GAED Medal Lecture 2022: Challenging the Dogma in Diabetic Neuropathy and Beyond

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Abstract

Dogma, according to the Britannica Dictionary, is "a belief or set of beliefs that is accepted by the members of a group without being questioned or doubted." Thus, in 2001, the heretical idea that corneal confocal microscopy (CCM)—an ophthalmic instrument—could be used to assess neurological disease truly challenged the dogma. The repurposing of CCM to study diabetic neuropathy and other neurodegenerative diseases is a wonderful illustration of being in the right time and place and having honest and open conversations between very different medical disciplines to 'challenged the dogma.' The Gulf Association of Diabetes and Endocrinology (GAED) Medal Lecture in 2022 and the European Association for the Study of Diabetes (EASD) Camillo Golgi Prize in 2019 have enabled me to tell my personal story in relation to the past, present, and future of CCM as a clinical tool to diagnose and predict neurodegeneration and identify nerve regeneration in clinical trials of new therapies for peripheral and central neurodegenerative diseases.

Keywords

- dogma
- corneal confocal microscopy
- ophthalmology
- neurological disease
- diabetic neuropathy
- neurodegenerative diseases
- The Gulf Association of Diabetes and Endocrinology Medal Lecture
- The European Association for the Study of Diabetes Camillo Golgi Prize
- clinical trials
- new therapies
- diabetes
- diagnosis

Discovering CCM

The optical principles of confocal microscopy were described by Minsky in 1955, who interestingly cited his rationale for developing this device "to better understand the interconnection of nerve cells."[1] The first functional microscope was developed by Petran et al[2] in 1960. However, it was not until 1988 that Dilly mounted Petran's device horizontally and examined his own cornea in vivo.[3] In the same week, my now long-standing friend and colleague Professor Nathan Efron (NE), an academic optometrist and international expert in contact lenses, learned about CCM at a biennial meeting of

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the International Society for Contact Lens Research, held in Kauai, Hawaii, USA. He sat in on a lecture by ophthalmologist Dwight Cavanagh, who had also developed a CCM with bioengineering colleagues at Georgetown University Medical Center, Washington, DC, USA. Renowned for his often flowery and effusive oratory, Cavanagh stepped up to the podium and commenced his lecture with a profound statement that stunned the audience: "It's very rare in one's life to ever be a part of something that's really fundamental in science that changes the way everything is done. I guess there are points like that in the history of science, when Isaac Newton saw the apple fall off the tree, when Galileo Galilei looked through his first telescope, and when Antonie van Leeuwenhoek found the little animals in pond water; but believe me ... confocal microscopy is one of those branch points in science, not just cell biology or ophthalmology or optometry or contact lenses. It is a new paradigm, a ... microscope that lets us see things nobody else has ever been able to see before."

Cavanagh then showed images of the cornea obtained using CCM, which NE described as "blew me away," and he immediately saw a potential application of CCM to study the ocular response to contact lens wear. However, it took almost 8 years for the first commercially available CCM—the Tomey ConfoScan Confocal Microscope Model P4 (Tomey, Erlangen, Germany) to come onto the market in 1996. NE migrated from Australia to take up a professorial appointment at the University of Manchester Institute of Science and Technology (UMIST) in the UK and, in 2000, gambled on using the remaining funds in his "start-up" budget to purchase a Tomey CCM. NE then started scanning the corneas of his colleagues and noted the presence of the sub-basal nerve plexus, which was known to exist from ex vivo histological studies but had never before been seen in the living human eye. Together with his graduate optometry student Laura Oliveira-Soto, he described the morphology of the sub-basal nerve plexus in healthy subjects.[4]

Translating CCM from Ophthalmology to Diabetes

NE published his paper describing the sub-basal nerve structure on May 1, 2001. He was then due for his annual diabetes review with his doctor, Professor Andrew Boulton, at the Manchester Royal Infirmary. Having gained weight and been less attentive with his glycemic control, more so to distract Professor Boulton, NE handed him a copy of the paper.[4] Andrew finished his clinic and came to my office, which was adjacent to his office, and asked me what I know about corneal confocal microscopy. I confess I knew nothing about CCM, but as with all good research registrars, I immediately deflected from my lack of knowledge on CCM and spoke with authority about a clinical trial I had read about that morning published in the prestigious NEJM showing how the topical application of nerve growth factor had miraculously healed neurotrophic corneal ulcers.[5] This was in stark contrast to a phase 3 clinical trial with NGF that had failed in patients with diabetic neuropathy.[6] I concluded that perhaps imaging corneal nerves might be relevant to diabetic neuropathy, especially for measuring nerve repair in clinical trials. Andrew wrote to NE, stating: "Turning now to your controcal [*sic*] microscope ... Rayaz Malik and I could possibly visit your Centre ... and think of some collaborative work in diabetic neuropathy."

