



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

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Abstract

Keywords

- ▶ dogma
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- ▶ The Gulf Association of Diabetes and Endocrinology Medal Lecture
- ▶ The European Association for the Study of Diabetes Camillo Golgi Prize
- ▶ clinical trials
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- ▶ diagnosis

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.

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 al² 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 subjects⁵ 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 al⁷ 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 al⁷ 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 paper⁹ 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 prognostic¹³ 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 adults²⁰ 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 UK³⁰ 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 neuropathy³⁵ 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 chemotherapy³⁷? 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 al⁴⁰ 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 al⁴³ 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 function⁴⁵; we have shown a significant loss of corneal nerves in patients with idiopathic small fiber neuropathy⁴⁶ 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 1A⁴⁸ 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 (GI₃)-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 al⁶⁴ 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 disease⁶⁶ 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 studies⁷²⁻⁷⁴ 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 lipids⁸⁹ 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 with⁹⁰ and without⁹¹ 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 diabetes⁹⁴ 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 analysis⁴⁴ 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 quantification¹⁰⁶ and disease severity classification in diabetic neuropathy.¹⁰⁷

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Conflict of Interest

None declared.

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References

- Minsky M. Memoir on inventing the confocal scanning microscope. *Scanning* 1988;10:128–138
- Petráň M, Hadravský M, Egger MD, Galambos R. Tandem-scanning reflected-light microscope. *J Opt Soc Am* 1968;58:661–664
- Dilly PN. Tandem scanning reflected light microscopy of the cornea. *Scanning* 1988;10:153–156
- Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. *Cornea* 2001;20(04):374–384
- Lambiase A, Rama P, Bonini S, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for corneal neurotrophic ulcers. *N Engl J Med* 1998;338(17):1174–1180
- Apfel SC, Schwartz S, Adornato BT, et al. Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: a randomized controlled trial. rhNGF Clinical Investigator Group. *JAMA* 2000;284(17):2215–2221
- Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Grönhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2000;41(10):2915–2921
- Malik RA, Newrick PG, Sharma AK, et al. Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* 1989;32(02):92–102
- Malik RA, Kallinikos P, Abbott CA, et al. Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. *Diabetologia* 2003;46(05):683–688
- Edwards K, Pritchard N, Vagenas D, Russell A, Malik RA, Efron N. Utility of corneal confocal microscopy for assessing mild diabetic neuropathy: baseline findings of the LANDMark study. *Clin Exp Optom* 2012;95(03):348–354
- Perkins BA, Lovblom LE, Bril V, et al. Corneal confocal microscopy for identification of diabetic sensorimotor polyneuropathy: a pooled multinational consortium study. *Diabetologia* 2018;61(08):1856–1861
- Gad H, Petropoulos IN, Khan A, et al. Corneal confocal microscopy for the diagnosis of diabetic peripheral neuropathy: A systematic review and meta-analysis. *J Diabetes Investig* 2022;13(01):134–147
- Perkins BA, Lovblom LE, Lewis EJH, et al. Corneal confocal microscopy predicts the development of diabetic neuropathy: A longitudinal diagnostic multinational consortium study. *Diabetes Care* 2021;44(09):2107–2114
- Tavakoli M, Mitu-Pretorian M, Petropoulos IN, et al. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes* 2013;62(01):254–260
- Azmi S, Jeziorska M, Ferdousi M, et al. Early nerve fibre regeneration in individuals with type 1 diabetes after simultaneous pancreas and kidney transplantation. *Diabetologia* 2019;62(08):1478–1487
- Tavakoli M, Ferdousi M, Petropoulos IN, et al. Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. *Diabetes Care* 2015;38(05):838–843
- Ferdousi M, Kalteniece A, Azmi S, et al. diagnosis of neuropathy and risk factors for corneal nerve loss in type 1 and type 2 diabetes: A corneal confocal microscopy study. *Diabetes Care* 2021;44(01):150–156
- Ferdousi M, Romanchuk K, Mah JK, et al. Early corneal nerve fibre damage and increased Langerhans cell density in children with type 1 diabetes mellitus. *Sci Rep* 2019;9(01):8758
- Gad H, Al-Jarrah B, Saraswathi S, et al. Corneal nerve loss in children with type 1 diabetes mellitus without retinopathy or microalbuminuria. *J Diabetes Investig* 2020;11(06):1594–1601
- Petropoulos IN, Green P, Chan AW, et al. Corneal confocal microscopy detects neuropathy in patients with type 1 diabetes without retinopathy or microalbuminuria. *PLoS One* 2015;10(04):e0123517
- Asghar O, Petropoulos IN, Alam U, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. *Diabetes Care* 2014;37(09):2643–2646
- De Clerck EEB, Schouten JSAG, Berendschot TTJM, et al. Reduced corneal nerve fibre length in prediabetes and type 2 diabetes: the Maastricht Study. *Acta Ophthalmol* 2020;98(05):485–491

