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Small Nerve Fibre Quantification in the Diagnosis of Diabetic Sensorimotor Polyneuropathy: Comparing Corneal Confocal Microscopy with Intraepidermal Nerve Fibre Density

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List of Abbreviations

AUC: area under the curve

CI: confidence interval

CNBD: corneal nerve branch density

CNFD: corneal nerve fibre density

CNFL: corneal nerve fibre length

CT: cold threshold

DSPN: diabetic sensorimotor polyneuropathy

eGFR: estimated glomerular filtration rate

HbA_{1c}: glycated hemoglobin

IENFD: intra-epidermal nerve fibre density

IGT: Impaired Glucose Tolerance

CCM: corneal confocal microscopy

NCS: nerve conduction studies

NDS: neuropathy disability score

PMNamp: peroneal motor nerve amplitude

PMNCV: peroneal motor nerve conduction velocity

ROC: receiver operating characteristic curve

SD: standard deviation

SSNamp: sural sensory nerve amplitude

SSNCV: sural sensory nerve conduction velocity

TC: Toronto Criteria

VPT: vibration perception threshold

WT: warm threshold

ABSTRACT

Quantitative assessment of small fibre damage is key to the early diagnosis, and assessment of progression or regression of Diabetic Sensorimotor Polyneuropathy (DSPN). Intraepidermal nerve fibre density (IENFD) is the current gold standard for quantifying small fibre neuropathy. Corneal confocal microscopy (CCM), an *in vivo* ophthalmic imaging modality, has the potential to be an objective image biomarker and non-invasive endpoint for small fibre quantification. 89 subjects (26 controls and 63 type 1 diabetic patients) with and without DSPN underwent detailed assessment of neuropathy including CCM and skin biopsy. Manual and automated corneal nerve fibre density (CNFD), branch density (CNBD) and length (CNFL) and IENFD were significantly reduced in diabetic patients without and particularly with DSPN compared to control subjects. The AUC under the ROC curve for identifying DSPN was: 0.79 for manual CNFD, 0.77 for automated CNFD and 0.66 for IENFD, which did not differ significantly (P=0.13). The sensitivity/specificity values were: 0.79/0.71, 0.64/0.79 and 0.53/0.77 for manual CNFD, automated CNFD and IENFD, respectively. CCM and IENFD are equivalent in their diagnostic ability to detect early small fibre neuropathy in DSPN.

Introduction

Diabetic Sensorimotor Polyneuropathy (DSPN) is one of most common long- term complications of diabetes. Up to 50% of diabetic patients suffer from it, and it is estimated that about one in six diabetic patients have chronic painful neuropathy (Abbott et al., 2011). Accurate detection and assessment of neuropathy would have a major medical, social and economic impact. Furthermore, due to difficulties with endpoints employed in clinical trials of DSPN (Dyck et al., 2007) there are currently no treatments for this condition (Malik, 2014b, Boulton et al., 2013).

Methods to quantify neuropathy include clinical scores based on symptoms and neurological tests, quantitative sensory testing (QST), electrophysiological measurements, in the form of nerve conduction studies (NCS), and IENFD in skin biopsy (Dyck et al., 2013b). The neurological examination involves an assessment such as the modified Neuropathy Disability Score (NDS) (Young et al., 1993), a composite score which assesses touch, temperature and vibration perception and reflexes, which requires expert clinical judgement, a strong element of subjectivity and hence poor reproducibility (Dyck et al., 2010). Neurophysiology is objective and reproducible and is currently considered to be the most reliable measurement for confirming the diagnosis of diabetic neuropathy and indeed represents an essential part of the Toronto Criteria (TC) to identify those with "Confirmed DSPN: the presence of an abnormality of NCS and a symptom or symptoms or a sign or signs of neuropathy" (Tesfaye et al., 2010). However, these measures mainly assess large nerve fibres, making them less sensitive to early signs of DSPN, which is more likely to involve small fibres (Malik, 2014a, Breiner et al., 2014). Small fibres can be assessed by quantifying thermal thresholds (Dyck et al., 2014) and Intra-Epidermal Nerve Fibre Density (IENFD) in skin biopsies (Lauria and Lombardi, 2012). Whilst QST assessment has been shown to have good repeatability (Dyck et al., 2014), IENFD is considered to be the most objective and quantitative for the diagnosis of small fibre neuropathy

(Hoeijmakers et al., 2012, Polydefkis et al., 2004). However, its invasive nature makes it unsuitable for repeated investigations (Lauria and Lombardi, 2012). Furthermore it has never been thoroughly validated in terms of its reliability for the diagnosis of DSPN in a large cohort of diabetic patients (Malik et al., 2011). Thus diabetic neuropathy currently lacks a non-invasive surrogate for accurately detecting small nerve fibre damage and repair. Several studies (Malik et al., 2003, Quattrini et al., 2007, Pritchard et al., 2014, Asghar et al., 2014, Tavakoli et al., 2013a) have shown that CCM is capable of making a quantitative assessment of small fibre damage and has the potential to be an ideal surrogate endpoint for DSPN (Malik, 2014a). Quantitative analysis using manual annotation of CCM images to identify fibres and branches is labour-intensive and subjective. However, a recently developed fully automated nerve fibre quantification method has been shown to have high correlation with the manually obtained measurements (Dabbah et al., 2010, Dabbah et al., 2011) and our recent study (Petropoulos et al., 2014) has compared manual and automated image analysis in a large cohort of diabetic patients. We have previously assessed both CCM and IENFD in the same patients and shown that the measures were related (Quattrini et al., 2007). However, to date there has been no attempt to directly compare the ability of CCM and IENFD in the diagnosis of DSPN.

