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ORIGINAL ARTICLE

Corneal confocal microscopy for the diagnosis of diabetic peripheral neuropathy: A systematic review and meta-analysis

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ABSTRACT

Introduction: Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic imaging technique that identifies corneal nerve fiber damage. Small studies suggest that CCM could be used to assess patients with diabetic peripheral neuropathy (DPN).

Aim: To undertake a systematic review and meta-analysis assessing the diagnostic utility of CCM for sub-clinical DPN (DPN^-) and established DPN (DPN^+).

Data sources: Databases (PubMed, Embase, Central, ProQuest) were searched for studies using CCM in patients with diabetes up to April 2020.

Study selection: Studies were included if they reported on at least one CCM parameter in patients with diabetes.

Data extraction: Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), and inferior whorl length (IWL) were compared between patients with diabetes with and without DPN and controls. Meta-analysis was undertaken using RevMan V.5.3.

Data synthesis: Thirty-eight studies including ~4,000 participants were included in this meta-analysis. There were significant reductions in CNFD, CNBD, CNFL, and IWL in DPN^- vs controls ($P < 0.00001$), DPN^+ vs controls ($P < 0.00001$), and DPN^+ vs DPN^- ($P < 0.00001$).

Conclusion: This systematic review and meta-analysis shows that CCM detects small nerve fiber loss in subclinical and clinical DPN and concludes that CCM has good diagnostic utility in DPN.

INTRODUCTION

Diabetic peripheral neuropathy (DPN) affects ~50% of patients with diabetes and leads to significant morbidity including neuropathic pain, erectile dysfunction, and foot ulceration[1]. Currently, the diagnosis of DPN in clinic relies on symptoms, loss of sensation to the 10 g monofilament, neurological examination, and occasionally electrophysiology[2]. However, these methods do not reliably detect small nerve fiber damage which occurs in early DPN[3].

In 2003, we showed that the ophthalmic technique of corneal confocal microscopy (CCM) can identify corneal small nerve fiber loss in patients with early and established DPN[4]. Subsequently we and others demonstrated good diagnostic utility for DPN[5–7], comparable to intra-epidermal nerve fiber density (IENFD)[8,9]. CCM also predicts incident DPN[8,10] and identifies individuals at higher risk of developing DPN[11]. However, some studies have failed to demonstrate corneal nerve fiber loss in patients with and without DPN[12,13], which has been attributed to a small sample size[13] and variances in image acquisition and analysis protocols[14].

We have undertaken a systematic review and meta-analysis to generate a definitive single estimate for the diagnostic utility of CCM in sub-clinical and clinical DPN.

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METHODS

Data sources and searches

This systematic review and meta-analysis is reported in accordance with MOOSE guidelines[15]. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on November 2020 (CRD42018093498). Four databases were chosen to search for this systematic review: PubMed, EMBASE (Ovid), CENTRAL, and web of science (WoS)- (1900-present). In the PubMed and CENTRAL database both Mesh subject headings and keywords were searched; in Embase-(1988-present) Emtree subject headings and keywords were utilized. Numerous terms were tested for relevancy and the final search strings for the three databases can be found in Table S1 in the supplement. Article language was limited to English and no date restrictions were set. A segment of the grey literature was searched through the use of dissertation and theses (ProQuest) and Clinicaltrials.gov. The databases were searched from inception to April 2020.

We included observational studies that reported on at least one of the following CCM parameters: corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), or inferior whorl length (IWL) in any of the following three groups: patients with type 1 and/or type 2 diabetes with diabetic peripheral neuropathy (DPN^+), without diabetic peripheral neuropathy (DPN^-), and controls. Cross-sectional and longitudinal observational studies were included in this systematic review and meta-analysis. Narrative reviews, systematic reviews, correspondence, and case reports were excluded. Study country, age, diagnosis (DPN^+, DPN^-, control), duration of diabetes, HbA1c, software used for image analysis, CNFD, CNBD, CNFL, and IWL were extracted when available. Studies using CCMetrics, ACCMetrics, ImageJ, and other morphometric software to quantify CNFD, CNBD, and CNFL were included. IWL was quantified using CCMetrics and ACCmetrics only. Data presented as median (IQR) were converted into mean ± SD using an online calculator and data presented as mean ± SEM were converted into mean ± SD using the RevMan calculator[16]. HbA1c presented in (%) was also converted into (mmol/mol) using the NGSP calculator, where NGSP % must be between 3 and 20[17]. Original studies that staged DPN as per the diabetic neuropathy study group in Japan (DNSGJ) were classified as: DPN^- for stage I, DPN^+ for stages II–V, for meta-analysis reporting purpose[18,19]. Stage I was reported as DPN^- and stages II–III were reported as DPN^+ in this study[20]. Patients classified according to the modified neuropathy disability score (NDS) were grouped as: scores between 0–2 (DPN^-) and 3–10 (DPN^+)[21,22]. No neuropathy was classified as DPN^- and mild-severe neuropathy was classified as DPN^+[23–26]. No differentiation was made for either painful or painless DPN and both were classified as DPN^+[27,28]. Where the vibration perception threshold (VPT) was used, <15V was classified as DPN^- and ≥15V as DPN^+[4].

Study selection

After the removal of duplicates, all citations were screened for relevance using the full citation, abstract, and indexing terms, before excluding studies deemed as irrelevant. Where there was a lack of consensus a third (senior) author was consulted. Duplicates were removed and the most recent and complete versions of the studies were reviewed for eligibility. Relevant studies were assessed by two reviewers (HG and INP) to assess eligibility according to the pre-specified inclusion and exclusion criteria. Full manuscripts of these potentially eligible citations were obtained. Two reviewers made the final inclusion and exclusion decisions independently and in the case of disagreement, a third reviewer was consulted to resolve any conflicts. A flow chart of search results was produced (Figure S1). A data collection tool was developed to extract the data from each study. Data verification was undertaken by two reviewers (HG and INP). In the event of missing data, the authors were emailed to obtain unpublished data.

Data extraction and quality assessment

The included studies were assessed using the Cochrane Collaborations tool for assessing the risk of bias (section 8.5)[29]. The tool categorizes the risk of bias into high, moderate, low, or unclear risk. This tool assessed six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias, where applicable. Quality assessment was undertaken by two reviewers (AK and GP). If the risk of bias of a study was unclear, the effect of removing the study was checked and relevant outcomes were reported (Table S2).

