Manuscript Number:

Title: Longitudinal assessment of neuropathy in type 1 diabetes using novel ophthalmic markers (LANDMark): study design and baseline characteristics

Article Type: Research Paper

Keywords: corneal confocal microscopy; diabetic neuropathy; LANDMark study; longitudinal trial; non-contact corneal aesthesiometry

Corresponding Author: Prof. Nathan Efron, DSc, PhD

Corresponding Author's Institution: Queensland University of Technology

First Author: Nicola Pritchard

Order of Authors: Nicola Pritchard; Katie Edwards; Cirous Dehghani; Hassan Fadavi; Maria Jeziorska; Andrew Marshall; Ioannis Ptopoulos; Georgios Ponirakis; Anthony Russell; Geoff Sampson; Ayda Shahidi; Sangeetha Srinivassan; Mitra Tavakoli; Dimitrios Vagenas; Rayaz Malik; Nathan Efron, DSc, PhD

Abstract: Aims: Corneal nerve morphology and corneal sensation threshold have recently been explored as potential surrogate markers for the evaluation of diabetic neuropathy. We present the baseline findings of the 'Longitudinal Assessment of Neuropathy in type 1 Diabetes using novel ophthalmic Markers'(LANDMark) study.

Methods: The LANDMark study is a 4-year, two-site, natural history study of three participant groups: type 1 diabetes with neuropathy (T1W), type 1 diabetes without neuropathy (T1WO) and control participants without diabetes or neuropathy. All participants undergo a detailed annual assessment of neuropathy including corneal nerve parameters measured using corneal confocal microscopy and corneal sensitivity measured using non-contact corneal aesthesiometry.

Results: 76 T1W, 166 T1WO and 154 control participants were enrolled into the study. Corneal sensation threshold (mbars) was significantly higher (i.e. sensitivity was lower) in T1W (1.0 ± 1.1) than T1WO (0.7 ± 0.7) and controls (0.6 ± 0.4) ($p < 0.001$), with no difference between T1WO and controls. Corneal nerve fibre length was lower in T1W (14.0 ± 6.4 mm/mm²) compared to T1WO (19.1 ± 5.8 mm/mm²) and controls (23.2 ± 6.3 mm/mm²) ($p < 0.001$). Corneal nerve fibre length was lower in T1WO compared to controls.

Conclusions: The LANDMark baseline findings confirm a reduction in corneal sensitivity only in Type 1 patients with neuropathy. However, corneal nerve fibre length is reduced even in Type 1 patients without neuropathy with an even greater deficit in Type 1 patients with neuropathy.

To The Editor
Diabetes Research and Clinical Practice

Monday, 11 November 2013

I am pleased to submit an article for consideration for publication in Diabetes Research and Clinical Practice, entitled: "Longitudinal assessment of neuropathy in type 1 diabetes using novel ophthalmic markers (LANDMark): study design and baseline characteristics".

Yours faithfully,

Nathan Efron

Diabetes Research and Clinical Practice

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Author's Name

Cirous Dehghani

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Signature

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Author's Name

Sangeetha Srinivasan

Signature

Sangeetha

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Author's Name

.....Ayala M. Shahidi.....

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Anthony RUSSELL

Signature

Anthony Russell

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Georgios Ponirakis

Signature

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...Dr Andrew Marshall.....

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Author's Name

Signature

R A MALIK.....

A handwritten signature in black ink, appearing to read 'R A Malik', is written over a faint, light blue grid background.

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Author's Name

.....Maria Jeziorska.....

Signature

....

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...Hassan Fadavi..... .....

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Author's Name

.....Katie Edwards.....

Signature

.....K. Edwards.....

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Author's Name

Dimitrios Vagenas

Signature

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Nicola Pritchard PhD^a, Katie Edwards PhD^a, Cirous Dehghani MSc^a, Hassan Fadavi MBChB^b, Maria Jeziorska PhD^b, Andrew Marshall MBChB^b, Ioannis N. Petropoulos PhD^b, Georgios Ponirakis MSc^b, Anthony W. Russell PhD^c, Geoff P. Sampson PhD^a, Ayda M. Shahidi PhD^a, Sangeetha Srinivasan MPhil^a, Mitra Tavakoli PhD^b, Dimitrios Vagenas PhD^a, Rayaz A. Malik PhD^b, Nathan Efron DSc^{a,*}

^a*Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, Queensland, Australia*

^b*Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester, UK*

^c*Princess Alexandra Hospital, Woolloongabba, Queensland, Australia*

[✉] This study was funded by the National Health and Medical Research Council of Australia (492730) and the Juvenile Diabetes Research Foundation International (8-2008-362). Support from the Manchester Wellcome Trust Clinical Research Facility is acknowledged.