- 23 Ziegler D, Papanas N, Zhivov A, et al; German Diabetes Study (GDS) Group. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. *Diabetes* 2014;63(07):2454–2463
- 24 Kalteniece A, Ferdousi M, Azmi S, et al. Corneal confocal microscopy detects small nerve fibre damage in patients with painful diabetic neuropathy. *Sci Rep* 2020;10(01):3371
- 25 Kalteniece A, Ferdousi M, Azmi S, et al. Corneal nerve loss is related to the severity of painful diabetic neuropathy. *Eur J Neurol* 2022;29(01):286–294
- 26 Maddaloni E, Sabatino F, Del Toro R, et al. In vivo corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in Type 1 diabetes. *Diabet Med* 2015;32(02):262–266
- 27 Wang H, Fan D, Wang W, Zhang S, Wang X. Early diagnosis of diabetic autonomic neuropathy by corneal confocal microscopy [article in Chinese]. *Zhonghua Yi Xue Za Zhi* 2015;95(35):2851–2856
- 28 Ishibashi F, Kojima R, Taniguchi M, Kosaka A, Uetake H, Tavakoli M. The preferential impairment of pupil constriction stimulated by blue light in patients with type 2 diabetes without autonomic neuropathy. *J Diabetes Res* 2017;2017:6069730
- 29 Misra SL, Craig JP, Patel DV, et al. In vivo confocal microscopy of corneal nerves: an ocular biomarker for peripheral and cardiac autonomic neuropathy in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2015;56(09):5060–5065
- 30 Tavakoli M, Begum P, McLaughlin J, Malik RA. Corneal confocal microscopy for the diagnosis of diabetic autonomic neuropathy. *Muscle Nerve* 2015;52(03):363–370
- 31 Azmi S, Ferdousi M, Alam U, et al. Small-fibre neuropathy in men with type 1 diabetes and erectile dysfunction: a cross-sectional study. *Diabetologia* 2017;60(06):1094–1101
- 32 Dhage S, Ho JH, Ferdousi M, et al. Small fibre pathology is associated with erectile dysfunction in men with type 2 diabetes. *Diabetes Metab Res Rev* 2020;36(03):e3263
- 33 Pritchard N, Edwards K, Russell AW, Perkins BA, Malik RA, Efron N. Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care* 2015;38(04):671–675
- 34 Lovblom LE, Halpern EM, Wu T, et al. In vivo corneal confocal microscopy and prediction of future-incident neuropathy in type 1 diabetes: a preliminary longitudinal analysis. *Can J Diabetes* 2015;39(05):390–397
- 35 Lewis EJH, Lovblom LE, Ferdousi M, et al. Rapid corneal nerve fiber loss: a marker of diabetic neuropathy onset and progression. *Diabetes Care* 2020;43(08):1829–1835
- 36 Dehghani C, Russell AW, Perkins BA, et al. A rapid decline in corneal small fibers and occurrence of foot ulceration and Charcot foot. *J Diabetes Complications* 2016;30(08):1437–1439
- 37 Ferdousi M, Azmi S, Petropoulos IN, et al. Corneal confocal microscopy detects small fibre neuropathy in patients with upper gastrointestinal cancer and nerve regeneration in chemotherapy induced peripheral neuropathy. *PLoS One* 2015;10(10):e0139394
- 38 Bennedsgaard K, Ventzel L, Andersen NT, et al. Oxaliplatin- and docetaxel-induced polyneuropathy: clinical and neurophysiological characteristics. *J Peripher Nerv Syst* 2020;25(04):377–387
- 39 Chiang JCB, Goldstein D, Trinh T, et al. A cross-sectional study of sub-basal corneal nerve reduction following neurotoxic chemotherapy. *Transl Vis Sci Technol* 2021;10(01):24
- 40 Stettner M, Hinrichs L, Guthoff R, et al. Corneal confocal microscopy in chronic inflammatory demyelinating polyneuropathy. *Ann Clin Transl Neurol* 2015;3(02):88–100
- 41 Motte J, Grüter T, Fisse AL, et al. Corneal inflammatory cell infiltration predicts disease activity in chronic inflammatory demyelinating polyneuropathy. *Sci Rep* 2021;11(01):15150