Data synthesis and analysis

Meta-analysis was performed in RevMan (version 5.3)[30]. Random effects meta-analysis was used in anticipation of heterogeneity due to differences in study population and type and duration of diabetes. The mean difference (MD) with a 95% confidence interval (CI) was calculated for CNFD, CNBD, CNFL, and IWL. The Chi-squared (χ^2) test was used to test for difference between subgroups. The I^2 statistic was calculated, which is derived from Cochrane's chi-squared test Q and is used to describe the percentage of between-study variations attributed to variability in the true exposure effect[29]. An I^2 value of 0–40% was classified as not important, 30–60% moderate, 50–90% substantial, and 75–100% considerable[29].

RESULTS

The search strategy identified 1,310 records (Figure S1). In total, 557 papers were screened on the basis of titles and abstracts, of which 508 were excluded, leaving 49 full text papers of which 38 were included in the meta-analysis.

Study characteristics

The studies were conducted in Canada[10,26,31–33], United Kingdom[4,8,9,21,24,25,28,34–42], Germany[27], Denmark[12], Australia[43–49], Japan[18,19,22,50], and China[23,51] (Table 1).

Table 1| | Characteristics of the included studies

Study	Country	Group	*n*	Age (years)	Duration of diabetes (years)	HbA1c% – mmol/mol	CCM Type	Software for image analysis	Assessment with CCM			
									CNFD	CNBD	CNFL	IWL
Ahmed *et al.*[31]	Canada	DPN+	33	50 ± 14.3	31.4 ± 13.5	8.7 ± 2.1–72	HRT-II	CCMetrics	√	√	√	
		DPN–	56	34.9 ± 14.8	17.6 ± 14	7.4 ± 1.3–57						
		Control	64	38.9 ± 17.6	N/A	NS						
Ostrovski *et al.*[32]	Canada	DPN+	13	56.2 ± 8.7	34.8 ± 13	8.5 ± 2.2–69	HRT-III	CCMetrics	√	√	√	
		DPN–	13	30.3 ± 13.7	10.7 ± 6.2	7.5 ± 1.3–58		ACCMetrics				
		Control	20	41.3 ± 17.3	N/A	5.5 ± 0.4–37						
Lovblom *et al.*[10]	Canada	DPN+	11	38 ± 16	21 ± 9	8.1 ± 1.6–65	HRT-III	CCMetrics	√	√	√	
		DPN–	54	34 ± 15	17 ± 12	7.6 ± 1.3–60						
Sivaskandarajah *et al.*[33]	Canada	DPN+	33	48.5 ± 13.7	32.3 ± 13.1	8.4 ± 1.6–68	HRT-III	CCMetrics	√	√	√	
		DPN–	63	32.7 ± 13.6	17.3 ± 12.2	7.5 ± 1.2–58						
		Control	64	38.3 ± 16.4	N/A	5.6 ± 0.4–38						
Hertz *et al.*[26]	Canada	DPN+	14	NS	NS	NS	HRT-III	CCMetrics	√	√	√	
		DPN–	12	NS	NS	NS						
		Control	20	41.4 ± 17.3	N/A	5.5 ± 0.4–37						
Alam *et al.*[9]	UK	DPN+	31	53.3 ± 11.9	37.2 ± 13.1	8.5 ± 1.5–69	HRT-III	CCMetrics	√	√	√	
		DPN–	30	38.8 ± 12.5	17.2 ± 12	8 ± 1.3–64						
		Control	27	41 ± 14.9	N/A	5.5 ± 0.3						
Azmi *et al.*[34]	UK	DPN+	29	61.9 ± 12.3	46 ± 13.9	8.3 ± 1.3	HRT-III	ACCMetrics	√	√	√	
		Control	32	47.7 ± 1.6	N/A	5.7 ± 0.6						
Chen *et al.*[35]	UK	DPN+	29	63 ± 12	19.9 ± 11.7	8.6 ± 3.6–70.4 ± 16	HRT-III	CCMetrics	√	√	√	
		DPN–	63	44 ± 15	20 ± 11.1	8 ± 4.1–63.9 ± 21.2		ACCMetrics				
		Control	84	46 ± 15	N/A	5.6–37.4 ± 3.5						
Brines *et al.*[21]	UK	DPN+	60	35.3 ± 14.3	35.3 ± 14.3	8.2 ± 1.3–66	HRT-III	ACCMetrics	√	√	√	
		DPN–	21	37.1 ± 16.5	17.9 ± 15.1	7.9 ± 1.3–63						
		Control	48	46.2 ± 16.9	N/A	5.7 ± 0.3–39						
Petropoulos *et al.*[36]	UK	DPN+	25	60.1 ± 10.2	24.8 ± 19.5	7.6 ± 1.5–60	HRT-III	CCMetrics	√		√	√
		DPN–	28	42.4 ± 14.7	16.2 ± 9.3	NS						
		Control	15	NS	N/A	5.4 ± 0.5–36						
Chen *et al.*[8]	UK	DPN+	17	59 ± 11	39 ± 14	8.5 ± 1.3–69	HRT-III	CCMetrics	√	√	√	
		DPN–	46	44 ± 13	23 ± 15	8.2 ± 1.4–66		ACCMetrics				
		Control	26	44 ± 15	N/A	5.5 ± 0.3–37						
Petropoulos *et al.*[37]	UK	DPN+	61	56.5 ± 13.2	35.33 ± 14.3	8.4 ± 1.8–68	HRT-III	CCMetrics	√	√	√	
		DPN–	50	44.2 ± 15.6	23 ± 14	7.9 ± 1.7–63						
		Control	47	52 ± 13.2	N/A	5.6 ± 0.3–38						
Petropoulos *et al.*[38]	UK	DPN+	100	NS	34.4 ± 17.3	7.9 ± 1.6–63	HRT-III	CCMetrics	√	√	√	
		DPN–	86	NS	24.2 ± 21.2	7.7 ± 1.6–61		ACCMetrics				
		Control	55	51.7 ± 11.4	N/A	5.5 ± 0.3–37						
Ponirakis *et al.*[39]	UK	DPN+	46	60.75 ± 8.9	36.5 ± 14.4	8.6 ± 0.4–70	HRT-III	CCMetrics	√	√	√	
		DPN–	64	45.5 ± 14.4	22.25 ± 13	7.62 ± 0.48–60						

Table 1 (*Continued*)