In this paper, we comprehensively evaluate both manually and automatically quantified CCM-derived measures of nerve fibre morphology and compare them with IENFD measurements according to the presence or absence of DSPN using the Toronto criteria.

Methods

Study Subjects

63 patients with type 1 Diabetes Mellitus and 26 controls were assessed for the presence and severity of DSPN between 2010 and 2011 based on the updated Toronto consensus criteria (Tesfaye et al., 2010). Informed written consent was obtained from all participants prior to their enrolment to the study. This research adhered to the tenets of the declaration of Helsinki and

was approved by the North Manchester Research Ethics Committee. Participants were excluded if they had a positive history of malignancy, connective tissue or infectious disease, deficiency of vitamin B_{12} or folate, chronic renal failure, liver failure, active diabetic foot ulceration and family history of peripheral neuropathy. Participants were also excluded if they had active ocular disease, systemic disease known to affect the cornea other than diabetes or chronic corneal pathologies. All participants underwent assessment of glycated haemoglobin (HbA_{1c}), high (HDL) and low (LDL) density lipoprotein cholesterol, triglycerides, body mass index (BMI) and renal status [estimated glomerular filtration rate (eGFR) and albumin to creatinine ratio (ACR)].

Peripheral Neuropathy Assessment

All study participants underwent an assessment of neurological deficits (Neuropathy Disability score (NDS)) (Young et al., 1993) and symptoms (Diabetic Neuropathy Symptom (DNS) score) (Meijer et al., 2002). Vibration perception threshold (VPT) was tested using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilfrod, Nottingham, UK). Cold (CT) and warm (WT) thresholds were established on the dorsolateral aspect of the left foot (S1) using the TSA-II NeuroSensory Analyser (Medoc Ltd., Ramat-Yishai, Israel). Electro-diagnostic studies were undertaken using a Dantec "Keypoint" system (Dantec Dynamics Ltd, Bristol, UK) equipped with a DISA temperature regulator to keep limb temperature constantly between 32-35°C. Sural sensory nerve amplitude (SSNamp), sural sensory nerve conduction velocity (SSNCV), peroneal motor nerve amplitude (PMNamp) and peroneal motor nerve conduction velocity (PMNCV) were assessed by a consultant neurophysiologist.

The Toronto Diabetic Neuropathy Expert Group (Tesfaye et al., 2010) recommendation was followed to define an individual to have neuropathy if he/she met both of the following criteria: (1) Abnormal nerve conduction – A PMNCV of <42 m/s; (2) a symptom or sign of neuropathy,

defined as ONE of the following: (a) DNS of 1 or more out of 4 (b) NDS of 3 or more out of 10.

For IENFD assessment a 3-mm punch skin biopsy was obtained from the dorsum of the foot and a bright-field immunohistochemistry protocol was used according to published guidelines (Lauria and Lombardi, 2012). Linear IENFD (number of fibres/millimetre) was established in at least four sections of 50μm thickness according to published counting rules (IENFD have to cross or originate at the dermal–epidermal junction, and secondary branches and fragments are not counted) (Polydefkis et al., 2004). The assessments were performed by two experts (MJ & RM) and cross-validated.

Manual and Automated quantification of corneal nerves

CCM images (Fig. 1) were captured from all participants using the Heidelberg Retina Tomograph Rostock Cornea Module (HRT-III) as described in (Petropoulos et al., 2014, Petropoulos et al., 2013). Their dimensions are 384×384 pixels with the pixel size of 1.0417μm. During a bilateral CCM scan over 100 images per patient were typically captured from all corneal layers and six sub-basal images from the right and left eyes were selected for analysis. Criteria for image selection were depth, focus position and contrast. A single experienced examiner, masked from the outcome of the medical and peripheral neuropathy assessment, quantified 1506 images of all study participants using purpose-written, proprietary software (CCMetrics®, M. A. Dabbah, Imaging Science, University of Manchester). The specific parameters measured per frame were: Corneal Nerve Fibre Density (CNFD: number of main fibres per mm²), Corneal Nerve Fibre Length (CNFL: total length of main fibres and branches per mm²) and Corneal Nerve Branch Density (CNBD: number of branches per mm²) in accordance with our previously published protocol (Petropoulos et al., 2014, Petropoulos et al., 2013). The main nerve fibres and branches are indicated in Fig. 1.