- 42 Pitarokoili K, Sturm D, Labedi A, et al. Neuroimaging markers of clinical progression in chronic inflammatory demyelinating polyradiculoneuropathy. *Ther Adv Neurol Disord* 2019;12:1756286419855485
- 43 Kemp HI, Petropoulos IN, Rice ASC, et al. use of corneal confocal microscopy to evaluate small nerve fibers in patients with human immunodeficiency virus. *JAMA Ophthalmol* 2017;135(07):795–800
- 44 Chen X, Graham J, Petropoulos IN, et al. Corneal nerve fractal dimension: a novel corneal nerve metric for the diagnosis of diabetic sensorimotor polyneuropathy. *Invest Ophthalmol Vis Sci* 2018;59(02):1113–1118
- 45 Freeman R, Gewandter JS, Faber CG, et al. Idiopathic distal sensory polyneuropathy: ACTION diagnostic criteria. *Neurology* 2020;95(22):1005–1014
- 46 Tavakoli M, Marshall A, Pitceathly R, et al. Corneal confocal microscopy: a novel means to detect nerve fibre damage in idiopathic small fibre neuropathy. *Exp Neurol* 2010;223(01):245–250
- 47 Egenolf N, Zu Altschilchesche CM, Krefß L, et al. Diagnosing small fiber neuropathy in clinical practice: a deep phenotyping study. *Ther Adv Neurol Disord* 2021;14:17562864211004318
- 48 Tavakoli M, Marshall A, Banka S, et al. Corneal confocal microscopy detects small-fiber neuropathy in Charcot-Marie-Tooth disease type 1A patients. *Muscle Nerve* 2012;46(05):698–704
- 49 Perini I, Tavakoli M, Marshall A, Minde J, Morrison I. Rare human nerve growth factor- β mutation reveals relationship between C-afferent density and acute pain evaluation. *J Neurophysiol* 2016;116(02):425–430
- 50 Pagovich OE, Vo ML, Zhao ZZ, et al. Corneal confocal microscopy: neurologic disease biomarker in Friedreich ataxia. *Ann Neurol* 2018;84(06):893–904
- 51 Barnett C, Alon T, Abraham A, et al. Evidence of small-fiber neuropathy in neurofibromatosis type 1. *Muscle Nerve* 2019;60(06):673–678
- 52 Plante-Bordeneuve V. Transthyretin familial amyloid polyneuropathy: an update. *J Neurol* 2018;265(04):976–983
- 53 Rousseau A, Cauquil C, Dupas B, et al. Potential role of in vivo confocal microscopy for imaging corneal nerves in transthyretin familial amyloid polyneuropathy. *JAMA Ophthalmol* 2016;134(09):983–989
- 54 Zhang Y, Liu Z, Zhang Y, et al. Corneal sub-basal whorl-like nerve plexus: a landmark for early and follow-up evaluation in transthyretin familial amyloid polyneuropathy. *Eur J Neurol* 2021;28(02):630–638
- 55 Politei JM, Durand C, Schenone AB. Small fiber neuropathy in Fabry disease: a review of pathophysiology and treatment. *J Inborn Errors Metab Screening* 2016;4:2326409816661351
- 56 Tavakoli M, Marshall A, Thompson L, et al. Corneal confocal microscopy: a novel noninvasive means to diagnose neuropathy in patients with Fabry disease. *Muscle Nerve* 2009;40(06):976–984
- 57 Bitirgen G, Turkmen K, Malik RA, Ozkagnici A, Zengin N. Corneal confocal microscopy detects corneal nerve damage and increased dendritic cells in Fabry disease. *Sci Rep* 2018;8(01):12244
- 58 Sharma S, Tobin V, Vas PRJ, Rayman G. The LDIFLARE and CCM methods demonstrate early nerve fibre abnormalities in untreated hypothyroidism: A prospective study. *J Clin Endocrinol Metab* 2018;103(08):3094–3102
- 59 Ramírez M, Martínez-Martínez LA, Hernández-Quintela E, Velazco-Casapía J, Vargas A, Martínez-Lavín M. Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy. *Semin Arthritis Rheum* 2015;45(02):214–219
- 60 Oudejans L, He X, Niesters M, Dahan A, Brines M, van Velzen M. Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia. *Sci Rep* 2016;6:23573