*Corresponding author: Professor Nathan Efron, ^aInstitute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, Queensland 4059, Australia. Tel: +617 3138 6401
E-mail address: n.efron@qut.edu.au

ABSTRACT

Aims: Corneal nerve morphology and corneal sensation threshold have recently been explored as potential surrogate markers for the evaluation of diabetic neuropathy. We present the baseline findings of the ‘Longitudinal Assessment of Neuropathy in type 1 Diabetes using novel ophthalmic Markers’(LANDMark) study.

Methods: The LANDMark study is a 4-year, two-site, natural history study of three participant groups: type 1 diabetes with neuropathy (T1W), type 1 diabetes without neuropathy (T1WO) and control participants without diabetes or neuropathy. All participants undergo a detailed annual assessment of neuropathy including corneal nerve parameters measured using corneal confocal microscopy and corneal sensitivity measured using non-contact corneal aesthesiometry.

Results: 76 T1W, 166 T1WO and 154 control participants were enrolled into the study. Corneal sensation threshold (mbars) was significantly higher (i.e. sensitivity was lower) in T1W (1.0 ± 1.1) than T1WO (0.7 ± 0.7) and controls (0.6 ± 0.4) ($p < 0.001$), with no difference between T1WO and controls. Corneal nerve fibre length was lower in T1W (14.0 ± 6.4 mm/mm²) compared to T1WO (19.1 ± 5.8 mm/mm²) and controls (23.2 ± 6.3 mm/mm²) ($p < 0.001$). Corneal nerve fibre length was lower in T1WO compared to controls.

Conclusions: The LANDMark baseline findings confirm a reduction in corneal sensitivity only in Type 1 patients with neuropathy. However, corneal nerve fibre length is reduced even in Type 1 patients without neuropathy with an even greater deficit in Type 1 patients with neuropathy.

Keywords: Corneal confocal microscopy, Diabetic neuropathy, LANDMark study, Longitudinal trial, Non-contact corneal aesthesiometry

1. Introduction

Diabetic neuropathy is a significant and prevalent complication, which leads to morbidity in the form of painful neuropathy, foot ulceration and lower extremity amputation [1]. Currently there are no disease modifying therapies approved by the USA Food and Drug Administration for diabetic neuropathy. Poor glycaemic control [2] and vascular risk factors [3] have been shown to be significant risk factors in the development of neuropathy. However, improved glycaemic control has been shown to prevent progression of diabetic neuropathy in type 1 but not type 2 diabetes [4,5].

For the approval of a new therapy a significant improvement in diabetic neuropathy needs to be shown. A variety of techniques are currently available for diagnosing diabetic neuropathy by directly and indirectly assessing nerve structure and function [3]; however, there may be significant limitations in their ability to define a therapeutic response [6]. It is perhaps for these reasons that defined end points for quantitative sensory testing and electrophysiology have not been able to show benefits of therapeutic interventions [7]. Poor diagnostic reproducibility has been demonstrated in the investigation of neurologic symptoms and deficits [8] and electrophysiology assesses large fiber damage; but does not assess small nerve fibers, which are damaged first [9], and demonstrate repair even in advanced neuropathy [6]. Examination of nerve fibre morphology using sural nerve biopsies [10] and intra-epidermal nerve fibre density using skin-punch biopsies [11] can accurately quantify nerve fiber damage and repair, but both procedures are invasive and uncomfortable for patients.

The cornea of the human eye is the only tissue in the body that facilitates direct optical imaging of unmyelinated nerve fibres. The laser scanning corneal confocal microscope (CCM), which became commercially available about a decade ago, allows examination of corneal nerve morphology at a cellular level (up to 500X magnification) [12]. Studies conducted over the past decade have sought to exploit this technique for assessing nerve damage [13]. The functional correlate of corneal nerve fibre damage is loss of corneal sensitivity, which can be measured using a non-contact corneal aesthesiometer (NCCA) [14]. Studies in diabetic patients have shown that increased severity of neuropathy (assessed using conventional measures of signs, symptoms, quantitative sensory testing and electrophysiology) is associated with reduced corneal nerve fibre length (CNFL) [13] and reduced corneal sensitivity [14]. Furthermore, deficits in CNFL have been shown to correlate with reduced intra-epidermal nerve fibre density in skin biopsy [15].