Study	Country	Group	*n*	Age (years)	Duration of diabetes (years)	HbA1c% – mmol/mol	CCM Type	Software for image analysis	Assessment with CCM			
									CNFD	CNBD	CNFL	IWL
Quattrini *et al.*[24]	UK	DPN+	44	59.3 ± 17.25	NS	8.01 ± 2.32–64	Confoscan-P4	Morphometric software	√	√	√	
		DPN–	10	43.5 ± 10.2	NS	7.16 ± 1.26–55						
		Control	15	55 ± 18.5	N/A	NS						
Tavakoli *et al.*[40]	UK	DPN+	67	59 ± 18.2	17.8 ± 29.55	8.2 ± 2.70–66	Confoscan-P4	Morphometric software	√	√	√	
		DPN–	34	55 ± 11.1	10.7 ± 10.6	8.1 ± 1.57–65						
		Control	17	55 ± 19.8	N/A	<6.5 < 48						
Tavakoli *et al.*[25]	UK	DPN+	96	59 ± 20	59 ± 20	8.30 ± 30.14–67	Confoscan-P4	CCMetrics	√	√	√	
		DPN–	42	57 ± 13	57 ± 13	7.88 ± 10.23–63						
		Control	26	53 ± 3	N/A	~5.8–40						
Kalteniece *et al.*[41]	UK	DPN+	69	62.08 ± 11.6	20.78 ± 17.8	7.19 ± 10.16–55	HRT-III	CCMetrics	√	√	√	√
		DPN–	47	46.9 ± 13.2	16.04 ± 12.2	7.72 ± 2.06–61						
		Control	22	50.32 ± 13.7	N/A	5.48 ± 00.42–36						
Kalteniece *et al.*[28]	UK	DPN+	140	65.09 ± 1.13	21.8 ± 2.05	7.5 ± 0.17–58	HRT-III	CCMetrics	√	√	√	√
		Control	30	61.2 ± 1.33	N/A	5.63 ± 00.06–38						
Malik *et al.*[4]	UK	DPN+	14	59.2 ± 9.9	23.4 ± 6.25	8.15 ± 1.3–66	Confoscan-P4	Morphometric software	√	√	√	
		DPN–	4	53 ± 18.5	21.3 ± 3.6	7.8 ± 0.8–62						
		Control	18	57.8 ± 11.5	N/A	<6.5–48						
Ponirakis *et al.*[42]	UK	DPN+	33	64.1 ± 1.79	37.6 ± 3.2	7.9 ± 0.26–63	HRT-III	ACCMetrics	√			
		DPN–	41	44.3 ± 2.19	23.3 ± 2.03	7.5 ± 0.18–58						
		Control	70	41.8 ± 1.63	N/A	5.29 ± 0.12–34						
Puttgen *et al.*[27]	Germany	DPN+	116	67.3 ± 9	17.6 ± 13	7.41 ± 1.3–57	HRT-III	CCMetrics ACCMetrics	√	√	√	
		Control	46	66 ± 5.2	N/A	5.44 ± 0.23–36						
Andersen *et al.*[12]	Denmark	DPN+	27	71.4 ± 3.1	12.2 ± 1.23	6.95 ± 0.48–52	HRT-III	ACCMetrics	√	√	√	
		DPN–	117	69.7 ± 2.7	11.67 ± 1.12	6.6 ± 0.33–49						
		Control	25	71.2 ± 0.69	N/A	5.5 ± 0.22–37						
Tummanapalli *et al.*[44]	Australia	DPN+	28	NS	NS	8.45 ± 0.5–69	HRT-III	ACCMetrics	√	√	√	√
		DPN–	35			7.59 ± 0.6–59						
		Control	34									
Dehghani *et al.*[47]	Australia	DPN+	13	NS	NS	NS	HRT-III	CCMetrics ACCMetrics			√	
		DPN–	20									
		Control	17									
Tummanapalli *et al.*[49]	Australia	DPN+	23	47 ± 15	22 ± 13	8.89 ± 1.9–74	HRT-III	ACCMetrics	√	√	√	√
		DPN–	27	32 ± 10	15 ± 9	7.83 ± 1.02–62						
		Control	29	37 ± 11	N/A	NS						
Tummanapalli *et al.*[43]	Australia	DPN+	35	51 ± 9.5	NS	8 ± 1.4–64	HRT-III	ACCMetrics	√	√	√	√
		DPN–	35	44.5 ± 11		8 ± 2–64						
Pritchard *et al.*[48]	Australia	DPN+	25	NS	NS	NS	HRT-III	CCMetrics			√	√
		DPN–	82	NS								
		Control	80	37.0 ± 17.8								

Table 1 (*Continued*)