- 61 Erkan Turan K, Kocabeyoglu S, Unal-Cevik I, Bezci F, Akinci A, Irkec M. Ocular surface alterations in the context of corneal in vivo confocal microscopic characteristics in patients with fibromyalgia. *Cornea* 2018;37(02):205–210
- 62 Evdokimov D, Frank J, Klitsch A, et al. Reduction of skin innervation is associated with a severe fibromyalgia phenotype. *Ann Neurol* 2019;86(04):504–516
- 63 Anjos R, Vieira L, Sousa A, Maduro V, Alves N, Candelaria P. Peripheral neuropathy in Parkinson disease: an in vivo confocal microscopy study. *Acta Ophthalmol* 2014;92. Doi: 10.1111/j.1755-3768.2014.2433.x
- 64 Kass-Iliyya L, Javed S, Gosal D, et al. Small fiber neuropathy in Parkinson's disease: A clinical, pathological and corneal confocal microscopy study. *Parkinsonism Relat Disord* 2015;21(12):1454–1460
- 65 Podgorny PJ, Suchowersky O, Romanchuk KG, Feasby TE. Evidence for small fiber neuropathy in early Parkinson's disease. *Parkinsonism Relat Disord* 2016;28:94–99
- 66 Misra SL, Kersten HM, Roxburgh RH, Danesh-Meyer HV, McGhee CN. Corneal nerve microstructure in Parkinson's disease. *J Clin Neurosci* 2017;39:53–58
- 67 Arrigo A, Rania L, Calamuneri A, et al. Early corneal innervation and trigeminal alterations in Parkinson disease: a pilot study. *Cornea* 2018;37(04):448–454
- 68 Avetisov SE, Karabanov AV, Surnina ZV, Gamidov AA. Changes in corneal nerves fibers in the early stages of Parkinson's disease according to in vivo confocal microscopy (preliminary report) [article in Chinese]. *Vestn Oftalmol* 2020;136(5. Vyp. 2):191–196
- 69 Lim SH, Ferdousi M, Kalteniece A, et al. Corneal confocal microscopy detects small fibre neurodegeneration in Parkinson's disease using automated analysis. *Sci Rep* 2020;10(01):20147
- 70 Che NN, Ding GX, Chen SY, et al. Measurement of corneal nerve fiber parameters in patients with Parkinson's disease [article in Chinese]. *Zhonghua Yi Xue Za Zhi* 2021;101(07):498–503
- 71 Lim SH, Ferdousi M, Kalteniece A, et al. Corneal confocal microscopy identifies Parkinson's disease with more rapid motor progression. *Mov Disord* 2021;36(08):1927–1934
- 72 Mikolajczak J, Zimmermann H, Kheirkhah A, et al. Patients with multiple sclerosis demonstrate reduced subbasal corneal nerve fibre density. *Mult Scler* 2017;23(14):1847–1853
- 73 Bitirgen G, Akpinar Z, Malik RA, Ozkagnici A. Use of corneal confocal microscopy to detect corneal nerve loss and increased dendritic cells in patients with multiple sclerosis. *JAMA Ophthalmol* 2017;135(07):777–782
- 74 Petropoulos IN, Kamran S, Li Y, et al. Corneal confocal microscopy: an imaging endpoint for axonal degeneration in multiple sclerosis. *Invest Ophthalmol Vis Sci* 2017;58(09):3677–3681
- 75 Ferrari G, Grisan E, Scarpa F, et al. Corneal confocal microscopy reveals trigeminal small sensory fiber neuropathy in amyotrophic lateral sclerosis. *Front Aging Neurosci* 2014;6:278
- 76 Fu J, He J, Zhang Y, et al. Small fiber neuropathy for assessment of disease severity in amyotrophic lateral sclerosis: corneal confocal microscopy findings. *Orphanet J Rare Dis* 2022;17(01):7
- 77 Gad H, Khan A, Akhtar N, et al. Corneal nerve and endothelial cell damage in patients with transient ischemic attack and minor ischemic stroke. *PLoS One* 2019;14(03):e0213319
- 78 Khan A, Kamran S, Akhtar N, et al. Corneal confocal microscopy detects a reduction in corneal endothelial cells and nerve fibres in patients with acute ischemic stroke. *Sci Rep* 2018;8(01):17333
- 79 Khan A, Akhtar N, Kamran S, et al. Corneal confocal microscopy identifies greater corneal nerve damage in patients with a recurrent compared to first ischemic stroke. *PLoS One* 2020;15(04):e0231987