Automated corneal nerve fibre quantification consists of two steps: (1) CCM image enhancement and nerve fibre detection and (2) quantification of the three morphometric parameters. As described in our earlier work (Dabbah et al., 2011), a dual-model feature descriptor combined with a neural network classifier was used to train the detection software to distinguish nerve fibres from the background (noise and underlying connective tissue). In the nerve fibre quantification process, all the end points and branch points of the detected nerve fibres are extracted and used to construct a connectivity map. Each segment in the connectivity map was then connected and classified as a main nerve fibre or branch (Fig. 2c).

To evaluate the effectiveness of using IENFD and manually and automatically generated CCM features to diagnose DSPN, we used the TC as ground truth to categorise the diabetic subjects into those with and without DSPN. Receiver Operating Characteristic (ROC) curves were generated by varying the decision thresholds.

RESULTS

Demographics, Metabolic and Anthropometric Assessment (Table 1).

The participant demographics and metabolic and anthropometric measurements in diabetic patients and control subjects are summarized in Table 1. The age was comparable between controls and diabetic patients with and without DSPN (Controls: 44±15, diabetic patients: 51±12). HbA1c ($P<0.0001$) was significantly higher in diabetic patients compared with control subjects with no difference between patients with and without DSPN. BMI was significantly higher in diabetic patients with DSPN compared to controls. Total cholesterol was significantly lower in diabetic patients without ($P=0.0025$) and with ($P<0.0001$) DSPN compared to control subjects. 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

and was also significantly higher in diabetic patients with DSPN compared to patients without DSPN, whilst diastolic blood pressure was comparable between groups.

NDS (Table 1)

The NDS was significantly greater in patients with DSPN compared to control subjects (P<0.0001) and diabetic patients without DSPN (P<0.0001), with no significant difference between controls and patients without DSPN.

Vibration Perception and Thermal Thresholds (Table 1)

VPT was significantly greater in patients with DSPN compared to patients without DSPN (P<0.0001) and control subjects (P<0.0001). CST was significantly lower in DSPN compared to patients without DSPN (P<0.0001) and control subjects (P<0.0001). WST was significantly greater in patients with DSPN compared to patients without DSPN (P<0.0001) and controls (P<0.0001).

Electrophysiology (Table 1)

Peroneal motor nerve conduction velocity was significantly lower in DSPN compared to controls (P<0.0001) and diabetic patients without DSPN (P<0.0001) and in patients without DSPN and controls (P<0.0001). Peroneal nerve amplitude was significantly lower in DSPN compared to patients without DSPN (P<0.0001) and controls (P<0.0001) and in patients without DSPN and controls (P=0.0041). Sural nerve conduction velocity and amplitude were significantly lower in DSPN (P<0.0001and P<0.0001respectively) compared with control subjects and diabetic patients without DSPN (P<0.0001 and P<0.0001 respectively). Sural nerve conduction velocity was also lower in diabetic patients without DSPN compared to controls (P<0.0001). This result in itself is unsurprising as we have used PMNCV as part of our definition of DSPN.

IENFD (Table 1)

IENFD was significantly reduced in diabetic patients with (P=0.002) and without (P=0.001) DSPN and was further reduced in those with DSPN compared to patients without DSPN (P=0.05) (Fig. 3, Fig. 4a). The median value of the control group is 9.35 and the 0.05 quantile is 4.31, which is consistent with previously published IENFD measurements (Lauria et al., 2010).

CCM (Table 1)

Manual CNFD was significantly reduced in diabetic patients with (P<0.0001) and without (P<0.0001) DSPN compared to control subjects and was further reduced in diabetic patients with DSPN compared to patients without DSPN (P<0.0001) (Fig. 3b). Manual CNBD was significantly reduced in diabetic patients with (P<0.0001) but not without (P=0.09) DSPN compared to control subjects. Manual CNFL was significantly reduced in diabetic patients with (P<0.0001) and without (P<0.0001) DSPN compared to control subjects and was further reduced in diabetic patients with DSPN compared to patients without DSPN (P=0.001).

Automated CNFD was significantly reduced in diabetic patients with (P<0.0001) and without (P<0.0001) DSPN compared to control subjects and was further reduced in diabetic patients with DSPN compared to patients without DSPN (P<0.0001) (Fig. 4c). Automated CNBD was significantly reduced in diabetic patients with (P<0.0001) and without (P<0.0001) DSPN compared to control subjects and was further reduced in diabetic patients with DSPN compared to patients without DSPN (P=0.002). Automated CNFL was significantly reduced in diabetic patients with (P<0.0001) and without (P<0.0001) DSPN compared to control subjects and was further reduced in diabetic patients with DSPN compared to patients without DSPN (P<0.0001) (Fig. 4d).