I must confess that when I met NE. I was skeptical, having believed the dogma I had been taught by my neurology professors in Aberdeen medical school, and stated, "but these are fifth cranial nerves, I am more interested in small peripheral nerves which are affected in diabetic neuropathy." Despite my initial reservations, I suggested we use CCM to assess a small cohort of patients with diabetic neuropathy to see how his ophthalmic technique compared to our established tests of diabetic neuropathy, which included symptoms, neurological examination, quantitative sensory testing, and nerve conduction. Recruitment was initially slow, as I relied on patients in my clinic making their own way across the city to the optometry department in UMIST for Panos Kallinikos, NE's PhD student, to undertake CCM. It was then that I had a conversation with Mike, one of the diabetes nurses, about improving recruitment. He asked me what car do I drive. I said a Porsche 944 Turbo. We finished the study in 3 months when I offered to drive the patients personally to UMIST in my Porsche! This was the beginning of an extremely fruitful collaboration with NE, which effectively translated the use of CCM from the world of ophthalmology/optometry to neurology, notwithstanding conceptual challenges by my neurology colleagues, especially when reviewing my papers and grants on CCM.

According Precedence

Laura Oliveira-Soto had described the anatomy of corneal stromal and sub-basal nerves in 14 healthy subjects[5] and submitted it to the journal *Cornea* in September 2000. They had concluded, "This study provides convincing evidence of the suitability of confocal microscopy to image corneal nerves," and in the discussion, had suggested: "Future studies should investigate the ... morphology and architecture of corneal nerves in ... diabetic patients ...."[5] In October 2000, Rosenberg et al[7] used a tandem scanning confocal microscope and demonstrated a progressive qualitative loss of long nerve fibers with increasing severity of diabetic neuropathy, concluding that "Confocal microscopy appears to allow early detection of beginning neuropathy."[7] In science, precedence must be accorded to those who publish first, so without a doubt, credit goes to Rosenberg et al[7] for being the first to describe corneal nerve loss in diabetic peripheral neuropathy.

I met NE and understood what CCM was in June 2001. We commenced our study in patients with diabetic neuropathy in August 2001, but from the beginning, we applied rigorous morphometric techniques to quantify the corneal nerves. I had learnt the importance of quantifying myelinated and unmyelinated nerve fibers in sural nerve biopsies in patients with diabetic neuropathy.[8] Our paper[9] was submitted to *Diabetologia* in November 2002 and challenged the dogma by concluding that "CCM is a rapid, noninvasive in vivo clinical examination technique, which accurately defines the extent

of corneal nerve damage and repair and acts as a surrogate measure of somatic neuropathy in diabetic patients. It could represent an advance to define the severity of neuropathy and expedite assessment of therapeutic efficacy in clinical trials of human diabetic neuropathy."[9]

CCM in Diabetic Neuropathy

Despite several rather negative reviews by my neurology colleagues, the Juvenile Diabetes Research Foundation (JDRF) awarded $53k to build on our pilot data showing that CCM could identify sub-clinical diabetic neuropathy and progressive worsening with increasing severity. Given the reluctance/hostility amongst some in the neurology community to accept that an ophthalmic instrument quantifying 'short cranial nerves' could reflect a dying back neuropathy affecting 'long sensory nerves,' we knew we had to secure further financial support to increase the size of the cohorts studied as well as establish longitudinal change and the response to interventions. We were fortunate to secure a RO1 grant for $1.5M from the NIDDK, alongside a large grant for almost $3M from the JDRF to conduct the LANDMARK study (Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic MARKers)."[10] Subsequent funding from the NIDDK enabled the establishment of an international consortium (Manchester, Brisbane, Calgary, Toronto), which combined CCM and detailed neuropathy assessment data from ~1,000 patients with type 1 and type 2 diabetes and impaired glucose tolerance. These data showed that CCM has excellent diagnostic[11,12] and prognostic[13] utility and could detect early nerve regeneration after simultaneous pancreas and kidney transplantation in patients with type 1 diabetes.[14,15]