Study	Country	Group	*n*	Age (years)	Duration of diabetes (years)	HbA1c% – mmol/mol	CCM Type	Software for image analysis	Assessment with CCM			
									CNFD	CNBD	CNFL	IWL
Edwards *et al.*[46]	Australia	DPN+	88	58 ± 9	23 ± 14	8.2 ± 1.7–66	HRT-III	CCMetrics		√	√	√
		DPN–	143	48 ± 16	14 ± 12	7.8 ± 1.2–62						
		Control	61	52 ± 14	N/A	5.4 ± 0.3–36						
Dehghani *et al.*[45]	Australia	DPN+	39	NS	NS	NS	HRT-III	ACCMetrics	√	√	√	
		DPN–	108	NS	NS							
		Control	60	NS	N/A							
Ishibashi *et al.*[18]	Japan	DPN+	55	56.4 ± 14.1	9.6 ± 16.3	8.03 ± 3.0–64	HRT-III	ImageJ	√	√	√	
		DPN–	23	48.1 ± 10.6	5.8 ± 5.8	7.7 ± 2.11–61						
		Control	28	50.2 ± 7.41	N/A	5.6 ± 0.26–38						
Ishibashi *et al.*[19]	Japan	DPN+	153	56.03 ± 10.3	12.4 ± 8.2	8.3 ± 3.5–67	HRT-III	ImageJ	√	√	√	
		DPN–	47	53.4 ± 7.54	10.5 ± 14.8	7.3 ± 1.4–56						
		Control	40	53.6 ± 12.65	N/A	5.7 ± 0.32–39						
Ishibashi *et al.*[22]	Japan	DPN+|	115	54.4 ± 19.1	7.9 ± 11.4	9.06 ± 4.4–76	HRT-III	ImageJ	√	√	√	
		DPN–	47	52.4 ± 9.6	5 ± 4.5	8.5 ± 1.4–69						
		Control	45	52.8 ± 4.7	N/A	5.5 ± 0.03–37						
Ishibashi *et al.*[50]	Japan	DPN+	18	59.4 ± 8.1	13.6 ± 10.61	9 ± 1.74–75	HRT-III	ImageJ	√	√	√	
		DPN–	57	54.4 ± 12.1	6.7 ± 6.34	9.1 ± 2.4–76						
		Control	42	53.1 ± 11.7	N/A	5.7 ± 0.4–39						
Li *et al.*[51]	China	DPN+	79	70.15 ± 7.34	12.58 ± 7.28	7.94 ± 1.86–63	HRT-II	CCMetrics ACCMetrics	√	√	√	
		DPN–	49	67.12 ± 6.01	9.79 ± 7.09	7.07 ± 0.96–54						
		Control	24	68.3 ± 5.19	N/A	5.88 ± 0.82–41						
Xiong *et al.*[23]	China	DPN+	79	70.3 ± 10	12.57 ± 10.2	7.95 ± 3.4–63	HRT-II	ImageJ	√	√	√	
		DPN–	49	67.12 ± 6.13	9.79 ± 7.14	7.07 ± 1.68–54						
		Control	24	68.63 ± 5.2	N/A	5.88 ± 0.83–41						
Pritchard *et al.*[70]	Australia, Canada, UK	DPN+	16	51 ± 14	29 ± 16	8 ± 1.1–64	HRT-III	CCMetrics			√	
		DPN–	74	42 ± 16	15 ± 12	7.9 ± 1.2–63						
Pritchard *et al.*[52]	Australia, UK	DPN+	48	57 ± 11	34 ± 16	8.6 ± 1.8–70	HRT-III	CCMetrics		√	√	
		DPN–	100	43 ± 16	20 ± 15	8 ± 1.2–64						
		Control	60	46 ± 15	N/A	5.5 ± 0.3–37						

Data are presented as mean ± SD. CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; IWL, inferior whorl length; NS, not stated, N/A, not applicable.

Figure 1 | (a) Forest plots of corneal nerve fiber density (CNFD) in patients with diabetic peripheral neuropathy (DPN^+) and without diabetic peripheral neuropathy (DNP^-). (b) Forest plots of corneal nerve fiber density (CNFD) in patients with diabetic peripheral neuropathy (DPN^+) and healthy control. (c) Forest plots of corneal nerve fiber density (CNFD) in patients without diabetic peripheral neuropathy (DNP^-) and healthy control.

Corneal nerve fiber density

DPN^+ vs DPN^-

Twenty-nine studies[4,8–10,12,18,19,21–26,31–33,35,37–44,50,51] with 3,214 (1,677 DPN^+ and 1,537 DPN^-) participants were included in the meta-analysis. The CNFD (fiber/mm^2) was significantly lower in the DPN^+ group compared with the DPN^- group (MD = −7.01, 95% CI −7.45 to 6.57, $P < 0.00001$) (CCMetrics (MD = −6.83, 95% CI −7.82 to −5.84, $P < 0.00001$), ACCMetrics (MD = −7.77, 95% CI −8.32 to −7.22, $P < 0.00001$), ImageJ (MD = −3.48, 95% CI −4.64 to −2.33, $P < 0.00001$), and morphometric software (MD = −11.40, 95% CI −15.42 to −7.38, $P < 0.00001$)). There was a significant difference in the magnitude of the CNFD reduction in the DPN^+ group between studies ($\chi^2 = 19.32$, $P = 0.0002$) (Figure 1a).

DPN^+ vs control

Twenty-nine studies[4,8,9,12,18,19,21–28,31–35,37,38,40,41,43–45,50,51] with 3377 (1994 DPN^+ and 1383 control) participants were included in the meta-analysis. The CNFD (fiber/mm^2) was significantly lower in the DPN^+ group compared with the controls (MD = −11.94, 95% CI −12.25 to −11.62, $P < 0.00001$) (CCMetrics (MD = −10.83, 95% CI −11.26 to −10.40, $P < 0.00001$), ACCMetrics (MD = −13.75, 95% CI −14.26 to −13.25, $P < 0.00001$), ImageJ (MD = −8.98, 95% CI −10.40 to −7.55, $P < 0.00001$), and morphometric software (MD = −22.26, 95% CI −27.67 to −16.85, $P < 0.00001$). There was a significant difference in the magnitude of the CNFD reduction in the DPN^+ group between studies ($\chi^2 = 15.50$, $P = 0.001$) (Figure 1b).

DPN^- vs control

Twenty-seven studies[4,8,9,12,18,19,21–26,31–33,35,37,38,40–45,50,51] with 3,035 (1,620 DPN^- and 1,415 control) participants were included in the meta-analysis. The CNFD (fiber/mm^2) was significantly lower in the DPN^- group compared with the controls (MD = −5.85, 95% CI −6.12 to −5.57, $P < 0.00001$) (CCMetrics (MD = −5.76, 95% CI −6.15 to −5.37, $P < 0.00001$), ACCMetrics (MD = −5.91, 95% CI −6.32 to −5.50], $P < 0.00001$), ImageJ (MD = −5.89, 95% CI −7.13 to −4.65, $P < 0.00001$), and morphometric software (MD = −11.07, 95% CI −16.34 to −5.80, $P < 0.0001$). There was no significant difference in the magnitude of the CNFD reduction in the DPN^- group between studies ($\chi^2 = 4.01$, $P = 0.26$) (Figure 1c).

Corneal nerve branch density

DPN^+ vs DPN^-

Thirty studies[4,8–10,12,18,19,21–26,31–33,35,37–41,43–46,50–52] with 3,552 (1,763 DPN^+ and 1,789 DPN^-) participants were included in the meta-analysis. The CNBD (branch/mm^2) was significantly lower in the DPN^+ group compared with the DPN^- group (MD = −3.36, 95% CI −4.11 to −2.61, $P < 0.00001$) (CCMetrics (MD = −10.37, 95% CI −12.56 to −8.18, $P < 0.00001$), and ACCMetrics (MD = −8.20, 95% CI −10.20 to −6.20, $P < 0.00001$). There was a significant difference in the extent of the CNBD reduction in the DPN^+ group between studies ($\chi^2 = 30.97$, $P < 0.00001$), (Figure 2a).