ROC analysis (Table 2)

The diabetic patients were categorised into those without (n=46) and with (n=17) DSPN. Table 2 shows the Area Under the ROC Curve (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, individually and in combination, as well as IENFD values. The highest AUC values among the manual and automated CCM measures were obtained for corneal nerve fibre density (CNFD) with AUC values of 0.79 and 0.77 respectively. Almost all individual CCM measurements resulted in higher AUC values than IENFD (0.66). Combining the three CCM features resulted in AUC values for both manual and automated measurement approximately equal to the best individual measurement. Sensitivity and specificity values are calculated at the equal error-rate point for purpose of consistency. For this measure of diagnostic performance also, CNFD provides the best discrimination (72% for manual measurement and 67% for automated measurement). Slightly higher values are obtained when the measures are combined (78% and 69% respectively), all exceeding the 65% achieved by IENFD.

In using IENFD to identify DSPN it is common to set a decision threshold for neuropathy at 2 standard deviations below the mean of the control group. Table 2 also shows the sensitivity/specificity values obtained by applying this threshold to each of the individual measurements (it cannot be applied to the combined CCM measures). Using this threshold, CNFD and CNFL result in better sensitivity/specificity combinations than IENFD: 0.79/0.71, 0.64/0.79 and 0.53/0.77, respectively. There was no statistical significant difference (P=0.13) between the ROC curves for manual CNFD and IENFD (Hanley and McNeil, 1982). However, CCM measurements show considerably less variability within the subject groups than IENFD measurements (Fig. 4) and larger area under the ROC values (Fig. 5).

Discussion

There is a need for surrogate end points of diabetic neuropathy, which accurately detect early disease, quantify disease progression and measure therapeutic response (Dyck et al., 2007). The current 'gold' standard for the diagnosis of neuropathy, neurophysiology is a robust measure, but has been shown to have poor reproducibility (Dyck et al., 2013a). Other measures of neuropathy such as symptoms and signs are also poorly reproducible (Dyck et al., 2010) whilst QST is reproducible but subjective (Dyck et al., 2014). Small fibre neuropathy has direct pathophysiological relevance to the main outcomes of pain and foot ulceration.

Skin biopsy assessment of IENFD has been proposed as a valid measure of diabetic neuropathy (Malik et al., 2011). Furthermore, skin biopsy detects early small nerve fibre damage even when electrophysiology and QST are still normal (Sumner et al., 2003, Singleton et al., 2001), suggesting that it could detect early neuropathy. It has recently been shown to be abnormal in IGT (Asghar et al., 2014) and recently diagnosed patients with Type 2 diabetes (Ziegler et al., 2014). IENFD has also been shown to increase with an improvement in metabolic risk factors in subjects with IGT (Smith et al., 2006), but not after combined pancreas and kidney transplantation in patients with Type 1 diabetes (Tavakoli et al., 2013a). However, the invasive nature of this technique limits its practical use as a diagnostic test and particularly when a repeat biopsy is required in longitudinal studies or clinical intervention trials.

CCM is a novel, rapid and readily reiterative technique, which quantifies small nerve fibres non-invasively and shows promise as a surrogate end point for neuropathy (Tavakoli et al., 2013b, Ziegler et al., 2014, Pritchard et al., 2014, Malik, 2014a, Sivaskandarajah et al., 2013, Halpern et al., 2013). A number of studies have shown the features extracted from CCM are associated with the severity of diabetic peripheral neuropathy (Petropoulos et al., 2014, Quattrini et al., 2007, Sivaskandarajah et al., 2013).

Because IENFD represents a measure of the most distal nerve fibres which are affected in DSPN, a natural assumption is that it should have a better diagnostic ability than CCM. However, a comparison between IENFD and CCM features for the individual diagnosis of DSPN has not been reported to date. In this paper, we present a comparison of nerve fibre features, quantified either manually or automatically from CCM images (CNFL, CNFD, and CNBD) with IENFD measurement in identifying DSPN in individuals. We show that automated, and hence more rapid and reproducible, quantification of CCM features show a high degree of consistency with those measured manually, confirming previously reported results indicating that these measures are equivalent (Dabbah et al., 2011, Petropoulos et al., 2014, Dehghani et al., 2014). The exception is the manually measured nerve branch density (CNBD), which has been found previously (Petropoulos et al., 2013) to be unreliable, due to the subjective judgement required in identifying branches. The algorithmic definition of branches in the automated measurement results in greater consistency, though this is the least useful individual automated CCM measurement. While both CCM and IENFD seek to measure small fibres, IENFD showed a poorer discrimination between those with and without DSPN. Furthermore, CCM measurements show considerably less variability within the subject groups than IENFD measurements. Interestingly, very low IENFD values were observed, even in control subjects.

Our results suggest that CCM provides a more consistent basis on which to assess DSPN than IENFD, and may be further preferred due to its non-invasive means of assessment. We conclude that CCM may be used as a non-invasive and objective test for small nerve fibre quantification in the assessment of DSPN.

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Table 1. Clinical measures and neuropathy assessment.