Age-adjusted normative values for CCM were established,[16] and we showed that the severity of, and risk factors for, corneal nerve loss differ between patients with type 1 and type 2 diabetes.[17] We also showed that corneal nerve loss occurs in children[18,19] and adults[20] with type 1 diabetes, before the development of diabetic retinopathy and microalbuminuria and in subjects with impaired glucose tolerance[21,22] and recently diagnosed type 2 diabetes.[23] Patients with painful diabetic neuropathy had evidence of greater corneal nerve loss, particularly at the inferior whorl,[24] and this was related to the severity of neuropathic pain.[25] Studies from Italy,[26] China,[27] Japan,[28] New Zealand,[29] and the UK[30] showed corneal nerve loss in patients with diabetic autonomic neuropathy. We also demonstrated corneal nerve fiber loss in men with type 1 and type 2 diabetes and erectile dysfunction.[31,32] Reduced corneal nerve fiber length (CNFL) has been shown to predict 4-year incident diabetic peripheral neuropathy,[33,34] and a more rapid decline in CNFL is associated with the development of clinical diabetic peripheral neuropathy[35] and Charcot foot with foot ulceration.[36]

CCM in Other Peripheral Neuropathies

Chemotherapy-induced peripheral neuropathy is characterized by pain and reduced quality of life, which can lead to dose reduction or discontinuation of chemotherapy. We showed corneal nerve loss in patients with gastro-esophageal cancer and nerve regeneration after the third cycle of platinum-based chemotherapy.[37] More recently, in a study from Denmark, corneal nerve morphology was found to be normal 5 years after adjuvant chemotherapy for breast and colon cancer.[38] Is this a surprise, given that we had previously shown active nerve regeneration after cycle 3 of chemotherapy[37]? Indeed, a study from Australia has shown corneal nerve loss in the central cornea and inferior whorl in patients 3-24 months after treatment with paclitaxel or oxaliplatin.[39] Stettner et al[40] showed significant corneal nerve loss and an increase in dendritic cells in patients with chronic inflammatory demyelinating poly neuropathy (CIDP), multifocal motor neuropathy, and monoclonal gammopathy of unknown significance, which was associated with the severity of disease and pain. Two subsequent longitudinal studies in CIDP have shown that increased corneal inflammatory cells can predict disease progression with high sensitivity.[41,42] Kemp et al[43] showed a loss of corneal nerve fibers in patients with HIV-associated neuropathy, and we further showed that the corneal nerve fractal dimension might differentiate HIV neuropathy from diabetic neuropathy.[44] Idiopathic small fiber neuropathy is characterized by painful neuropathic symptoms and small fiber dysfunction/damage with preserved large fiber function[45]; we have shown a significant loss of corneal nerves in patients with idiopathic small fiber neuropathy[46] with an increased detection rate of this condition using CCM.[47]

Corneal nerve loss has been reported in patients with Charcot Marie Tooth Disease Type 1A[48] and severe peripheral neuropathy associated with a rare nerve growth factor-β mutation.[49] We have also demonstrated corneal nerve loss in patients with Friedreich's ataxia and related it to the number of GAA trinucleotide repeats as well as clinical disability assessed using the Scale for the Assessment and Rating of Ataxia and Friedreich's Ataxia Rating Scale.[50] In a cohort of 51 patients with neurofibromatosis type 1, 4 (8%) had abnormal nerve conduction studies, 7 (13%) had abnormal thermal thresholds, 11 (22%) had abnormal intraepidermal nerve fiber density, but 27 (52%) had reduced corneal nerve fiber length.[51] Transthyretin familial amyloid polyneuropathy is a fatal inherited disorder characterized by progressive neuropathy and cardiomyopathy.[52] In 15 patients with this disease, a reduction in CNFL was related to the neuropathy impairment score of the lower limbs, autonomic dysfunction, sensory nerve action potential, and intra-epithelial nerve fiber density (IENFD).[53] CNFL could be measured in all participants, while sural nerve amplitude and IENFD could only be measured in 73% and 27% of patients, respectively. This lack of a floor effect increases the utility of CNFL compared to IENFD in longitudinal and interventional studies of amyloid neuropathy. Recently, a study from China has confirmed and extended these findings by showing corneal nerve loss in the central and inferior whorl regions with an AUC for CNFL and IWL of 88.0% and 89.3%, respectively, for the diagnosis of familial amyloid neuropathy.[54]