DPN^+ vs control

Thirty studies[4,8,9,12,18,19,21–28,31–35,37,38,40,41,43–46,50,51] with 3,460 (2,072 DPN^+ and 1,388 control) participants were included in the meta-analysis. The CNBD (branch/mm^2) was significantly lower in the DPN^+ group compared with the controls (MD = −11.00, 95% CI −11.65 to −10.35, $P < 0.00001$) (CCMetrics (MD = −20.87, 95% CI −22.05 to −19.68, $P < 0.00001$), ACCMetrics (MD = −7.34, 95% CI −8.35 to −6.32, $P < 0.00001$), ImageJ (MD = −4.79, 95% CI −6.05 to −3.53, $P < 0.0001$), and morphometric software (MD = −21.81, 95% CI −26.61 to −17.01, $P = 0.0003$)). There was a significant difference in the magnitude of the CNBD reduction in the DPN^+ group between studies ($\chi^2 = 30.98$, $P < 0.00001$) (Figure 2b).

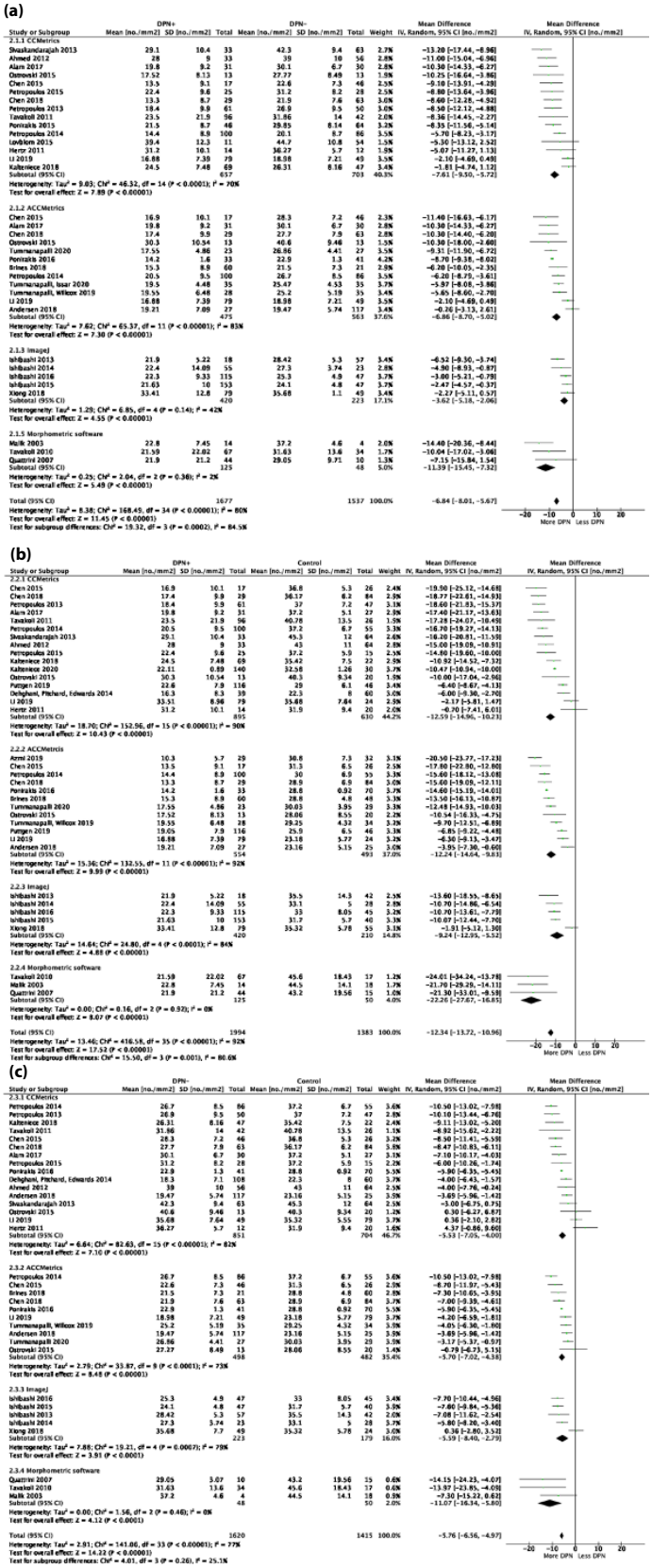
DPN^- vs control

Twenty-six studies[4,8,12,18,19,21–24,26,31–33,35,37,38,40,41,43–46,50–52] with 2,813 (1,606 DPN^- and 1,207 control) participants were included in the meta-analysis. The CNBD (branch/mm^2) was significantly lower in the DPN^- group compared with the controls (MD = −6.37, 95% CI −7.31 to −5.44, $P < 0.00001$) (CCMetrics (MD = −11.08, 95% CI −13.40 to −8.75, $P < 0.00001$), ACCMetrics (MD = −11.17, 95% CI −13.46 to −8.88, $P < 0.00001$), ImageJ (MD = −3.34, 95% CI −4.52 to −2.17, $P < 0.0001$), and morphometric software (MD = −16.26, 95% CI −21.14 to −11.37, $P = 0.007$)). There was a significant difference in the magnitude of the CNBD reduction in the DPN^- group between studies ($\chi^2 = 33.32$, $P < 0.00001$) (Figure 2c).

Corneal nerve fiber length

DPN^+ vs DPN^-

Thirty-four studies[4,8–10,12,18,19,21–26,31–33,35,37–41,43–48,50–53] with 3,868 (1,855 DPN^+ and 2,013 DPN^-) participants were included in the meta-analysis. The CNFL (mm/mm^2) was significantly lower in the DPN^+ group compared with the DPN^- group (MD = −3.08, 95% CI −3.58 to −2.58, $P < 0.00001$) (CCMetrics (MD = −3.74, 95% CI −4.49 to −2.99,