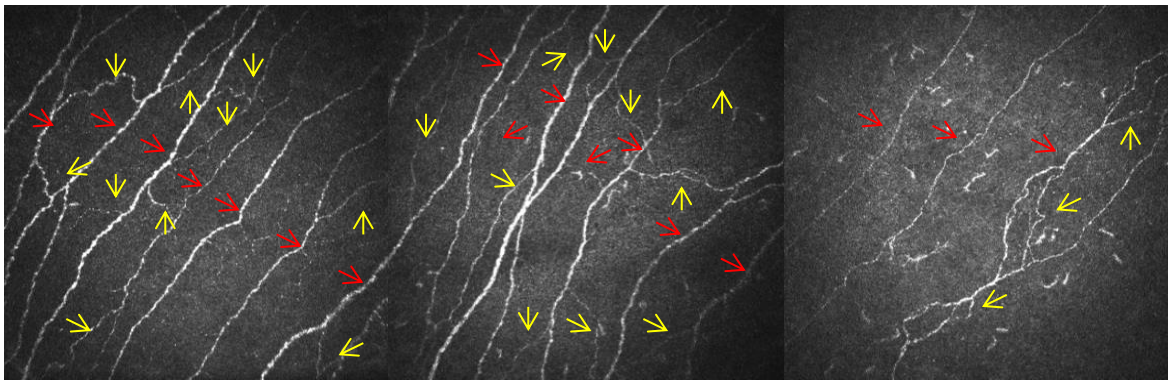
Variable	Control (n=26)	DSPN (-) (n=46)	DSPN (+) (n=17)
Age	44±15	44±13	59±11
Duration of Diabetes	n/a	23±15	39±14
HbA_{1c} (%) / mmol/mol ‡	5.5±0.3 37.1±3.5	8.2±1.4 62.2±24.1 ¶	8.5±1.3 69.3±14.3 ¶
BMI (Kg/m^2) *	26.8±4.0	26.4±4.5	27.5±3.5 ¶
Total Cholesterol (mmol/l)	5.0±0.8	4.4±0.9¶	4.3±0.9¶
HDL (mmol/l)	1.5±0.3	1.6±0.5	1.6±0.4
Triglycerides (mmol/l)	1.4±0.7	1.2±0.7	1.3±0.6
BP (mm Hg) Systolic † / Diastolic	126.7±16.3 70.2±9.1	130.3±17.8 ¶ 71.6±9.6	141.1 ±25.2 ¶§ 73.0 ±9.8
VPT (V) ‡	6.0±5.5	7.6±5.5	25.2±13.4 ¶§
WT † / CT † (ºC)	36.4±2.0 28.8±1.6	38.7±3.6 ¶ 27.1±2.7 ¶	43.5±4.6 ¶§ 16.8±10.6 ¶§
PMNCV (m/s) ‡	49.1±3.4	43.9±3.1 ¶	31.0 ±9.5 ¶§
SSNCV (m/s) ‡	50.9± 3.9	45.3± 5.2 ¶	37.8 ±6.8 ¶§
PMNamp (µV) ‡	6.0± 2.4	6.0± 8.3	1.6± 1.6 ¶§
SSNamp (µV) ‡	19.7± 8.3	12.5± 6.9 ¶	4.3± 3.5 ¶§
IENFD*	9.8 ±3.7	7.0 ±5.0 ¶	5.0 ±5.5 ¶§
MCNFD‡	36.8±5.3	28.3±7.2 ¶	16.9±10.1 ¶§
MCNBD*	92.8±36.4	56.1±30.3 ¶	48.2±32.9 ¶
MCNFL†	26.7±3.7	20.2±5.1 ¶	14.8±8.3 ¶§
ACNFD‡	31.3±6.5	22.6±7.3 ¶	13.5±9.1 ¶§
ACNBD†	44.6±17.2	26.2±15.1 ¶	15.4±12.1 ¶§
ACNFL‡	17.7± 2.8	13.4±3.3 ¶	8.8±4.7 ¶§

Results are expressed as mean ± SD, statistically significant differences using ANOVA/ Kruskal-Wallis: *p<0.05, ¿ P<0.01, † P<0.001, ‡ P < 0.0001 Post hoc results for DSPN (+) significantly different from ¶ control subjects and § DSPN (-). N/A: not applicable for this group. M (manual), A (automated).

Table 2. AUC, 95% confidence interval values and sensitivity-specificity for manual and automated CCM for the diagnosis of DSPN.

