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REVIEW ARTICLE

Corneal confocal microscopy: Recent progress in the evaluation of diabetic neuropathy

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ABSTRACT

The present brief review discusses recent progress with corneal confocal microscopy for the evaluation of diabetic sensorimotor polyneuropathy. Corneal confocal microscopy is a new, non-invasive and reproducible diagnostic modality, and it can also be easily applied for patient follow up. It enables new perspectives of studying the natural history of diabetic sensorimotor polyneuropathy, severity of nerve fiber pathology and documenting early nerve fiber regeneration after therapeutic intervention. It shows moderate to high sensitivity and specificity for the timely diagnosis of diabetic sensorimotor polyneuropathy. Currently, corneal confocal microscopy is mainly used in specialized centers, but deserves more widespread application for the assessment of diabetic sensorimotor polyneuropathy. Finally, further progress is required in terms of technical improvements for automated nerve fiber quantification and for analysis of larger images.

INTRODUCTION

Chronic diabetic sensorimotor polyneuropathy (DSPN) is the most common diabetic complication involving the nervous system[1,2]. Its diagnosis mainly rests on careful clinical examination including sensory and motor modalities[1], while new practical tests have been developed during the past 15 years to improve its diagnosis in clinical reality[3,4]. *In vivo* corneal confocal microscopy (CCM) of the human eye is one of these new diagnostic modalities[5]. It is best used in the expert setting, and enables early demonstration of nerve fiber loss[5]. The aim of the present brief review was to discuss recent progress with CCM in the evaluation of DSPN.

Search Strategy

We carried out an electronic search through the PubMed, Embase and Google scholar databases up to 10 January 2015 using the following key words in various combinations: 'complications,' 'cornea,' 'corneal confocal microscopy,' 'diabetes,' 'diabetic,' 'diagnosis,' 'neuropathy,' 'polyneuropathy' and 'small-fiber.' Articles written in English were studied in full, whereas those written in other languages were only studied in abstract form.

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Corneal Nerve Fibers and CCM

The cornea of the human eye harbors a multitude of nerve fibers originating from the ophthalmic division of the trigeminal nerve, and is organized in three main groups: the sub-basal plexus, the sub-epithelial plexus and the stromal nerves[6]. Of these, it is the sub-basal nerve plexus lying underneath the basal epithelium that has received the most attention by CCM for the evaluation of diabetic neuropathy[7]. In addition, corneal Langerhans cells, which represent antigen-presenting cells, have attracted some interest during the study of diabetic neuropathy by CCM[8].

In regard to the technique involved, CCM makes use of a light beam, which passes through an aperture and is appropriately focused by an objective lens into the examined cornea layer[9,10]. At the same time, all light coming from other points is eliminated by a beam splitter and a photodetection device[9,10]. Three methodological variants have been developed: the tandem scanning CCM (TSCM), the slit-scanning CCM (SSCM) and the more recent laser scanning CCM (LSCM)[7–12]. SSCM and LSCM have been used for the detection of corneal nerve pathology in diabetic patients with and without DSPN[7]. The former enables rapid visualization of all points along the axis of a given slit[7,9,10,12]. The latter has been used more recently, and employs a laser beam as a light source, offering higher resolution and clearer visualization of corneal epithelium and stroma[7,9,10,12,13]. Even more recent technological progress in

LSCM includes automated software, higher magnification lenses, real-time images, and 3-D reconstruction[14–19].

Role of CCM in the Assessment of Diabetic Neuropathy

The following parameters have been mainly used in the assessment of corneal nerve pathology: (i) corneal nerve fiber density (CNFD), defined as the total number of major nerves per mm^2; (ii) corneal nerve fiber length (CNFL), defined as the total length of all nerve fibers and branches (mm/mm^2); (iii) corneal nerve branch density (CNBD), defined as the number of branches emanating from major nerves per mm^2; and (iv) corneal nerve fiber tortuosity (CNFTo), mathematically calculated as total nerve fiber curvature reflecting the variability of nerve fiber directions[7]. Importantly, these parameters, especially CNFL[7], have now proved to be highly reproducible[20–22]. More recently, Petropoulos *et al.*[23] have shown that manual CNFD and automated CNFL yielded the highest diagnostic performance for DSPN. An interesting novel parameter is tortuosity-standardized CNFL[24]. With this approach, standardized CNFL shows higher correlations with established measures and risk factors for DSPN as compared with classical CNFL[24].