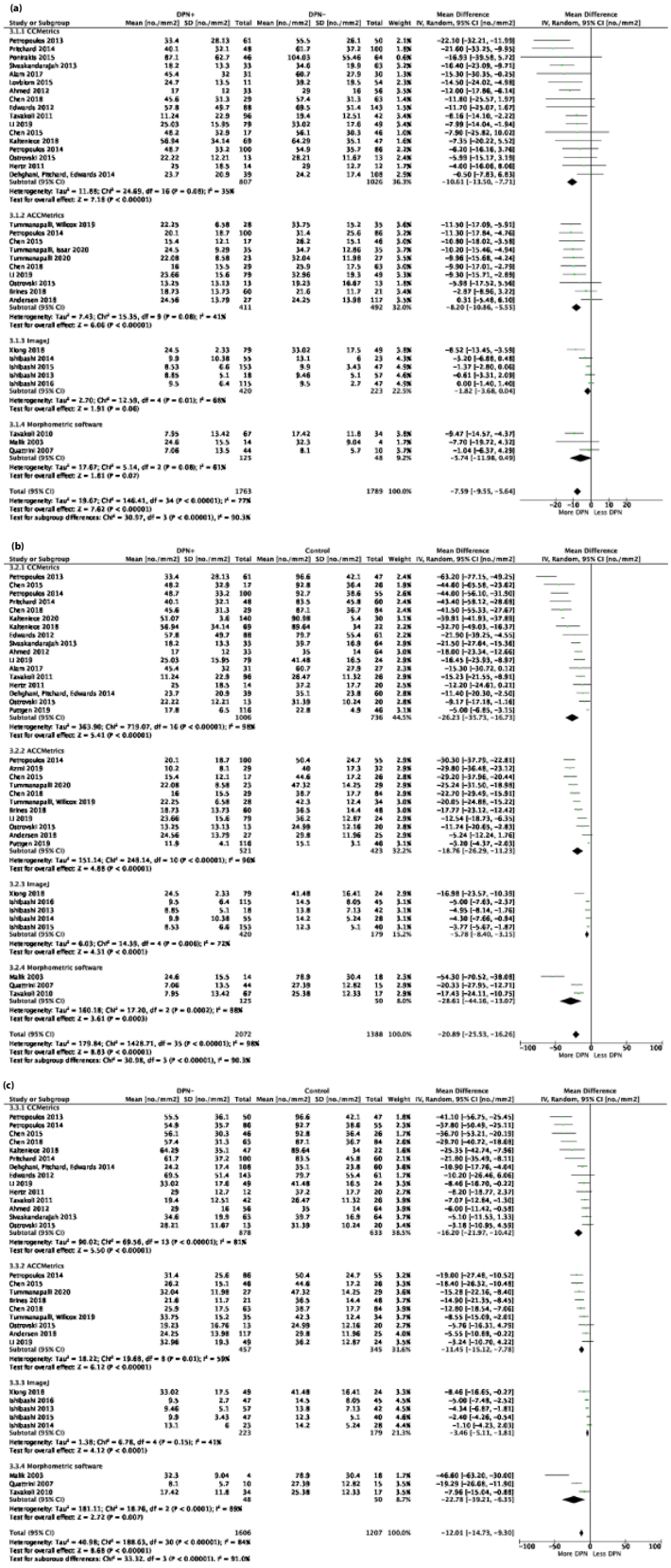


Figure 2 | (a) Forest plots of corneal nerve branch density (CNBD) in patients with diabetic peripheral neuropathy (DPN^+) and without diabetic peripheral neuropathy (DNP^-). (b) Forest plots of corneal nerve branch density (CNBD) in patients with diabetic peripheral neuropathy (DPN^+) and healthy control. (c) Forest plots of corneal nerve branch density (CNBD) in patients without diabetic peripheral neuropathy (DNP^-) and healthy control.

$P < 0.00001$), ACCMetrics (MD = −2.80, 95% CI −3.57 to −2.04, $P < 0.00001$), ImageJ (MD = −1.57, 95% CI −2.06 to −1.09, $P < 0.00001$), and morphometric software (MD = −3.49, 95% CI −5.63 to −1.35, $P = 0.001$). There was a significant difference in the magnitude of the CNFL reduction in the DPN^+ group between studies ($\chi^2 = 25.42$, $P < 0.00001$) (Figure 3a).

DPN^+ vs control

Thirty-two studies[4,8,9,12,18,19,21–26,28,31–35,37,38,40,43–47,50–52] with 3,459 (2,036 DPN^+ and 1,423 control) participants were included in the meta-analysis. The CNFL (mm/mm^2) was significantly lower in the DPN^+ group compared with the controls (MD = −6.05, 95% CI −6.77 to −5.34, $P < 0.00001$) (CCMetrics (MD = −6.91, 95% CI −8.06 to −5.76, $P < 0.00001$), ACCMetrics (MD = −5.49, 95% CI −7.03 to −3.95, $P < 0.00001$), ImageJ (MD = −4.14, 95% CI −4.72 to −3.56, $P < 0.00001$), and morphometric software (MD = −6.07, 95% CI −8.64 to −3.50, $P < 0.00001$). There was a significant difference in the magnitude of CNFL reduction between studies ($\chi^2 = 19.59$, $P = 0.0002$) (Figure 3b).

DPN^- vs control

Thirty studies[4,8,9,12,18,19,21–26,31–33,35,37,38,40,41,43–48,50–52] with 3,149 (1,786 DPN^- and 1,363 control) participants were included in the meta-analysis. The CNFL (mm/mm^2) was significantly lower in the DPN^- group compared with the controls (MD = −2.87, 95% CI −3.34, −2.40, $P < 0.00001$) (CCMetrics (MD = −3.12, 95% CI −4.06 to −2.19, $P < 0.00001$), ACCMetrics (MD = –2.63, 95% CI −3.43 to −1.83, $P < 0.00001$), ImageJ (MD = −2.78, 95% CI −3.35 to −2.22, $P < 0.00001$), and morphometric software (MD = −2.68, 95% CI −3.48 to −1.88, $P < 0.00001$). There was no difference in the magnitude of the CNFL reduction in the DPN^- group between studies ($\chi^2 = 0.72$, $P = 0.87$), (Figure 3c).

Inferior whorl length

DPN^+ vs DPN^-

Six studies[8,41,43,44,48] with 459 (205 DPN^+ and 254 DPN^-) participants were included in the meta-analysis. The IWL (mm/mm^2) was significantly lower in the DPN^+ group compared with the DPN^- group (MD = −4.11, 95% CI −5.10 to −3.12, $P < 0.00001$) (CCMetrics (MD = −3.42, 95% CI −5.47 to −1.36, $P = 0.001$), and ACCMetrics (MD = −4.40, 95% CI −5.53 to −3.28, $P < 0.00001$). There was no significant difference in the magnitude of the CNFL reduction in the DPN^+ group between studies ($\chi^2 = 0.68$, $P = 0.41$), (Figure 4a).