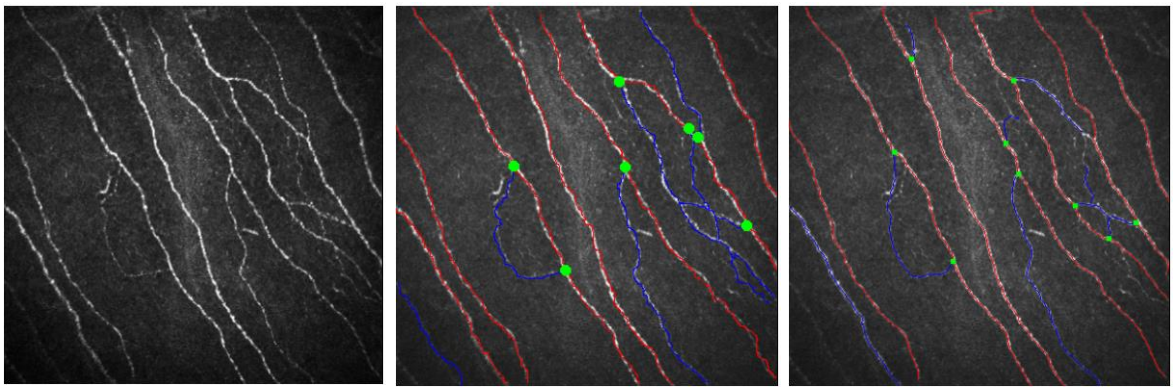
CCM and IENFD	**AUC**	**95% CI**	**Sensitivity Specificity at equal error rate**	**Sensitivity /Specificity at mean±2SD (Threshold)**
MCNFD	0.8165	[0.68 0.95]	0.76	0.82/0.71 (24.0)
MCNFL	0.6969	[0.54 .085]	0.71	0.59/0.74 (16.5)
MCNBD	0.5889	[0.43 0.75]	0.53	0.17/0.96 (15.0)
ACNFD	0.7980	[0.66 0.93]	0.82	0.59/0.83 (15.5)
ACNFL	0.7711	[0.63 0.91]	0.70	0.59/0.80 (10.5)
ACNBD	0.7020	[0.55 0.86]	0.59	0.29/0.98 (4.0)
IENFD	0.6598	[0.50 0.82]	0.65	0.53/0.76 (3.30)

Figures



(a) (b) (c)

Fig. 1: Corneal confocal microscopy images of the subbasal nerve plexus from a control (a), T1D without DPN (b), and T1D with DPN (c) showing the reduction in corneal nerves in those with DSPN. Red arrows indicate main nerve fibres (to calculate CNFD); Yellow arrows indicate branch fibres (to calculate CNBD).



(a) (b) (c)

Fig. 2: (a) Original CCM image (b) Manually quantified CCM image (c) Automatically quantified CCM image. Red lines represent main nerve fibres, blue lines are branches and green spots indicate branch points on the main nerve trunks.

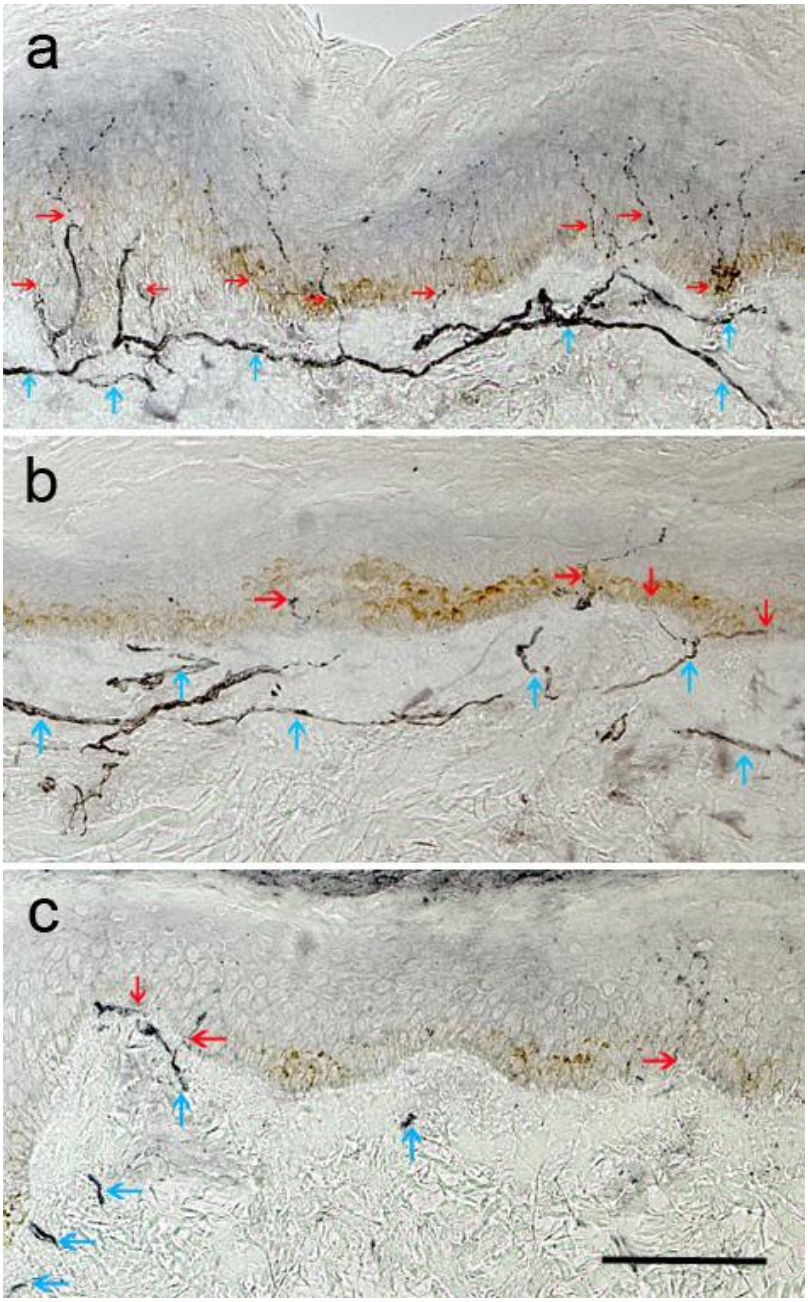
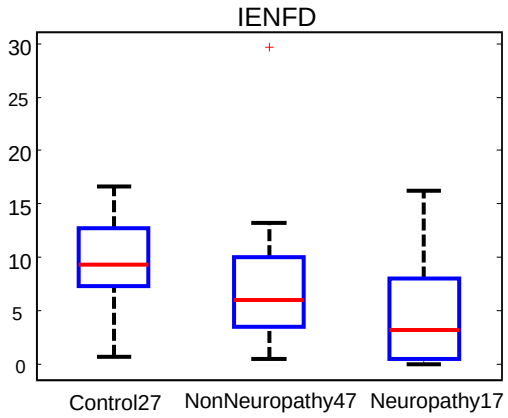
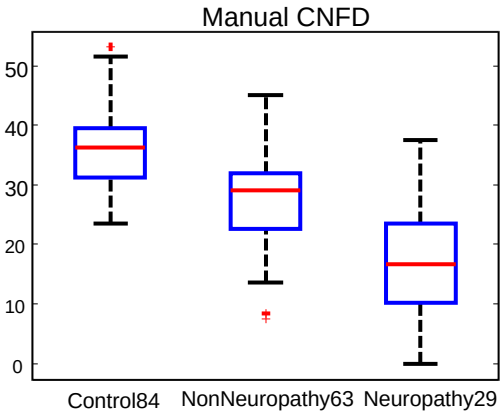