In CCM studies, diagnosis and staging of DSPN has rested on clinical examination, mainly neuropathic deficits, occasionally also nerve conduction studies (NCS)[5,8,25–31]. Other measures of DSPN have included intra-epidermal nerve fiber density (IENFD) and the Neuropad indicator test of sudomotor function[5,27,32,33]. Of particular note, Halpern *et al.*[34] have recently shown in patients with type 1 diabetes that CNFL was diagnostically valid against various DSPN definitions, exhibiting the highest diagnostic performance against NCS criteria. Furthermore, CCM has been compared against measures of cardiac autonomic neuropathy (CAN)[27,31,35]. Other works have studied the association of CCM with diabetic retinopathy[36–39] and reduced corneal sensation[5,8,28,29,31,38].

We will now briefly discuss the role of CCM in several aspects related to diabetic neuropathies. Recent studies are summarized in Table 1. Earlier works have been reviewed in more detail previously[7].

Role in Detecting DSPN

Given the fact that corneal nerve fiber pathology is more severe in the presence of DSPN[5,7,8,25–28,33], it has been suggested to use CCM for the diagnosis of this complication[7,8]. Of particular note, CCM is sensitive enough to detect corneal nerve fiber perturbations as early as in recently diagnosed diabetes[5] (Figure 1) and before clinical neuropathic deficits develop[33], encouraging the hope that this modality might prove useful for the earliest diagnosis of DSPN.

In this context, some works have examined the sensitivity and specificity of CCM for the detection of DSPN[28,32,40]. Sensitivity and specificity are summarized in Table 2. Compared with IENFD, CNFD was more specific but less sensitive[32]. Of note, Ahmed *et al.*[40] identified the two best diagnostic cut-off points: (i) CNFL $\leq$14.0 mm/mm^2 with 85% sensitivity, 84% specificity, positive likelihood ratio of 5.3 and negative likelihood ratio of 0.18; and (ii) CNFL $\geq$15.8 mm/mm^2 with 91% sensitivity, 93% specificity, positive likelihood ratio of 8.5 and negative likelihood ratio of 0.16[40].

Role in Staging for DSPN Severity

CCM parameters typically deteriorate progressively with increasing severity of neuropathy[5,8,25–29,33,41–43]. CNFD, CNBD and CNFL show a significant negative correlation with neuropathic deficits[8,27,29,41,44–46] and Neuropad response[33,47], as well as a positive correlation with upper[5] and lower extremity nerve conduction velocities[5,29,48–50]. Edwards *et al.*[45] found that CNFL and CNBD most strongly correlated with NCS attributes and modestly with the other tests of neuropathy. There is also evidence for a negative correlation between corneal nerve fiber pathology and small-fiber dysfunction: impairment of pain/thermal perception[27,48,49] and diminution of axon reflex-mediated neurogenic vasodilatation in response to cutaneous heating (laser Doppler imaging flare [LDI_{FLARE}])[49]. In type 1 diabetes, every 1-mm/mm^2 reduction of CNFL was linked with a 0.61°C reduction of cold perception threshold and a 0.07-cm^2 reduction of the LDI_{FLARE} area[49]. Furthermore, a positive correlation between CNFD and IENFD has been found in some[27,31,50], but not in all[5] studies.