Small fiber neuropathy is a hallmark of Fabry's disease due to globotriaosylceramide (GI_3)-induced nerve damage.[55] We

were the first group to show corneal nerve loss using the first-generation Tomey ConfoScan in patients with Fabry disease.[56] More recently, in a detailed study with colleagues from Turkey using a Heidelberg HRT III CCM, we have shown that corneal nerve loss correlated with the total Mainz severity score index.[57] In a study of patients with hypothyroidism, CNFD was reduced and improved after 12 months of treatment with levothyroxine.[58] In a study from Mexico of patients with fibromyalgia, stromal nerve thinning and a reduction in sub-basal nerve fiber density was related to a variety of pain descriptors.[59] In a subsequent very detailed phenotyping study from the Netherlands, corneal nerve loss was identified in 51% of patients with fibromyalgia and related to central sensitization.[60] A study from Turkey has reported a reduction in CNFL, which correlated with the 'widespread pain index' in patients with fibromyalgia.[61] We have recently confirmed abnormalities in multiple small fiber tests including a comparable reduction in IENFD and corneal nerves in a large cohort of 117 women with fibromyalgia.[62]

CCM in Central Neurodegenerative Disease

A number of centers, including ours have explored whether corneal nerve loss could act as a surrogate marker of neurodegeneration in central neurodegenerative diseases. In our initial study of 25 patients with Parkinson's disease (PD), we showed reduced corneal sensitivity and corneal nerve fiber density, branch density, and length.[63] Kass-Iliyya et al[64] showed corneal nerve loss in patients with PD and related it to the unified PD rating scale and autonomic dysfunction. Another study in 26 newly diagnosed patients with PD showed a reduction in corneal nerve parameters, with normal nerve conduction and IENFD.[65] Corneal nerve loss has also been related to the severity of cognitive dysfunction in patients with Parkinson's disease[66] and associated with altered white matter diffusion properties of the trigeminal nerve.[67] Recently, a significant decrease in the directional anisotropy coefficient and an increase in the directional symmetry coefficient of corneal nerve fibers has been demonstrated in patients with PD.[68] We have confirmed the loss of corneal nerve fibers in a large cohort of 98 participants with PD.[69] In a recent study from China, CNFD showed excellent diagnostic performance for CCM, with an AUC of 0.96 for PD and corneal nerve fiber parameters correlated with the severity of motor symptoms measured using the H-Y stage, UPDRS-III, and UPDRS-total.[70] Furthermore, we have shown that a lower CNFL predicts progressive worsening of UPDRS-III over 12 months in patients with PD.[71] CCM could therefore complement the diagnostic toolbox for pre-motor Parkinson's disease.

Four recent studies[72–74] have demonstrated a significant reduction in sub-basal corneal nerve density in patients with multiple sclerosis. Corneal nerve loss was not related to disease type and optic neuritis but correlated with disease severity and was paralleled by a reduction in retinal nerve fiber layer thickness and an increase in corneal immune cells.[73] A small study demonstrated corneal nerve loss, which was associated with the bulbar function disability score in patients with amyotrophic lateral sclerosis (ALS).[75] More recently, in a study of 64 patients with ALS, nerve loss was found in the central cornea, especially the inferior whorl and was associated with bulbar involvement as well as disease severity and progression.[76] Corneal nerve loss occurs in patients with transient ischemic attack and minor stroke,[77] major stroke,[78] and recurrent stroke,[79] and is associated with the presence of white matter hyperintensities.[80] We have shown corneal nerve fiber loss in patients with mild cognitive impairment and dementia, which was associated with the degree of cognitive impairment and physical disability.[81,82] Furthermore, we have recently shown that the severity of cerebral ischemia was associated with cognitive impairment, brain atrophy, and corneal nerve loss in subjects with mild cognitive impairment and dementia.[83]

