPT) were tested on the dorsolateral aspect of the nondominant foot (S1) using a TSA-II NeuroSensory Analyzer (Medoc, Ramat Yishai, Israel). Electrodiagnostic studies were undertaken by a consultant neurophysiologist using a Dantec ‘Keypoint’ system (Dantec Dynamics, Bristol, UK) equipped with a thermistor (DISA temperature regulator, Denmark) to keep the limb temperature at 32°C–35°C and sural nerve conduction velocity (SNCV) was tested.

Ophthalmic assessment
Examinations of the anterior ocular segment using slit-lamp biomicroscopy and CCM examination using laser scanning CCM (Heidelberg Retina Tomograph III Rostock Cornea Module; Heidelberg Engineering, Heidelberg, Germany) were performed for both eyes according to our established protocol.

Six images (three per eye) from the central sub-basal nerve plexus and four images (two per eye) from the inferior whorl region were selected and manually quantified using CCMetrics (The University of Manchester, Manchester, UK). Images were selected by a single expert in a masked fashion taking into account the quality, depth and variability following our previously established protocol. Corneal nerve fiber density (CNFD; total number of main nerves per square millimeter) (no./mm²), corneal nerve branch density (CNBD; total number of branches per square millimeter) (no./mm²), CNFL (total length of main nerves and nerve branches per square millimeter) (mm/mm²), IWL (total length of nerves per square millimeter) (mm/mm²) and average nerve fiber length (ANFL=CNFL+IWL/2) (mm/mm²) were quantified.

Statistical analysis
Analysis was carried out using SPSS V.22.0 for Windows. The Shapiro-Wilk test was employed to assess whether data were normally distributed. Fisher’s exact test was used to test the association between two categorical variables. Analysis of variance and analysis of covariance with least significant difference correction were used for multiple comparisons between groups. All data were expressed as mean±SEM. P<0.05 was considered significant. Graphs were created using GraphPad Prism (V.7.0c for Windows; GraphPad Software, La Jolla, California, USA). Receiver operating characteristic (ROC) curves were used to define the optimum cut-off points with the highest sensitivity and specificity in the diagnosis of DPN, and Youden’s index (J=sensitivity+specificity−1) was measured.

RESULTS

Clinical, demographic and laboratory findings
All participant groups were matched for gender (p=0.4) and ethnicity (p=0.3). Age (p=0.003), duration of diabetes (p=0.01), HbA1c (p<0.0001), BMI (p=0.04) and low-density lipoprotein cholesterol (p<0.0001) were significantly different between all participant groups. There was no difference in high-density lipoprotein cholesterol (p=0.4) or triglycerides (p=0.7) between groups (table 1).

Peripheral neuropathy assessment
VPT was significantly higher in patients with no diabetes (12.2±1.24, p=0.02), mild (15.05±1.2, p=0.0001) and moderate to severe (25.56±1.35, p=0.0001) neuropathy compared with controls (7.1±1.80) (table 2). CPT was significantly lower only in patients with mild (19.52±1.47,
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p=0.02) and moderate to severe (18.99±1.55, p=0.01) neuropathy compared with controls (25.38±0.96). WPT was significantly higher in patients with no (41.65±0.6, p=0.01), mild (43.47±0.6, p<0.0001) and moderate to severe (43.62±0.7, p<0.0001) neuropathy compared with controls (38.87±0.9). SNCV was significantly lower in patients with no (43.14±1.56, p=0.001), mild (43.5±1.69, p=0.002) and moderate to severe (40.19±1.79, p=0.001) neuropathy compared with controls (52.09±2.1).

**Corneal confocal microscopy**

CNFD was significantly lower in patients with no (26.61±1.05, p<0.0001), mild (24.47±1.09, p<0.0001) and moderate to severe (22.4±1.14, p<0.0001) neuropathy compared with controls (33.71±1.3) (figure 1, table 2). CNBD was significantly lower in patients with no (64.07±4.39, p=0.01), mild (58.49±4.76, p=0.002) and moderate to severe (45.60±4.50, p<0.0001) neuropathy compared with controls (81.52±5.54). CNFL was significantly lower in patients with mild (20.84±1.004, p=0.01) and moderate to severe (19.27±1.04, p=0.001) neuropathy compared with controls (25.07±1.27). IWL was significantly lower in patients with no (24.9±1.26, p=0.001), mild (22.28±1.31, p<0.0001) and moderate to severe (19.03±1.36, p<0.0001) neuropathy compared with controls (31.69±1.66). ANFL was significantly lower in patients with no (24.1±0.98, p=0.009), mild (21.56±1.02,
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p<0.0001) and moderate to severe (19.15±1.06, p<0.0001) neuropathy compared with controls (28.38±1.3).

Diagnostic utility of different measures of DPN

The ROC curves (table 3) for quantitative sensory testing, nerve conduction and CCM for the diagnosis of diabetic neuropathy (NDS >3) (figure 2) revealed comparable area under the curve (AUC) but variable sensitivities and specificities for VPT (AUC 0.79, sensitivity 55%, specificity 90%), WPT (AUC 0.67, sensitivity 50%, specificity 76%), CPT (AUC 0.64, sensitivity 80%, specificity 47%), SNCV (AUC 0.70, sensitivity 76%, specificity 54%), CNFD (AUC 0.71, sensitivity 58%, specificity 83%), CNBD (AUC 0.70, sensitivity 69%, specificity 65%), CNFL (AUC 0.68, sensitivity 64%, specificity 67%), IWL (AUC 0.72, sensitivity 70%, specificity 65%) and ANFL (AUC 0.72, sensitivity 71%, specificity 66%).