Manifestation Patterns

Corneal nerve fiber pathology has been found to be symmetrical[46], similar to DSPN in general[1,2], except in patients with very severe DSPN[46]. In both diabetes types, corneal nerve fibers might be affected before DSPN becomes clinically manifest[5,33,51]. In type 1 diabetes, CNFL even among patients without DSPN might be lower than among controls[43,51]. Stem *et al.*[43] have carried out an interesting comparison between the two types of diabetes. In type 1 diabetes without DSPN, CNFL was significantly reduced compared with controls, whereas among patients with type 2 diabetes, only those with severe DSPN showed a significant reduction of CNFL compared with controls[43]. Thus, it has been argued that CNFL might be reduced in type 1 diabetes earlier than in type 2 diabetes[43]. A further interesting observation in recently diagnosed diabetic patients is that they frequently show abnormal CNFD with concomitant normal IENFD and vice versa, suggesting that small nerve fibers are *not* simultaneously affected in all organs[5].

Role in Impaired Glucose Tolerance

An initial small series of subjects with small-fiber neuropathy showed perturbations in CNFL, CNFD, CNBD and CNFTo, but there were no differences in corneal parameters between subjects with and those without impaired glucose tolerance (IGT)[29]. However, these observations were based on just eight IGT subjects[29]. Indeed, a larger series including 37 subjects with IGT and 20 age-matched control subjects found that the former showed significant reductions in CNFD, CNBD, CNFL and IENFD along with other manifestations of predominantly small-fiber

Table 1 | Recent studies on corneal confocal microscopy for the evaluation of diabetic polyneuropathy