The potential morbidity, patient distress and financial costs associated with diabetic neuropathy and associated complications [16] could be minimized if tests applying direct quantification of structural and functional measures prove effective at diagnosing and monitoring the progression of diabetic neuropathy as those at risk can be targeted early for improved risk factor control. In this regard, CCM and NCCA have been demonstrated to have good diagnostic capability for diagnosing diabetic neuropathy (CCM – 82% sensitivity, 52% specificity [17]; NCCA – 70% sensitivity, 75% specificity [18]).

The few longitudinal studies of diabetic neuropathy that have been undertaken show a progressive deterioration of neuropathy over time in patients with type 1 [19] and type 2 [20]

diabetes, and the placebo arms of several clinical trials of diabetic neuropathy show a monotonic worsening in electrophysiology and quantitative sensory testing [21]. We are conducting a longitudinal assessment of patients with type 1 diabetes using novel ophthalmic markers (LANDMark), the aims of which are to (a) observe the natural history of diabetic neuropathy using these novel non-invasive ophthalmic tests, (b) prospectively characterize changes in structural (CCM) and functional (NCCA) corneal parameters in individuals with and without diabetic neuropathy, (c) determine the predictive validity of CCM and NCCA, and (d) identify demographic and metabolic risk factors associated with these changes over time. In this paper, we describe the study design and summarize the baseline characteristics of participants enrolled in the LANDMark study.

2. Materials and methods

2.1. Study design

The LANDMark study is an investigator-masked, prospective, longitudinal, controlled, natural history (observational) study conducted at two sites – Brisbane, Australia and Manchester, UK. A minimum cohort of 404 participants were intended to be enrolled (202 per site) according the inclusion/exclusion criteria, and followed annually for four subsequent years (i.e. 5 examinations in total). All participants were examined at baseline and will be examined annually thereafter. Ethical clearance was granted by partner hospitals, universities and other relevant national research ethics committees in Australia and the UK. Written informed consent was obtained from all participants and the study is being carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

2.2. *Setting and participants*

Individuals with type 1 diabetes (age range 14 to 80) were recruited from the Centre for Diabetes and Endocrine Research at Princess Alexandra Hospital and Mater Hospitals in Brisbane, and the Manchester Diabetes Centre, Manchester Royal Infirmary, as well as community and other clinics at both sites. Sample size was determined using the expected effect size in the two primary outcome variables – corneal nerve fibre length and corneal sensation threshold – taking into account at least 40% attrition and multisite variability. Individuals were excluded if they had a history of ocular trauma or surgery, ocular disease or systemic disease affecting the cornea; systemic disease other than diabetes (e.g. malignant disease, congestive heart failure, major psychosis); history of neuropathy of non-diabetic cause, current diabetic foot ulcer or infection or participation in any interventional research trial. A group of non-diabetic control participants was also included and two specific exclusion criteria applied were: (a) positive glutamic acid decarboxylase antibody status, or (b) neuropathy according to the Toronto criteria [3].

An individual was considered to have neuropathy if they met the following ‘Toronto criteria’ [3]: (a) abnormal nerve conduction, based on age-matched controls at the site, and (b) a symptom or sign of neuropathy, defined as one or more of the following: (i) diabetic neuropathy symptom score of $\geq 1/4$ [22], or (ii) neuropathy disability score of $\geq 3/10$ [23].

2.3. *Masking and retention*

The order of testing was randomized where practical, and all procedures were conducted by trained individuals according to standard operating procedures. Investigators were masked as to

the group assignment of participants. All data analyses were performed in a masked fashion. For example, a masked investigator analysed CCM images in large batches, with the images only being identifiable by number codes; thus, these measurements were made without reference to NCCA or neuropathy results or participant history, which otherwise could have compromised masking. Retention of individuals was maximised through strategies of flexibility, accessibility, personalized attention, and feedback.