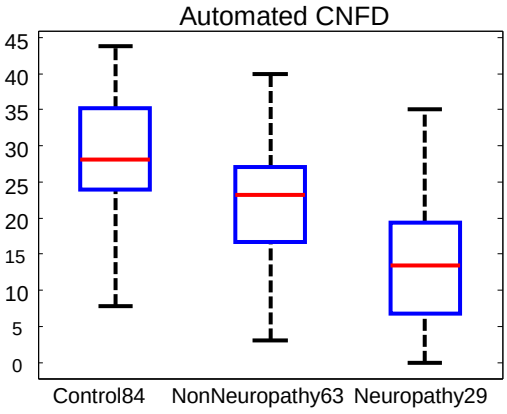
Fig. 3: Immunohistochemical study (neuronal marker PGP9.5) in sections from skin biopsies from dorsum of the foot from a healthy subject (a), a patient with T1 diabetes without neuropathy (b), and with neuropathy (c). Note the depletion of IENFD (red arrows) and reduction of subepidermal nerve plexus (blue arrows) in b and c, with both features more severe in a patient with neuropathy (c). Original magnification x200, scale bar = 100 μm.


IENFD
30
25
20
15
10
5
0
Control27
NonNeuropathy47
Neuropathy17

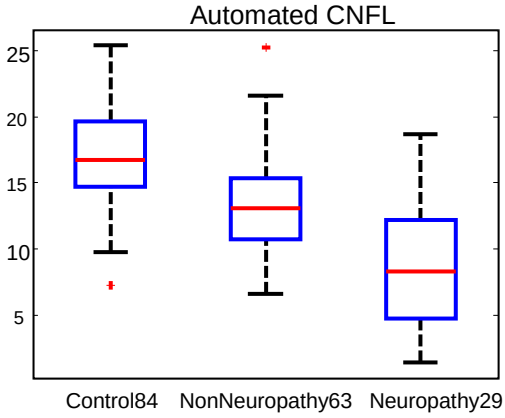
(a)


Manual CNFD
50
40
30
20
10
0
Control84
NonNeuropathy63
Neuropathy29

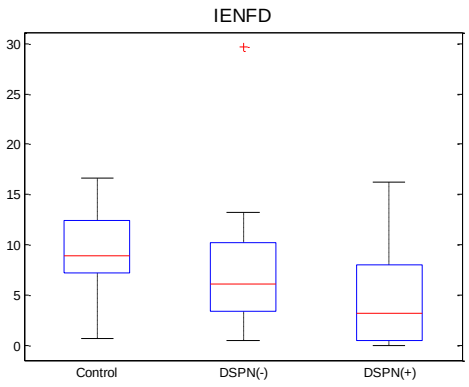
(b)

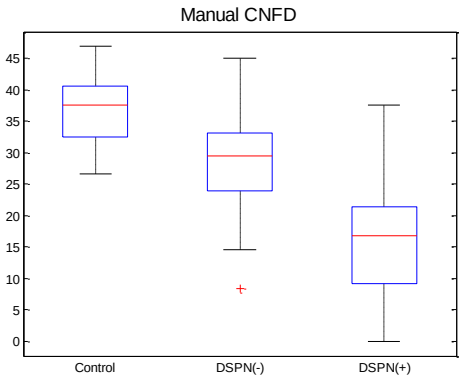

Automated CNFD
45
40
35
30
25
20
15
10
5
0
Control84
NonNeuropathy63
Neuropathy29