Authors [Ref.]	*n* (DM/controls)	Main findings
Ziegler *et al.*[5]	86 recently diagnosed T2DM/48	1) Reductions in T2DM vs controls: CNFL-MNF ($P = 0.001$), CNFD-MNF ($P < 0.001$), CNBD-MNF ($P < 0.001$), CNCP ($P = 0.006$), IENFD ($P < 0.001$), NCS ($P < 0.001$), QST ($P < 0.001$) and VR ($P = 0.006$) 2) CNFD-MNF among T2DM: reduced below the 2.5th percentile in 21% 3) IENFD among T2DM: reduced below the 2.5th percentile in 14% 4) Vast majority of patients with abnormal CNFD: concomitantly normal IENFD 5) Vast majority of patients with abnormal IENFD: concomitantly normal CNFD
Petropoulos *et al.*[23]	186/55	1) Increasing DSPN severity: significant reduction in manual and automated CNFD ($P < 0.0001$), CNBD ($P < 0.001$), and CNFL ($P < 0.0001$) 2) Manual and automated analysis: correlated for CNFD ($r = 0.9$, $P < 0.0001$), CNFL ($r = 0.89$, $P < 0.0001$) and CNBD ($r = 0.75$, $P < 0.0001$) 3) Highest diagnostic performance: manual CNFD and automated CNFL
Edwards *et al.*[24]	231/61	1) Tortuosity standardized CNFL vs classical CNFL in DM: better in showing differences between DSPN and no DSPN 2) Tortuosity standardized CNFL in DM: 70.5 ± 27.3 (DSPN) vs 84.9 ± 28.7 mm/mm^2 (no DSPN), $P < 0.001$, ROC area under the curve = 0.67 3) Classical CNFL in DM: 15.9 ± 6.9 (DSPN) vs 18.4 ± 6.2 mm/mm^2 (no DSPN), $P = 0.004$, ROC area under the curve = 0.64 4) Tortuosity standardized CNFL vs classical CNFL: 94.3 ± 27.1 (DM without DSPN) vs 84.9 ± 28.7 mm/mm^2 (controls) ($P = 0.028$) 5) Classical CNFL: 20.1 ± 6.3 (DM without DSPN) vs 18.4 ± 6.2 mm/mm^2 (controls) ($P = 0.084$) 6) Tortuosity standardized CNFL vs classical CNFL in DM: stronger correlations with DSPN attributes 7) Tortuosity standardized CNFL vs classical CNFL in DM: stronger correlations with risk factors for DSPN
Halpern *et al.*[34]	89 T1DM/0	1) Comparable areas under the ROC curve for CNFL against various definitions of DSPN (except for clinical definition) 2) DSPN definitions including NCS: optimal CNFL threshold 14 mm/mm^2 3) Clinical DSPN definition: optimal CNFL threshold 15.4 mm/mm^2
Maddaloni *et al.*[35]	36 T1DM/20	1) T1DM vs controls: 45.4 ± 20.2 vs 92.0 ± 22.7 fibers/mm^2 ($P < 0.001$), more tortuous corneal nerve fibers ($P = 0.022$), 15.1 ± 3.5 vs 20.6 ± 5.0 beadings ($P < 0.001$) 2) In T1DM, CAN vs no CAN: CNFD 32.8 ± 16.4 vs 51.7 ± 18.9 fibers/mm^2 ($P = 0.008$); CNFL 5.5 ± 2.4 vs 9.2 ± 3.8 mm/mm^2 ($P = 0.005$) 3) In T1DM, NS differences between CAN vs no CAN: branching grade (1.4 ± 0.8 vs 1.9 ± 0.7, $P = 0.06$), nerve tortuosity (36.4 vs 64%; $P = 0.159$), nerve beadings (14.8 ± 4.2 vs 15.3 ± 3.2, $P = 0.719$)
Zhivov *et al.*[39]	18 T2DM/20	1) Corneal sensation: 59 ± 18 mm in healthy volunteers and 43 ± 11 mm in T2DM ($P < 0.001$) 2) Reductions in T2DM vs controls: component pixels ($P < 0.001$), skeleton pixels ($P < 0.001$), component ratio ($P < 0.001$), single nerve fibers ($P < 0.001$), single nerve fibers per component ($P < 0.001$), total fiber length ($P < 0.001$), CNFD ($P < 0.001$), connectivity points ($P < 0.001$), number of branches ($P < 0.001$), homogeneity of component pixels ($P = 0.001$) and average single fiber length ($P = 0.08$) 3) T2DM: NS differences in the aforementioned CCM nerve parameters between patients with DR and those without DR
Stem *et al.*[43]	25 T1DM without DSPN and 18 T2DM with DSPN/9	1) Severe DSPN: lower CNFL vs controls (12.5 ± 6.1 mm/mm^2 vs 20.7 ± 2.2 mm/mm^2, $P = 0.009$) 2) T1DM without DSPN: lower CNFL vs controls (15.1 ± 4.7 mm/mm^2 vs 20.7 ± 2.2 mm/mm^2, $P = 0.033$)
Petropoulos *et al.*[46]	111/47	1) CNFD, CNBD and CNFL: symmetrical pathology (except in patients with severe DSPN) 2) CNFD: significant ($P < 0.001$) reduction between controls and DM with increasing DSPN severity 3) CNBD: significant ($P < 0.001$) reduction between controls and DM with increasing DSPN severity 4) CNFL: significant ($P < 0.001$) reduction between controls and DM with increasing DSPN severity
Ishibashi *et al.*[47]	78 T2DM/28	1) DSPN vs no DSPN: reductions in CNFD ($P < 0.001$), CNFL ($P < 0.001$) and beading frequency ($P < 0.0001$) with increased CNFTo ($P < 0.0001$) 2) Sudomotor function: negative correlations with CNFD ($P < 0.002$) and CNBD ($P < 0.01$) 3) Sweat gland duct size: correlated with triglycerides ($P < 0.02$), uric acid ($P < 0.01$), CNBD ($P < 0.03$), sudomotor function ($P < 0.03$) and DSPN severity ($P < 0.03$)

Table 1 | (*Continued*)