2.4. Corneal nerve morphology

Images of the sub-basal nerve plexus of the cornea were captured from one eye (the side of hand dominance) of each participant using a CCM (Heidelberg Retinal Tomograph III with Rostock Cornea Module; Heidelberg Engineering GmB, Germany), after anaesthetizing the cornea. For each participant, a minimum of three of the clearest images at the level of the sub-basal nerve plexus of the central cornea were analysed using semi-automated software (CCMetrics, University of Manchester) to calculate CNFL in mm/mm^2 and corneal nerve branch density (CNBD) in $\text{number}/\text{mm}^2$ [24].

2.5. Corneal sensation threshold

Corneal sensation threshold was measured using an NCCA designed and constructed for the Institute of Health and Biomedical Innovation based on the original work of Murphy et al [25]. The eye on the side of hand dominance was measured using a stimulus duration of 0.9 seconds in a quiet room without distractions. The participant was instructed to fixate on a target, the nozzle tip distance was positioned 10 mm from the central cornea and sensation threshold was

determined using a modified Garcia-Perez staircase technique, according to the procedure described previously by Pritchard et al [18].

2.6. *Measures of neuropathy*

Symptoms were assessed using the diabetic neuropathy symptom score [22], where 0 indicates no neuropathy and 1-4 points indicates increasing severity of neuropathy.

Neurological deficits were evaluated by determining the neuropathy disability score [23], which involved measurement of vibration perception, pin prick perception, temperature perception and the presence or absence of ankle reflexes using a tendon hammer applied to both lower limbs. A score is derived ranging from 0–10, whereby a neuropathy disability score of ≤ 2 indicates no neuropathy.

The Neuropad[®] (miro Verbandstoffe GmbH, Wiehl, Germany) [36] was applied to the plantar aspect of the big toe and after 10 minutes, the percentage blue area was estimated. A 100% pink pad indicates a normal, non-neuropathic response and an abnormal response was indicated when the pad remained blue or was patchy.

The monofilament test was performed using a 10-gram nylon filament and was applied to 3 predetermined points on the sole of the foot on the hand-dominant side.

Vibration thresholds were measured using a Medoc VSA-3000 Vibratory Sensory Analyzer (Medoc Advanced Medical Systems, Ramat-Yishai, Israel) in Brisbane, and a Horwell

Neuroaesthesiometer (Scientific Laboratory Supplies, Nottingham, UK) in Manchester.

Assessments were made on the hand-dominant side of the dorsolateral aspect of the foot. Warm and cold sensation and pain thresholds were determined at both sites using the Medoc TSA-II NeuroSensory Analyzer.

Nerve conduction studies were performed using a Neuropack S1 EMG/Evoked Potential Measuring System (Nihon Kohden, Tokyo, Japan) in Brisbane and a Dantec Keypoint System (Dantec Dynamics Ltd, Bristol, UK) in Manchester. Peroneal and sural nerve conduction velocities, amplitudes and latencies were recorded on the hand-dominant side in Brisbane (87% right) and the left side in Manchester.

2.7. Diabetic retinopathy

Retinal fundus images (3-field) were taken at each visit with a dilated pupil and each image was graded by an ophthalmologist according to the Early Treatment of Diabetic Retinopathy Study scale [27] or the National Screening Committee, UK.

2.8. Skin punch biopsy

Intraepidermal nerve fibre density was performed on a subset of 207 individuals at the Manchester site. Two punch skin biopsies (3mm in diameter) were performed on the dorsum of the foot approximately 2 cm above the second metatarsal head under local anaesthesia (1% lignocaine). The biopsy site was closed using Steri-strips, and the specimen was fixed, cryoprotected and processed as previously described [15]. Intraepidermal nerve fibre linear

density, i.e. the number of fibres per millimeter of epidermal length, expressed as intraepidermal nerve fibres per millimeter, was recorded.

2.9. *Risk factors*

Blood pressure, body mass index, waist circumference, alcohol and tobacco consumption, renal function, HbA_{1c}, lipid profile and red blood cell B₁₂ and folate were assessed.

2.10. *Statistical methods*

Descriptive statistics for the total cohort and for each group are presented. For quantitative variables, data are presented as mean $\pm$ standard deviation unless otherwise stated. Differences between quantitative variables with a normal distribution were tested using a generalized linear model or with an analysis of variance (IBM SPSS Statistics Version 19, Armonk, NY). Posthoc tests were performed with Least Significant Difference' (LSD) post hoc test. The non-parametric Kruskal-Wallis test was used to analyse non-normally distributed data. The Chi-squared test was used to analyse categorical data.