- 80 Kamran S, Khan A, Salam A, et al. Cornea: a window to white matter changes in stroke; corneal confocal microscopy a surrogate marker for the presence and severity of white matter hyperintensities in ischemic stroke. *J Stroke Cerebrovasc Dis* 2020;29(03):104543
- 81 Ponirakis G, Al Hamad H, Sankaranarayanan A, et al. Association of corneal nerve fiber measures with cognitive function in dementia. *Ann Clin Transl Neurol* 2019;6(04):689–697
- 82 Al-Janahi E, Ponirakis G, Al Hamad H, et al. Corneal nerve and brain imaging in mild cognitive impairment and dementia. *J Alzheimers Dis* 2020;77(04):1533–1543
- 83 Ponirakis G, Elstouhy A, Al Hamad H, et al. Association of cerebral ischemia with corneal nerve loss and brain atrophy in MCI and dementia. *Front Neurosci* 2021;15:690896
- 84 Kinard KI, Smith AG, Singleton JR, et al. Chronic migraine is associated with reduced corneal nerve fiber density and symptoms of dry eye. *Headache* 2015;55(04):543–549
- 85 Shetty R, Deshmukh R, Shroff R, Dedhiya C, Jayadev C. Subbasal nerve plexus changes in chronic migraine. *Cornea* 2018;37(01):72–75
- 86 Lee JI, Böcking T, Holle-Lee D, et al. Corneal confocal microscopy demonstrates corneal nerve loss in patients with trigeminal neuralgia. *Front Neurol* 2020;11:661
- 87 O'Neill F, Marshall A, Ferdousi M, Malik RA. Corneal confocal microscopy detects small-fiber neuropathy in burning mouth syndrome: a cross-sectional study. *J Oral Facial Pain Headache* 2019;33(03):337–341
- 88 Mehra S, Tavakoli M, Kallinikos PA, et al. Corneal confocal microscopy detects early nerve regeneration after pancreas transplantation in patients with type 1 diabetes. *Diabetes Care* 2007;30(10):2608–2612
- 89 Tavakoli M, Kallinikos P, Iqbal A, et al. Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. *Diabet Med* 2011;28(10):1261–1267
- 90 Adam S, Azmi S, Ho JH, et al. Improvements in diabetic neuropathy and nephropathy after bariatric surgery: a prospective cohort study. *Obes Surg* 2021;31(02):554–563
- 91 Azmi S, Ferdousi M, Liu Y, et al. Bariatric surgery leads to an improvement in small nerve fibre damage in subjects with obesity. *Int J Obes* 2021;45(03):631–638
- 92 Dahan A, Dunne A, Swartjes M, et al. ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density. *Mol Med* 2013;19(01):334–345
- 93 Culver DA, Dahan A, Bajorunas D, et al. Cibinetide improves corneal nerve fiber abundance in patients with sarcoidosis-associated small nerve fiber loss and neuropathic pain. *Invest Ophthalmol Vis Sci* 2017;58(06):BIO52–BIO60
- 94 Brines M, Dunne AN, van Velzen M, et al. ARA 290, a non-erythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes. *Mol Med* 2015;20(01):658–666
- 95 Lewis EJH, Perkins BA, Lovblom LE, Bazinet RP, Wolever TMS, Brill V. Effect of omega-3 supplementation on neuropathy in type 1 diabetes: A 12-month pilot trial. *Neurology* 2017;88(24):2294–2301
- 96 Britten-Jones AC, Kamel JT, Roberts LJ, et al. Investigating the neuroprotective effect of oral omega-3 fatty acid supplementation in type 1 diabetes (nPROOFS1): A randomized placebo-controlled trial. *Diabetes* 2021;70(08):1794–1806
- 97 Ponirakis G, Abdul-Ghani MA, Jayyousi A, et al. Effect of treatment with exenatide and pioglitazone or basal-bolus insulin on diabetic neuropathy: a substudy of the Qatar Study. *BMJ Open Diabetes Res Care* 2020;8(01):e001420
- 98 Pritchard N, Edwards K, Efron N. Non-contact laser-scanning confocal microscopy of the human cornea in vivo. *Cont Lens Anterior Eye* 2014;37(01):44–48
- 99 Edwards K, Pritchard N, Gosschalk K, et al. Wide-field assessment of the human corneal subbasal nerve plexus in diabetic