Authors [Ref.]	*n* (DM/controls)	Main findings
Dehghani *et al.*[48]	147 T1DM/60	1) DSPN vs controls: significant ($P = 0.01$) linear decline of CNFD, in association with age ($P = 0.04$) and T1DM duration ($P = 0.03$) 2) DSPN: modest correlation between CNBD and peroneal conduction velocity ($r = 0.38$, $P = 0.05$) 3) DSPN: modest correlation between CNFL and CDT ($r = 0.40$, $P = 0.03$)
Sivaskandarajah *et al.*[49]	96 T1DM/64	1) In T1DM, DSPN vs no DSPN: lower CDT values ($P < 0.0001$), smaller LDI_{FLARE} areas ($P = 0.0002$), and lower HRV values ($P < 0.0001$) 2) In T1DM, reduction of CNFL by every 1 mm/mm^2: association with a 0.61°C lower CDT, a 0.07 cm^2 lower LDI_{FLARE} area, and a 1.78% lower HRV 3) CNFL in T1DM: significant positive correlations with CDT ($P = 0.0002$), LDI_{FLARE} ($P = 0.002$) and HRV ($P < 0.0001$) 4) CNFD in T1DM: significant positive correlations with CDT ($P = 0.002$), LDI_{FLARE} ($P = 0.0002$) and HRV ($P = 0.003$) 5) CNBD in T1DM: significant positive correlations with CDT ($P = 0.0002$), LDI_{FLARE} ($P = 0.01$) and HRV ($P = 0.001$) 6) CNFTo in T1DM: no association with small-fiber function
Dehghani *et al.*[54]	0/64	1) Age: significant ($P = 0.02$) linear CNFL reduction by 0.05 mm/mm^2 per added year 2) CNFL: NS change in over 36 months ($P = 0.41$)
Pritchard *et al.*[51]	242 T1DM (76 with DSPN, 166 without DSPN)/154	1) CNFL: lower in T1DM with DSPN (14.0 ± 6.4 mm/mm^2) vs T1DM without DSPN (19.1 ± 5.8 mm/mm^2) and controls (23.2 ± 6.3 mm/mm^2) ($P < 0.001$) 2) CNFL: lower in T1DM without DSPN (19.1 ± 5.8 mm/mm^2) vs controls (23.2 ± 6.3 mm/mm^2) ($P < 0.001$) 3) CNBD: lower in T1DM with DSPN (40.1 ± 32.1 branches/mm^2) vs T1DM without DSPN (61.7 ± 37.2 branches/mm^2) and controls (83.5 ± 45.8 branches/mm^2) ($P < 0.001$) 4) CNBD: lower in T1DM without DSPN (61.7 ± 37.2 branches/mm^2) vs controls (83.5 ± 45.8 branches/mm^2) ($P = 0.001$)
Asghar *et al.*[52]	37 IGT/20	1) IGT vs controls: significantly increased NSP ($P < 0.001$), McGill pain index ($P < 0.001$), NDS ($P = 0.001$), VPT ($P = 0.002$), WDT ($P = 0.006$) and CDT ($P = 0.03$) 2) IGT vs controls: reductions in IENFD ($P = 0.03$), CNFD ($P < 0.001$), CNBD ($P = 0.002$) and CNFL ($P = 0.05$)
Pritchard *et al.*[55]	90 T1DM without DSPN	1) Development of DSPN after 4 years: associations with lower CNFL ($P = 0.041$) 2) Development of DSPN after 4 years: associations with longer T1DM duration ($P = 0.002$), higher triglycerides ($P = 0.023$), retinopathy ($P = 0.008$), nephropathy ($P = 0.001$), higher NDS ($P = 0.037$), lower CDT ($P = 0.001$), higher WDT ($P = 0.008$), higher VPT ($P = 0.003$), impaired monofilament response ($P = 0.003$), NCS impairments ($P < 0.05$) 3) CNFL cut-off of 14.1 mm/mm^2: 63% sensitivity and 74% specificity for the prediction of DSPN after 4 years
Azmi *et al.*[56]	49 T1DM (18 CSII, 31 MDI)/40	T1DM CSII vs T1DM MDI: increase in CNFD ($P = 0.05$), CNBD ($P = 0.006$) and CNFL ($P = 0.003$), NS difference in VPT, CDT, WDT, NCS or IENFD
Brines *et al.*[57]	48 T2DM/55	ARA 290 vs placebo: improvement of neuropathic symptoms ($P = 0.037$), increase in CNFD ($P = 0.02$) and reduction in HbA1c ($P = 0.002$)
Tavakoli *et al.*[58]	34/18	1) CNFD (best cut-off <23.26 nerves per mm^2): 86% sensitivity and 78% specificity for the diagnosis of DAN (AUC = 0.915, $P = 0.0001$) 2) CNBD (best cut-off <19.53 branches per mm^2): 100% sensitivity and 56% specificity for the diagnosis of DAN (AUC = 0.889, $P = 0.0001$) 3) CNFL (best cut-off <4.78 mm/mm^2): 86% sensitivity and 78% specificity for the diagnosis of DAN (AUC = 0.907, $P = 0.0001$) 4) CNFD, CNBD, CNFL: significant ($P < 0.001$) correlations with CASS and COMPASS