3. **Results**

Of 449 individuals recruited with type 1 diabetes mellitus and healthy controls, 396 were deemed to be eligible for participation in the LANDMark study. Table 1 shows the baseline demographic and clinical characteristics for the three groups studied: 'type 1 diabetes with neuropathy' (T1W), 'type 1 diabetes without neuropathy' (T1WO) and 'no diabetes or neuropathy' controls. Approximately half (53%) of the commencing cohort were Caucasian of European descent and the remaining 47% were of Asian, South East Asian, Middle Eastern, or other ethnic origin.

3.1. *Clinical measures*

Clinical measures relating to the three groups are shown in Table 1. Both age and duration of diabetes were 14 years greater in T1W versus T1WO. Supine systolic and diastolic blood pressure was 13 and 2 mmHg greater in T1W than T1WO, respectively. Waist circumference was 6 cm greater in T1W than T1WO. A significantly higher proportion of T1W had retinopathy (75%) compared with T1WO (45%).

Height, body mass index, alcohol consumption and number of cigarettes per day did not differ between groups. There were no significant differences in any clinical measures between the T1WO and controls.

Antihypertensive or antianginal medications were being taken by 45% of T1W, 17% of T1WO and 4% of controls. Approximately double the number of T1W took analgesics and anti-inflammatory medicine compared to T1WO and controls.

3.2. *Metabolic profile*

Compared to T1WO, T1W had 0.6% higher HbA_{1c}, 0.6 mg/mmol higher albumin creatinine ratio, 16 mL/min lower estimated glomerular filtration rate, 0.2 mmol/L higher total cholesterol and 0.3 mmol/L higher triglycerides. HbA_{1c} was higher and triglycerides were lower in T1WO compared to controls (Table 2).

3.3. Neuropathy assessment

All measures of neuropathy indicated greater disease severity in T1W compared to T1WO and controls, with the exception of warm pain threshold, which did not differ between groups (Table 3). Neuropathy measures were significantly more severe in the T1WO group than in controls in respect of neuropathy disability score, cold threshold, vibration threshold, peroneal and sural conduction velocity and monofilament sensibility.

3.4. Ophthalmic measures

Outcomes of ophthalmic assessments are shown in Figure 1. Corneal sensation threshold (mbars) was significantly higher in T1W (1.0 ± 1.1) than T1WO (0.7 ± 0.7) and controls (0.6 ± 0.4) ($p < 0.001$), with no difference between T1WO and controls (Tukey's HSD, $p = 0.482$). Corneal nerve fibre length (mm/mm^2) was significantly lower in T1W (14.0 ± 6.4) than T1WO (19.1 ± 5.8) and controls (23.2 ± 6.3) ($p < 0.001$); post-hoc testing showed that CNFL in T1WO was significantly lower than in controls (Tukey's HSD $p < 0.001$). CNBD ($\text{branches}/\text{mm}^2$) was significantly lower in T1W (40.1 ± 32.1) than T1WO (61.7 ± 37.2) and controls (83.5 ± 45.8) ($p < 0.001$). Furthermore post-hoc testing showed T1WO had a significantly lower CNBD compared to controls (Tukey's HSD $p = 0.001$).

3.5. *Site differences*

Duration of diabetes, blood pressure, height, alcohol consumption and ethnicity were different between the two sites, regardless of group. All metabolic test parameters showed differences between sites, with the exception of urine albumin-creatinine ratio and HDL cholesterol. Most measures of neuropathy showed more severe disease at the Manchester site compared with the Brisbane site. Warm and cold sensation threshold and cold pain threshold were not different between sites. Regardless of group, corneal sensation threshold was 0.2 mbars higher (i.e. poorer sensitivity) and corneal nerve fibre length was 4 mm/mm² greater (i.e. better nerve morphology) at the Manchester site compared to the Brisbane site, representing a 30% and 22% difference, respectively.