In patients with migraine, corneal nerve fiber density and length are reduced,[84] particularly in those with chronic migraine and photophobia.[85] Corneal nerve fiber density and length are also reduced in patients with trigeminal neuralgia.[86] In a study of 17 patients with burning mouth syndrome, we observed a significant reduction in corneal nerve fiber density and length and an increase in Langerhans cell density.[87]

CCM in Clinical Trials

Almost 4 years after our original paper speculating that CCM could "expedite assessment of therapeutic efficacy in clinical trials of human diabetic neuropathy,"[9] we showed early corneal nerve fiber regeneration 6 months after simultaneous pancreas and kidney (SPK) transplantation in patients with type 1 diabetes.[88] Subsequently, we showed corneal nerve regeneration 24 months after an improvement in glycemia, blood pressure, and lipids[89] and 12 months after SPK transplantation, but with no change in symptoms, neurophysiology, quantitative sensory testing or skin biopsy, the very tests that are accepted and dogmatically endorsed by the FDA.[14] We have shown an increase in CNFL at 12 months, followed by an improvement in neuropathic symptoms at 24 months and neurophysiology at 36 months after SPK transplantation.[15] We have also recently shown an improvement in corneal nerve morphology in obese subjects with[90] and without[91] diabetes, after bariatric surgery. A novel first-in-class peptide (ARA290-Cibinetide), which reduces tissue inflammation, improved corneal nerve fiber density and length in patients with sarcoidosis-related neuropathy[92,93] and type 2 diabetes[94] and was paralleled by an improvement in pain scores. In a subsequent Phase 2b study, improvement in corneal nerve morphology strongly correlated with the expression of GAP-43^{+} in skin biopsies, indicating intraepidermal nerve fiber repair and with an improvement in pain intensity after 28 days.[93] In a trial of seal oil omega-3 polyunsaturated fatty acid in patients with type 1 diabetes, there was a significant 29% increase in CNFL, with no change in nerve conduction velocity and sensory function over 12 months.[95] In a randomized placebo-

controlled trial of omega-3 fatty acid in patients with type 1 diabetes, after 180 days, there was a significant increase in corneal nerve fiber length, but no change in thermal thresholds, autonomic function, or nerve conduction studies.[96] In a randomized clinical trial of once-weekly GLP-1 agonist exenatide or basal-bolus insulin over 12 months, we have recently shown corneal nerve regeneration with no change in vibration perception or sudomotor function.[97]

Future Developments of CCM

Several slit scanning in vivo CCMs are commercially available, including Tomey Corporation (Cambridge, MA, USA), Nidek Technologies (Gamagori, Japan), and Helmut Hund (Wetzlar, Germany), but they have limited image resolution for the sub-basal nerve plexus. The laser CCM (Heidelberg Retina Tomograph III Rostock Corneal Module, Heidelberg Engineering GmbH, Heidelberg, Germany) is a contact CCM utilizing laser scanning that is capable of generating high-resolution images of corneal epithelial cells, keratocytes, endothelial cells, and the sub-basal nerve plexus. Looking to the future, a non-contact CCM would make image acquisition easier and facilitate more widespread uptake amongst practitioners; a non-contact attachment for the Heidelberg instrument has been developed,[98] but image acquisition of the corneal sub-basal nerve plexus using this first-generation set-up is very difficult.[98]

A limitation of CCM is the small field of view, and some centers have used wide-field imaging to create maps of the sub-basal nerve plexus.[99–101] The main morphological parameters quantified include corneal nerve fiber density, branch density, fiber length, and inferior whorl length.[102] Corneal nerve fractal dimension analysis[44] may help to differentiate neuropathies of differing etiology.[103] To expedite unbiased corneal nerve quantification, CCMetrics and ACCMetrics are freely available software for manual and automated quantification of sub-basal corneal nerves.[104,105] Novel artificial intelligence-based algorithms have also been developed for fully automated corneal nerve quantification[106] and disease severity classification in diabetic neuropathy.[107]

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Conflict of Interest
None declared.

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