(c)


Automated CNFL
25
20
15
10
5
Control84
NonNeuropathy63
Neuropathy29

(d)


IENFD
30
25
20
15
10
5
0
Control
DSPN(-)
DSPN(+)


Manual CNFD
45
40
35
30
25
20
15
10
5
0
Control
DSPN(-)
DSPN(+)

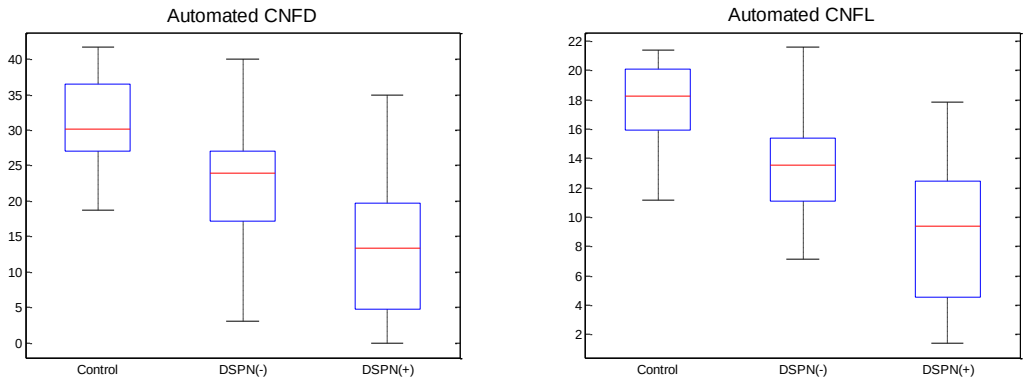


Fig. 4: Boxplot of (a) IENFD (b) Manual CNFD values (c) Automated CNFD values (d) Automated CNFL values grouped into controls, non-neuropathic and neuropathic groups, based on Toronto criteria.

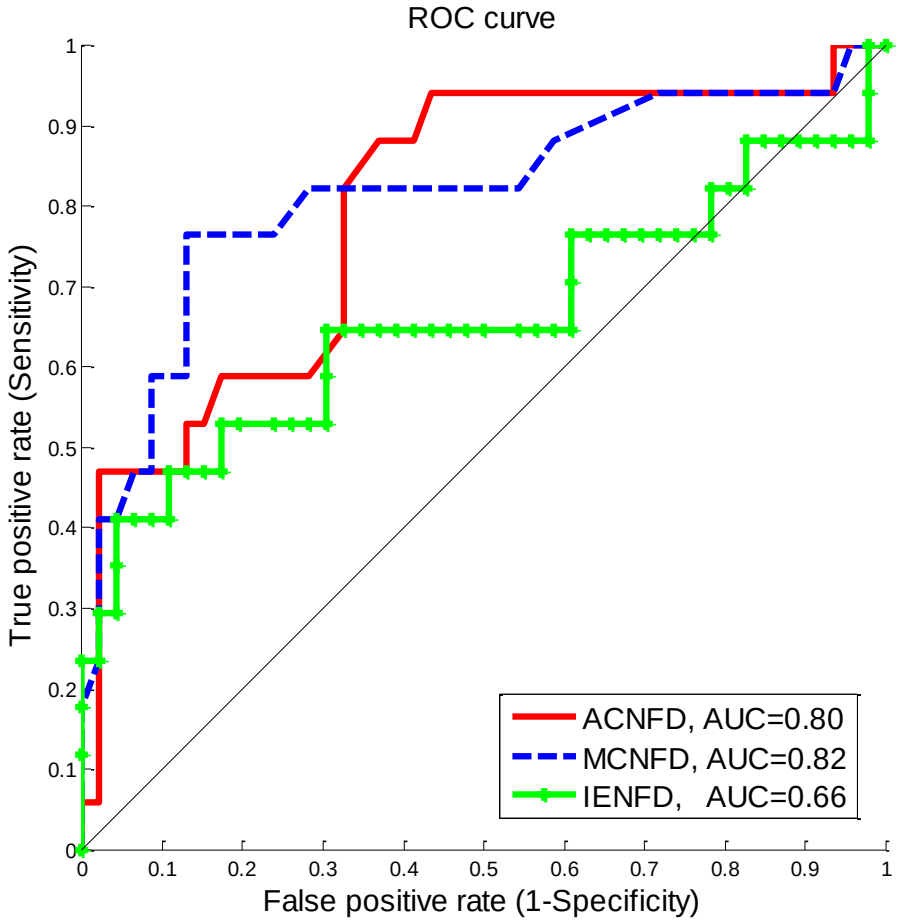


Fig. 5: ROC curves of using ACNFD (red solid line), MCNFD (blue dashed line) and IENFD (green solid line with dots) to discriminate neuropathic group from non-neuropathic group.