4. Discussion

The primary objective of the LANDMark study is to observe the natural history of diabetic neuropathy, applying two novel, non-invasive ophthalmic tests, which may serve as useful markers of this potentially debilitating condition. We present the baseline findings at the two study sites, Brisbane, Australia and Manchester, UK. The LANDMark study cohort of 396 participants was recruited over approximately 1.5 years. The application of strict inclusion and exclusion criteria has resulted in a valid cohort, as judged by comparison of these baseline findings with those of diabetic participants examined in other large clinical trials. For example, in the LANDMark study, HbA_{1c} was 8.6% at baseline compared to 8.3% in the Diabetes Control and Complications/Epidemiology of Diabetes Interventions and Complications Study [28]. Similarly, total cholesterol was 4.4 mmol/L in the present study versus 4.8 mmol/L (185 mg/dL)

in the study of Lorbeer et al [29]. Controls in the LANDMark cohort had a mean HbA_{1c} of 5.6% compared to 5.3% in the study of Lorbeer et al [29].

4.1. *Between-group differences in ophthalmic measures*

CNFL was greater in the controls compared to patients with type 1 diabetes (23.2 vs. 13.8 to 19.1 mm/mm²). The measures of CNFL and CNBD reported in our control group are similar to those reported by researchers using the same technology in individuals without diabetes [30-32].

Several authors have reported corneal nerve parameters of individuals with diabetes using the same Heidelberg technology as used in this study [33-35]. CNFL of diabetic participants in the present study (17.4 mm/mm²) is similar to that reported by Ahmed et al [33] and Hertz et al [34] (9.5 to 16.7 mm/mm²), but higher than reported by Ishibashi et al [35], Tavakoli [36] and Messmer et al [37] (9.7 to 11.4 mm/mm²). This discrepancy is likely due to differences in sample sizes, severity of neuropathy of the cohorts examined, acquisition mode using CCM, number of images analysed per participant, field of view of the acquisition lens, operator technique and software applied to analyse images.

Corneal sensation threshold in the present study (average 0.61 mbars in controls) was similar to that reported in other studies, using similar instrumentation, investigating healthy individuals without diabetes [14,38-40]. The corneal sensation threshold for T1WO in the current study (0.95 mbars), was similar to that reported by Murphy et al [40], but somewhat lower than reported by other studies, where measures were on average 1.5 mbars [17,38,39]. This discrepancy may be due to diabetic participants in those studies having a greater severity of neuropathy.

4.2. *Limitations of the study*

Some site differences are to be expected due to recruitment and cohort differences, instrumentation and investigator preferences, despite common standard operating procedures adopted by both sites. Observer and laboratory differences are not uncommon in multi-centre studies and in fact broaden the sample to represent real-world data. These differences are viewed as improving the generalisability of the outcomes of the study. The generalisability (external validity) of the trial findings should be sound given the large population and the range of ethnicities and participant ages at the two sites.

The variables expected to show differences between sites, but analyzed together, include all nerve conduction results and corneal sensation thresholds. The variables that were not analyzed together – due to differences in instrumentation and technique of these tests – include monofilament and vibration sensation threshold.

Participant age and duration of diabetes were each 2 years greater at the Manchester site than the Brisbane site. We would have expected these differences to influence the severity of neuropathy; however, this was not the case. Another limitation of the LANDMark study is that, despite our best efforts, we were unable to ensure masking of all clinical measurement procedures. Intermittent failure to mask the investigators from the diabetic and/or neuropathic status of the individuals being examined may be a source of potential bias.

In conclusion, CCM and NCCA have the potential to supplement the clinical repertoire of endocrinologists and neurologists, perhaps with increased involvement from eye care professionals, in diagnosing and managing diabetic neuropathy.

Conflicts of interest

All authors declare that they have no conflict of interests.

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Table 1 – Clinical demographics of the LANDMark cohort at baseline (mean $\pm$ standard deviation, range [in parenthesis], n), of the following groups of study participants: type 1 diabetes with neuropathy (T1W), type 1 diabetes without neuropathy (T1WO) and control participants without diabetes or neuropathy.