DPN^+ vs control

Six studies[8,28,41,43,44,48] with 520 (310 DPN^+ and 210 control) participants were included in the meta-analysis. The IWL (mm/mm^2) was significantly lower in the DPN^+ group compared with the controls (MD = −10.36, 95% CI −13.30 to −7.42, $P < 0.00001$) (CCMetrics (MD = −11.62, 95% CI −15.97 to −7.28, $P < 0.00001$), and ACCMetrics (MD = −8.32, 95% CI −9.40 to −7.24, $P < 0.00001$)). There was no significant difference in the extent of the IWL reduction in the DPN^+ group between studies ($\chi^2 = 2.08$, $P = 0.15$), (Figure 4b).

DPN^- vs control

Five studies[8,41,43,44,48] with 399 (219 DPN^- and 180 control) participants were included in the meta-analysis. The IWL (mm/mm^2) was significantly lower in the DPN^- group compared with the controls (MD = −3.81, 95% CI −4.56 to −3.06, $P < 0.00001$) (CCMetrics (MD = −4.43, 95% CI −5.56 t0 −3.29, $P = 0.003$), and ACCMetrics (MD = −3.34, 95% CI −4.33 to −2.34, $P < 0.00001$). There was no significant difference in the extent of IWL reduction in the DPN^- group between studies ($\chi^2 = 2.11$, $P = 0.15$), (Figure 4c).

DISCUSSION

In this large systematic review and meta-analysis of over 3,000 participants, CCM demonstrated a consistent reduction in four major corneal nerve parameters in patients with DPN compared with healthy controls and those without DPN. Furthermore, we demonstrate a lesser but significant reduction in all corneal nerve parameters in patients without DPN compared with controls, suggesting that CCM detects early sub-clinical DPN. This is consistent with the demonstration of corneal nerve loss in subjects with impaired glucose tolerance[54], recently diagnosed type 2 diabetes[55] and children with type 1 diabetes[56]. The greater corneal nerve loss in patients with DPN compared with those without DPN is consistent with studies showing that corneal nerve loss is associated with the severity of DPN[4,24,51,57,58] and has good sensitivity and specificity for diagnosing DPN[5–7]. Both CNFD and IENFD have a comparable diagnostic performance for DPN[8,9,59], although in a study of patients with recently diagnosed type 2 diabetes there were differences in the extent of small nerve fiber damage between CCM and skin biopsy[57]. Additionally, a reduction in corneal nerve parameters is associated with incident DPN[10,53,60] and greater corneal nerve loss[41], and augmented nerve branching[27] occurs in patients with painful diabetic neuropathy. CCM could act as a biomarker as defined by the NIH Biomarkers

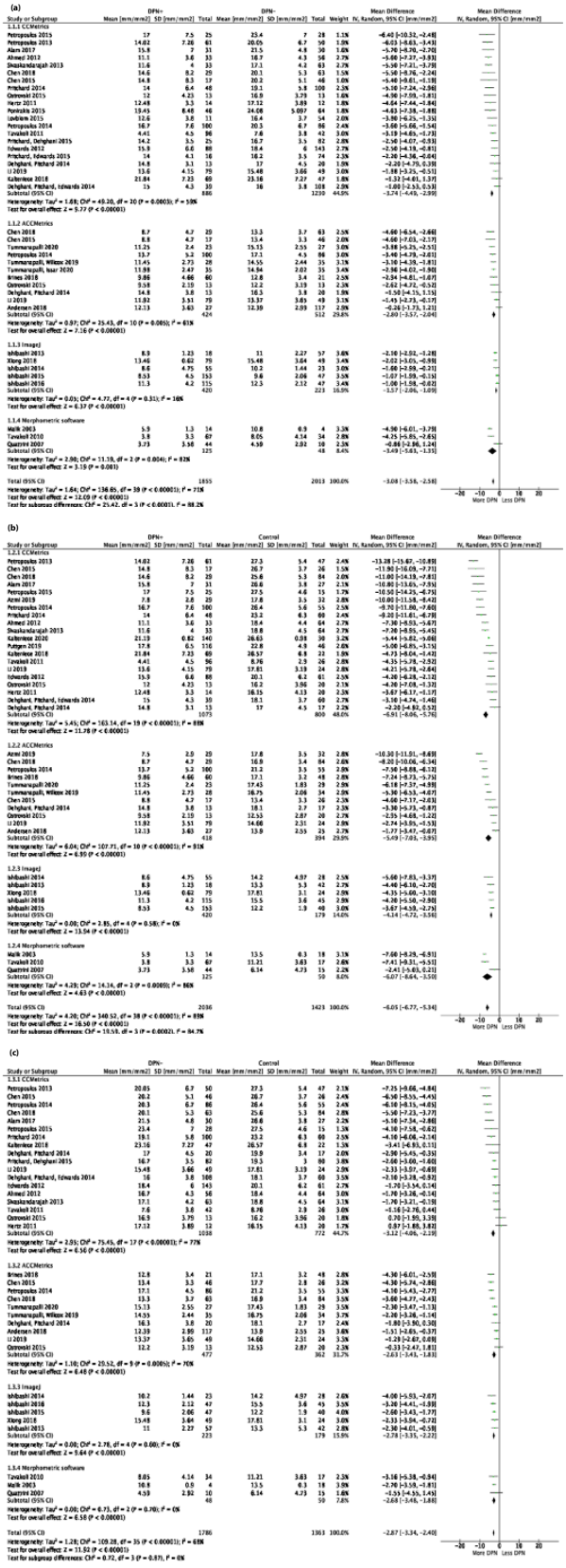


Figure 3 | (a) Forest plots of corneal nerve fiber length (CNFL) in patients with diabetic peripheral neuropathy (DPN^{+}) and without diabetic peripheral neuropathy (DNP^{-}). (b) Forest plots of corneal nerve fiber length (CNFL) in patients with diabetic peripheral neuropathy (DPN^{+}) and healthy control. (c) Forest plots of corneal nerve fiber length (CNFL) in patients without diabetic peripheral neuropathy (DNP^{-}) and healthy control.

Definitions Working Group[61]; it is non-invasive, easily measured, and produces rapid results with high sensitivity[5–7]. It allows the detection of subclinical DPN, and there is minimal overlap in corneal nerve parameters between patients with and without DPN and healthy people. In addition, CCM identifies those at risk of developing DPN[10,11,53].