- neuropathy using a novel mapping technique. *Cornea* 2012;31(09):1078–1082
- 100 Kheirkhah A, Muller R, Mikolajczak J, et al. Comparison of standard versus wide-field composite images of the corneal subbasal layer by in vivo confocal microscopy. *Invest Ophthalmol Vis Sci* 2015;56(10):5801–5807
- 101 Allgeier S, Maier S, Mikut R, et al. Mosaicking the subbasal nerve plexus by guided eye movements. *Invest Ophthalmol Vis Sci* 2014;55(09):6082–6089
- 102 Petropoulos IN, Ferdousi M, Marshall A, et al. The inferior whorl for detecting diabetic peripheral neuropathy using corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2015;56(04):2498–2504
- 103 Petropoulos IN, Al-Mohammed A, Chen X, et al. The utility of corneal nerve fractal dimension analysis in peripheral neuropathies of different etiology. *Transl Vis Sci Technol* 2020;9(09):43
- 104 Petropoulos IN, Alam U, Fadavi H, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2014;55(04):2071–2078
- 105 Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Fully automated, semiautomated, and manual morphometric analysis of corneal subbasal nerve plexus in individuals with and without diabetes. *Cornea* 2014;33(07):696–702
- 106 Williams BM, Borroni D, Liu R, et al. An artificial intelligence-based deep learning algorithm for the diagnosis of diabetic neuropathy using corneal confocal microscopy: a development and validation study. *Diabetologia* 2020;63(02):419–430
- 107 Salahouddin T, Petropoulos IN, Ferdousi M, et al. Artificial intelligence-based classification of diabetic peripheral neuropathy from corneal confocal microscopy images. *Diabetes Care* 2021;44(07):e151–e153