ARA 290, a peptide derived from erythropoietin; AUC, area under the curve; CAN, cardiac autonomic neuropathy; CASS, composite autonomic scoring scale; CCM, corneal confocal microscopy; CDT, cooling detection threshold; CNBD, corneal nerve fiber branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CNFTo, corneal nerve fiber tortuosity; CNCP, corneal nerve connecting points; COMPASS, composite autonomic symptom scale; CSII, continuous subcutaneous insulin infusion; DAN, diabetic autonomic neuropathy; DR, diabetic retinopathy; DSPN, diabetic polyneuropathy; HbA1c, glycated hemoglobin; HRV, heart rate variability; IENFD, intra-epidermal nerve fiber density; IGT, impaired glucose tolerance; LDI_{FLARE}, laser Doppler imaging flare; MDI, multiple daily insulin injections; MNF, major nerve fibers; NCS, nerve conduction study; NDS, neuropathy disability score; NS, not significant; NSP, neuropathy symptom profile; QST, quantitative sensory testing; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; VPT, vibration perception threshold; VR, Valsalva ratio; WDT, warm detection threshold.

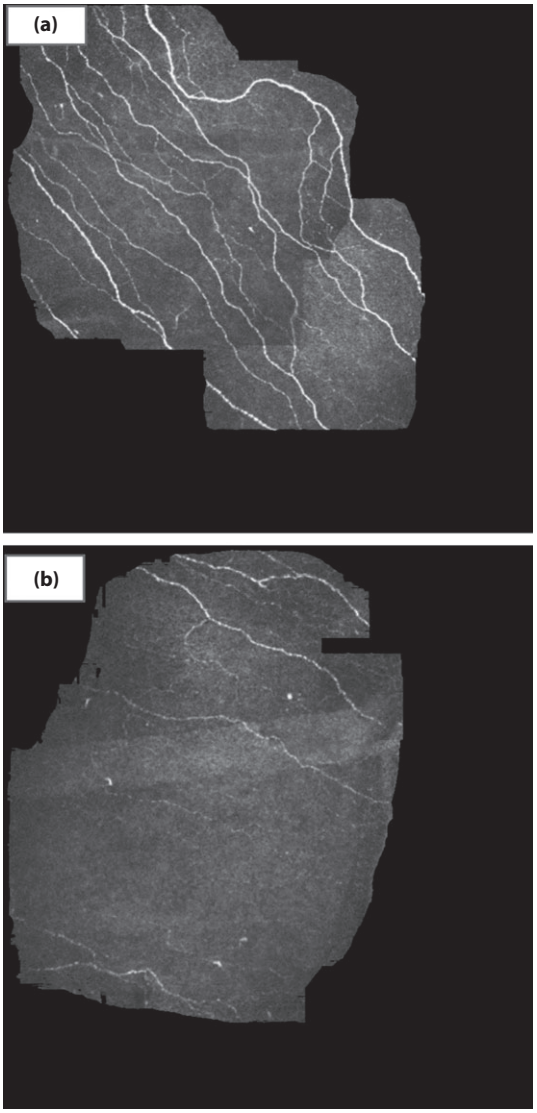

Figure 1 | Corneal confocal microscopy showing the sub-basal nerve plexus. (a) Normal structure corneal nerve fibers in a healthy subject. (b) Loss of corneal nerve fibers in a recently diagnosed subject with type 2 diabetes.

neuropathy[52]. These findings add to the growing knowledge on the early beginning of neuropathy, as early as in prediabetes[53].