Characteristic	T1W (A)	T1WO (B)	Controls (C)	P Group Difference
Site n (Brisbane/Manchester)	48/26	100/68	60/94	< 0.001
Sex (n/%F)	33/45%	83/49%	84/54%	0.346
Age (yrs)	57 $\pm$ 11 (20-80) 74	43 $\pm$ 16 (14-78) 168	46 $\pm$ 15 (15-77) 154	< 0.001 A vs B, C
Duration of diabetes (yrs)	34 $\pm$ 16 (1-58) 74	20 $\pm$ 15 (1-60) 165	n/a	< 0.001
Height (cm)	170 $\pm$ 9 (143-189) 74	170 $\pm$ 10 (150-194) 167	168 $\pm$ 10 (144-193) 151	0.169
Weight (kg)	81 $\pm$ 21 (46-172) 74	77 $\pm$ 15 (48-139) 167	74 $\pm$ 16 (43-131) 151	0.016 A vs C
BMI	28 $\pm$ 6	26 $\pm$ 4	26 $\pm$ 5	0.033

	(18-48) 74	(18-40) 167	(15-47) 151	A vs B, C
Waist circumference (cm)	95 ± 17 (33-139) 73	89 ± 14 (26-123) 163	89 ± 14 (59-134) 148	0.009 A vs B, C
Systolic BP (mmHg) (supine)	136 ± 22 (85-207) 73	123 ± 17 (88-184) 166	122 ± 17 (90-180) 150	< 0.001 A vs B, C
Diastolic BP (mmHg) (supine)	75 ± 10 (54-101) 73	71 ± 8 (48-100) 166	72 ± 9 (52-97) 150	0.031 A vs B, C
Cigarettes (number per day)	2 ± 5 (0-30) 71	1 ± 3 (0-20) 162	1 ± 3 (0-25) 143	0.326
Alcohol (units per week)	6 ± 9 (0-56) 67	5 ± 6 (0-30) 159	4 ± 6 (0-22) 140	0.146
Retinopathy (n)				
No apparent retinopathy	15	67		
Mild non-proliferative	19	41		
Moderate non-proliferative	19	15		
Severe non-proliferative	1	1		
Proliferative	3	0		
			n/a	<0.001 (Chi ²)

Table 2 – Metabolic profile of the LANDMark cohort at baseline (mean $\pm$ standard deviation, range [in parenthesis], n), of the following groups of study participants: type 1 diabetes with neuropathy (T1W), type 1 diabetes without neuropathy (T1WO) and control participants without diabetes or neuropathy.

Characteristic	T1W (A)	T1WO (B)	Controls (C)	P Group Difference
HbA _{1c} (%)	8.6 $\pm$ 1.8 6.1-13.9 72	8.0 $\pm$ 1.2 5.6-12.2 160	5.5 $\pm$ 0.3 4.6-6.4 143	< 0.001 A vs B, C B vs C
HbA _{1c} (mmol/mol)	70 $\pm$ 19 43-128 74	64 $\pm$ 13 38-110 158	37 $\pm$ 4 27-46 143	
Albumin/creatinine ratio (mg/mmol)	28 $\pm$ 76 0-418 49	5 $\pm$ 36 0-398 124	1 $\pm$ 2 0-11 130	< 0.001 A vs B, C
eGFR (mL/min)	65 $\pm$ 23 1- 91 55	81 $\pm$ 14 2-91 115	83 $\pm$ 10 41-91 127	< 0.001 A vs B, C
Total cholesterol (mmol/L)	4.8 $\pm$ 1.2 3.0-8.5 73	4.6 $\pm$ 0.9 2.8-8.6 166	5.2 $\pm$ 1.0 2.8-9.2 142	< 0.001 A vs C
HDL	1.7 $\pm$ 0.6	1.6 $\pm$ 0.4	1.5 $\pm$ 0.4	0.013

(mmol/L)	0.8-3.7 73	0.7-2.7 166	0.7-2.7 141	A vs C
LDL (mmol/L)	2.5 ± 0.9 0.8-5.1 72	2.5 ± 0.8 0.6-6.3 166	3.1 ± 1.0 0.0-6.7 143	< 0.001 A vs C
Triglycerides (mmol/L)	1.4 ± 1.0 1-7 73	1.1 ± 0.6 0-4 166	1.3 ± 0.7 0-4 142	0.003 B vs A, C

Table 3 – Neuropathy findings of the LANDMark cohort at baseline (mean $\pm$ standard deviation, range [in parenthesis], n), of the following groups of study participants: type 1 diabetes with neuropathy (T1W), type 1 diabetes without neuropathy (T1WO) and control participants without diabetes or neuropathy.