DISCUSSION

Recommendations for the diagnosis of diabetic neuropathy require the presence of symptoms and signs and abnormal nerve conduction studies or a measure of small fiber damage if nerve conduction study (NCS) is normal.24 25 Abnormal monofilament testing and VPT reflect large nerve fiber damage and predict an increased risk of diabetic foot ulceration,26 while thermal sensory thresholds and inner epidermal nerve fiber density identify early small nerve fiber dysfunction27 and degeneration,28 respectively. CCM is a rapid, non-invasive ophthalmic technique for the quantification of small nerve fiber damage in DPN.29 30 Studies have shown a relationship between central corneal nerve loss and the severity of diabetic neuropathy20 31–34 with good sensitivity and specificity for the diagnosis of DPN.19 33 In a small early study using wide-field mapping in patients

Table 3 Diagnostic performance of quantitative sensory testing, nerve conduction and CCM in the diagnosis of diabetic peripheral neuropathy

| Neuropathy measure | AUC  | P value | Sensitivity (%) | Specificity (%) | Youden's cut-off point |
|--------------------|------|---------|-----------------|-----------------|------------------------|
| VPT                | 0.79 | <0.0001 | 55              | 90              | 17.37                  |
| WPT                | 0.67 | <0.001  | 50              | 76              | 44.2                   |
| CPT                | 0.64 | <0.002  | 80              | 47              | 27.3                   |
| SNCV               | 0.70 | <0.01   | 76              | 54              | 45.9                   |
| CNFD               | 0.71 | <0.0001 | 58              | 83              | 23.95                  |
| CNBD               | 0.70 | <0.0001 | 69              | 65              | 58.54                  |
| CNFL               | 0.68 | <0.0001 | 64              | 67              | 21.6                   |
| IWL                | 0.72 | <0.0001 | 70              | 65              | 24.4                   |
| ANFL               | 0.72 | <0.0001 | 71              | 66              | 23.4                   |

ANFL, average nerve fiber length; AUC, area under the curve; CCM, corneal confocal microscopy; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CPT, cold perception threshold; IWL, inferior whorl length; SNCV, sural nerve conduction velocity; VPT, vibration perception threshold; WPT, warm perception threshold.
with diabetes with and without neuropathy, we showed greater corneal nerve loss at the inferior whorl region.\textsuperscript{35} Subsequently, in a cohort of patients with and without DPN, we demonstrated that IWL assessment enhanced the diagnostic ability of CCM\textsuperscript{14, 30} and it was more markedly reduced in patients with painful diabetic neuropathy.\textsuperscript{16, 36} More recently, in a longitudinal study in patients with DPN, we have shown more rapid corneal nerve loss at the inferior whorl compared with the central cornea.\textsuperscript{31} Furthermore, IWL rather than CNFL reduction has been associated with increased glucose variability and time above range.\textsuperscript{37}

The current study demonstrates progressive loss of corneal nerves at the central and inferior whorl region with increasing severity of DPN.\textsuperscript{4, 33} Moreover, in patients without neuropathy we show that IWL was reduced but CNFL was still preserved, consistent with an early distal axonopathy in DPN.\textsuperscript{38} We also show progressive abnormality in the VPT, CPT, WPT and SNCV with increasing severity of DPN. In particular we show an early increase in the VPT of patients without DPN, which may reflect the early involvement of the Pacinian corpuscles.\textsuperscript{39}

For the diagnosis of DPN we show comparable AUCs for quantitative sensory testing, nerve conduction and CCM. However, the sensitivity and specificity of these tests vary and may reflect the limitations of NDS in identifying early DPN. Thus VPT had a relatively low sensitivity but high specificity, while SNCV and CPT, more precise measures of large myelinated fibers, had higher sensitivity but lower specificity. However, CNFD had comparable sensitivity and specificity with VPT, while the other measures of corneal nerve fiber loss, particularly IWL and ANFL, had better sensitivity and specificity in the diagnosis of DPN. Perkins \textit{et al.}\textsuperscript{6} assessed 998 patients with diabetes and showed comparable AUC and sensitivity and specificity in the diagnosis of DPN using the Toronto criteria. Pritchard \textit{et al.}\textsuperscript{2} have reported 90% sensitivity and 50% specificity for CNFL compared with 80% specificity and 60% specificity for IWL; however, they used fully automated software to analyze CCM images, which underestimates corneal nerve parameters compared with the manual analysis used in this study.

A limitation in the interpretation of our findings is that NDS is a relatively crude and subjective measure to diagnose and stratify the severity of DPN. Indeed, this may explain why the group designated as having no neuropathy already have abnormal VPT, CPT, SNCV and corneal nerve loss. Nevertheless, this is the largest study to date to demonstrate comparable utility of a range of CCM measures compared with quantitative sensory testing and nerve conduction studies, which are established measures of DPN.

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**Contributors** MF researched data, performed the statistical analysis and wrote the manuscript. AK researched data and wrote the manuscript. SA, INP, AW, LD, SD, GP, UA and AM researched data. CGF, GL and HS reviewed and revised the manuscript. RAM designed the study and reviewed and revised the manuscript. RAM is the guarantor of this work and had full access to the data of the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors alone are responsible for the content and writing of the paper.

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**Data availability statement** Data are available upon reasonable request. The data sets generated and analyzed during the study are available from the corresponding author on a reasonable request.

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**Figure 2** ROC curves for CNFL (mm/mm\textsuperscript{2}), IWL (mm/mm\textsuperscript{2}) and ANFL (mm/mm\textsuperscript{2}). ANFL, average nerve fiber length; CNFL, corneal nerve fiber length; IWL, inferior whorl length; ROC, receiver operating characteristic.
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