Natural History

In healthy individuals, age induced a slight but significant ($P = 0.02$) linear diminution of CNFL: this was calculated as a linear decrease by 0.05 mm/mm^2 for every added year of age[54]. Despite this, however, CNFL remained fairly constant over 36 months[54]. In type 1 diabetes, after a 4-year follow up there was a significant reduction of CNFD in association with age ($P = 0.04$) and type 1 diabetes duration ($P = 0.03$)[48]. Impressively, small changes in corneal nerve fibers are indicative of clinical DSPN after 4 years in type 1 diabetes[55]: lower CNFL has been found to be associated with DSPN after 4 years ($P = 0.041$). A CNFL cut-off of 14.1 mm/mm^2 could predict DSPN after 4 years with 63% sensitivity and 74% specificity[55].

Effects of Therapeutic Interventions

There is evidence that CCM can objectify early nerve fiber improvements. In an observational study enrolling 25 diabetic patients with mild/moderate DSPN, improved cholesterol levels after 24 months were linked to significant improvements in CNFD, CNBD and CNFTo, and glycated hemoglobin reduction was significantly correlated with the increase in CNFD[30]. In another setting, simultaneous pancreas and kidney transplantation in patients with type 1 diabetes induced significant improvements in CNFD and CNFL at 6 months, as well as in CNFD, CNFL, CNBD at 12 months[31,50]. Among all diagnostic modalities investigated, only CCM showed improvement after 12 months[31]. Very recently, the effect of continuous subcutaneous insulin infusion on DSPN was compared with that of multiple insulin injections in type 1 diabetes[56]. After 24 months, significant increases in CNFD ($P = 0.05$), CNBD ($P = 0.006$) and CNFL ($P = 0.003$) were noted only in the group under continuous subcutaneous insulin infusion, despite suboptimal and comparable glycemic control in both treatment arms[56]. Arguably, the stability of glycemic control accomplished with continuous subcutaneous insulin infusion exerted a beneficial effect on small nerve fibers, and CCM was accurate enough to show this effect[56]. Brines *et al.*[57] have shown that administration of ARA 290, a peptide derived from erythropoietin, for 28 days could significantly improve neuropathic symptoms ($P = 0.037$) and CNFD ($P = 0.02$) in type 2 diabetes.

Associations with Retinopathy and Corneal Sensation

Corneal nerve fiber pathology has been found to be associated with both the presence and severity of diabetic retinopathy (background vs proliferative retinopathy)[36–38]. Conversely, Zhivov *et al.*[39] have reported no difference in corneal nerve morphology between patients with vs without diabetic retinopathy. Patients with DSPN frequently have reduced corneal sensation as well, and a positive correlation between CNFD and corneal sensation has been reported[41]. Tavakoli *et al.*[8] have reawakened the interest in the immune component of DSPN by showing a significant increase of corneal Langerhans cells in diabetic patients as well as an inverse correlation between these cells and the clinical severity of DSPN. The authors' interpretation was that the increase of Langerhans cells in early DSPN pointed to the role of immune mechanisms in the first steps of its pathogenesis, although other factors became later more decisive in advanced DSPN[8].