Characteristic	T1W (A)	T1WO (B)	Controls (C)	P Group Difference
Diabetic neuropathy symptom score (0-4)	1.5 $\pm$ 1.3 0-4 72	0.2 $\pm$ 0.6 0-4 167	0.1 $\pm$ 0.3 0-3 144	<0.001* A vs B, C**
Neuropathy disability score (0-10)	4.2 $\pm$ 3.2 0-10 73	1.1 $\pm$ 1.8 0-8 166	0.3 $\pm$ 0.8 0-5 149	<0.001* A vs B, C** B vs C**
Cold sensation threshold (°C)	20.6 $\pm$ 9.4 0.0-31.3 69	27.1 $\pm$ 4.6 6.0-31.5 165	28.5 $\pm$ 2.3 18.3-33.2 150	<0.001* A vs B, C** B vs C**
Warm sensation threshold (°C)	42.9 $\pm$ 4.1 33.9-50.0 69	37.8 $\pm$ 3.7 33.0-47.5 165	37.1 $\pm$ 3.4 28.5-47.0 150	<0.001* A vs B, C**
Cold pain threshold (°C)	5.9 $\pm$ 9.2 0-49.9 69	11.8 $\pm$ 9.3 0-29.0 164	11.4 $\pm$ 8.8 0-29.4 149	<0.001* A vs B, C**

Warm pain threshold (°C)	47.4 ± 8.4 0-50.0 69	46.1 ± 3.4 36.9-50.0 164	45.3 ± 3.9 19.9-50.0 149	<0.001* A vs B, C** B vs. C
Vibration threshold ^a (Hz)	30.1 ± 30.4 1.1-130.0 47	8.7 ± 10.5 0.7-47.7 100	7.0 ± 8.1 0.7-42.2 60	<0.001* A vs B, C**
Vibration threshold ^b (V)	27.3 ± 13.5 3.5-51.0 26	9.2 ± 7.6 2.0-35.0 66	5.2 ± 4.5 0.5-27.0 89	<0.001* A vs B, C** B vs C**
Peroneal conduction velocity; ankle to FH ^c (m/s)	35.3 ± 8.4 19.0-50.9 72	45.6 ± 4.8 20.0-57.4 162	48.8 ± 4.9 21.1-60.3 143	<0.001* A vs B, C** B vs C**
Sural conduction velocity; calf to ankle ^d (m/s)	30.9 ± 11.3 5-50 70	42.2 ± 5.7 31-54 161	48.1 ± 6.1 31-61 144	<0.001* A vs B, C** B vs C**
Neuropad (n) Abnormal Patchy Normal	 8 30 35	 6 39 121	 0 31 114	 <0.001 (Chi ²)
Monofilament ^e (responses/3)	2.3 ± 1.0 0-3 50	2.9 ± 0.5 0-3 97	3.0 ± 0.2 2-3 60	<0.001* A vs B, C**
Monofilament ^f	6.6 ± 4.0	8.7 ± 3.0	10 ± 0.0	<0.001*

(responses/10)	0-10	0-10	10-10	A vs B, C**
	20	61	86	B vs C**
Intraepidermal nerve fibre density ^g	4.47±4.77	6.98±4.68	10.29±3.29	0.006
(number/mm)	0-11.66	2.85-16.24	4.96-16.63	A vs B, C**
	14	48	33	B vs C**

a Brisbane - Medoc VSA-3000 (Hz)

b Manchester – Horwell Bioesthesiometer (volts)

c Brisbane - where there was no response, the following substituted data were used: Peroneal CV 20 m/s; sural CV 31 m/s, reflecting lowest values recorded in this laboratory

d Manchester - where there was no response, the following substituted data were used: Peroneal CV 19 m/s; sural CV 5 m/s, reflecting lowest values recorded in this laboratory

e Brisbane - responses out of 3

f Manchester - responses out of 10

g Data from Manchester only

* Kruskal-Wallis test; ** Mann-Whitney U test

Figure Legend

Figure 1

Short title: Baseline corneal parameters of the LANDMark cohort

Detailed caption: Corneal nerve fibre length (A), corneal nerve branch density (B), and corneal sensation threshold (C) in participants with type 1 diabetes (T1) with and without neuropathy, and non-diabetic control participants, of the LANDMark cohort at baseline. Data shown are mean $\pm$ standard error.

Figure 1A
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Figure 1B
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