The outcomes of the current review extend considerably the findings of a previous systematic review and meta-analysis showing a reduction in CNFD, CNBD, and CNFL in patients with and without DPN compared with controls from 13 studies with 1,680 participants[62] and a more recent trial sequential meta-analysis which showed a reduction in CNFD, CNBD, and CNFL in patients with and without DPN compared with controls in 13 studies with 1,830 participants[14].

In the present review we have included IWL which has the potential to detect earlier nerve damage[36,63,64], especially in patients with painful diabetic neuropathy[28,41].

The reliability of establishing a single estimate for the effect size of corneal nerve outcome measures from all the published studies may be affected by the inclusion of the same subjects from several studies, type of CCM used to acquire the images, the mode of image acquisition, and the image analysis tool used to quantify corneal nerve parameters. Our analysis showed that the type of software used for image analysis had no significant influence on the heterogeneity of corneal nerve outcomes. Whilst the corneal nerve measure was lower when using automated (ACCMetrics) compared with manual (CCMetrics, ImageJ) software, the magnitude of difference in corneal nerve parameters between groups was comparable[38,65].

Our sensitivity analysis shows no evidence of significant bias or heterogeneity (Doc S1). This was expected, given that there may be differences in corneal nerve parameters between patients with type 1 and type 2 diabetes[5,7,13] and in relation to HbA1c[66] and glycemic variability[67], presence of metabolic syndrome[68] and hypertension or hyperlipidemia[7,69].

CONCLUSIONS

Corneal confocal microscopy is a rapid, non-invasive and reproducible imaging technique to quantify small nerve fiber damage. Our systematic review and meta-analysis provides robust evidence that corneal confocal

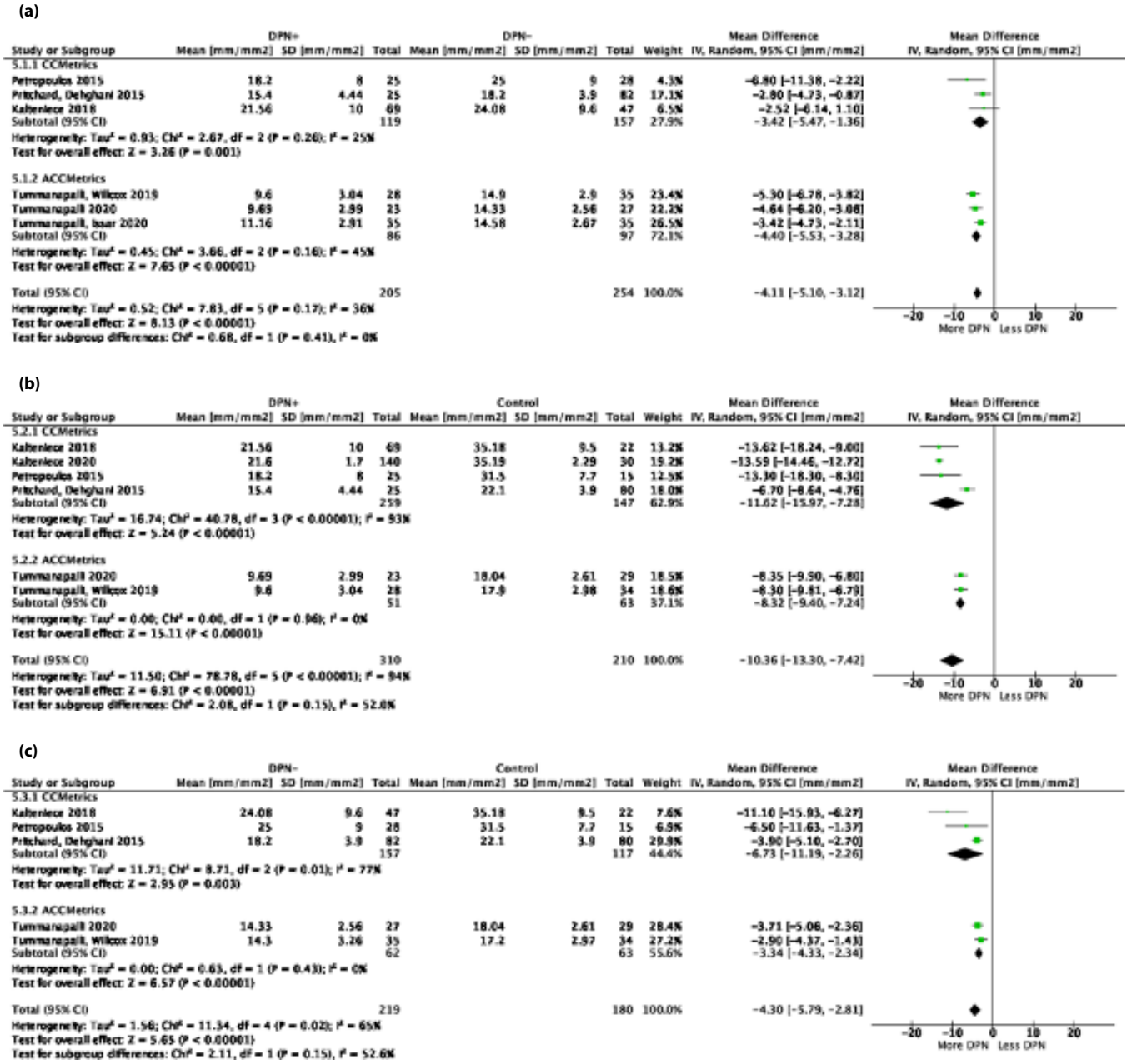


Figure 4 | (a) Forest plots of inferior whorl length (IWL) in patients with diabetic peripheral neuropathy (DPN^+) and without diabetic peripheral neuropathy (DNP^-). (b) Forest plots of inferior whorl length (IWL) in patients with diabetic peripheral neuropathy (DPN^+) and healthy control. (c) Forest plots of inferior whorl length (IWL) in patients without diabetic peripheral neuropathy (DNP^-) and healthy control.

microscopy can be used to diagnose sub-clinical and established DPN.

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DISCLOSURE

Approval of the research protocol: N/A.
Informed Consent: N/A.
Approval date of Registry and Registration No. of the study/-trial: N/A.
Animal Studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Flowchart of the included studies.
Table S1 | Search details
Table S2 | Risk of bias assessment for non-randomized studies
Doc S1 | Methods. Risk of bias and sensitivity analysis.