Role in Detecting Autonomic Neuropathy

CCM parameters show a positive correlation with heart rate variability on deep breathing[27,49]. In type 1 diabetes, every 1-mm/mm^2 reduction of CNFL has been reported to be linked with a reduction of heart rate variability by 1.78%[49]. In a study

Table 2 | Sensitivity and specificity of corneal confocal microscopy and skin biopsy

Tavakoli *et al.*[28]				
	CNFD		CNBD	
Diagnosis	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
DSPN	82	52	91	45
At-risk foot	71	64	71	71
Quattrini *et al.*[32]				
	CNFD		IENFD	
Diagnosis	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
DSPN	56	75	78	56
At-risk foot	63	72	88	54
Small-fiber neuropathy	50	84	69	63
Severe small-fiber neuropathy	86	69	86	46

CCM, corneal confocal microscopy; CNBD, corneal nerve fiber branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; DSPN, diabetic polyneuropathy; IENFD, intra-epidermal nerve fiber density.

comparing 36 type 1 diabetic patients with 20 age- and sex-matched controls, the former showed fewer ($P < 0.001$) and more tortuous corneal nerve fibers ($P = 0.022$), and fewer beadings ($P < 0.001$) than the latter[35]. Among patients with type 1 diabetes, CNFD was significantly lower ($P = 0.008$) in the presence of CAN, and this difference remained significant after adjustment for age, sex, type 1 diabetes duration, insulin dosage and severity of DSPN[35]. Similarly, CNFL was significantly lower in the presence of CAN ($P = 0.005$), and this difference remained significant after adjustment for type 1 diabetes duration, insulin dosage and severity of DSPN, but it lost significance after adjustment for age and sex[35]. Tavakoli *et al.*[58] have recently reported that CNFD, CNBD and CNFL yielded very high (86–100%) sensitivity and moderate to high specificity (56–78%) for the diagnosis of autonomic neuropathy (Table 1). Furthermore, these parameters showed significant ($P < 0.001$) correlations with the gravity of autonomic symptoms5[8].

Future Perspectives

There has been considerable progress with CCM for the evaluation of DSPN, and more knowledge is still accumulating. Future improvements should be mainly pursued in the following areas.

Widespread Use of CCM

For the time being, CCM is mainly used in specialized centers[7]. Given the evidence for its moderate to high sensitivity and specificity for the diagnosis of DSPN, it is reasonable that this modality could be of increased clinical utility, once technology and know-how become more widely available. Further arguments in favor of its wider availability include its non-invasive nature and the ability to repeat the examination for patient follow up[30,31,50,56,57].

Normative Database

Especially if CCM becomes more widely used, we need a database of normative values, similar to the one reported for IENFD[59]. Such normal values are now for the first time becoming available[60], but their application in clinical practice is awaited.

Revisiting the Efficacy of Pathogenetic Treatments Through CCM

The effect of neuroprotective, disease-modifying agents on peripheral nerve structure can now be revisited utilizing CCM. This suggestion is based on the ability of CCM to visualize nerve fiber regeneration[30,31,50,56,57].

More Experience in Children and Adolescents

After the interesting pilot study by Sellers *et al.*[61], additional experience in diabetic children and adolescents is highly welcome.

Technical Improvements in Automated Nerve Fiber Quantification and Image Analysis

Improved automated fiber measurement[10,23] and wider-area image analysis[5,17,19] are expected to increase accuracy and to enable the acquisition of more representative images.

CONCLUSIONS

There are now more than 10 years of experience with CCM for the evaluation of DSPN[7,62]. The advantages of CCM include its non-invasive nature, its high reproducibility[20–22] and its easy application for patient follow up[30,31,50,56,57]. CCM opens new perspectives of studying the natural history of DSPN, staging nerve fiber pathology[5,8,25–29,33,41–43,45,46,49] and documenting incipient nerve fiber regeneration after therapeutic intervention[30,31,50,56,57]. Importantly, it is useful for the early detection of nerve pathology[5,33] and high-risk foot[32] with moderate to high sensitivity and specificity[28,32,34,40].

Based on this ample evidence, more widespread application of CCM for the evaluation of DSPN can be advocated. Such a broad utilization should serve both diagnostic and prognostic

purposes in terms of DSPN evaluation at baseline and/or after therapeutic interventions[5,30,31,33,50,56,57]. Importantly, normal values for CCM parameters are now becoming available[60] and need to be applied in practice. Finally, technical improvements in automated nerve fiber quantification and wider-area image analysis[5,10,17,19,23] are more than welcome to increase diagnostic performance.

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