# Corneal confocal microscopy meets continuous glucose monitoring: a tale of two technologies

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Zhao *et al*[1] from Shanghai, China, have undertaken a detailed clinical study in a cohort of 206 asymptomatic patients with type 2 diabetes utilizing advanced *in vivo* nerve imaging with corneal confocal microscopy (CCM-Heidelberg HRT III RCM) and continuous glucose monitoring (CGM-iPro2 system) over 7 days. The study provides important insights into the relationship between relatively short-term glucose perturbation over 7 days and corneal nerve loss in diabetic neuropathy. Furthermore, it reinforces the role of CCM in detecting early sub-clinical nerve loss, as almost one-third of asymptomatic patients had corneal nerve fiber loss, which was independently associated with glucose time in range (TIR) (percentage of time within the glucose range of 3.9–10.0 mmol/L) but not with quartiles of hemoglobin A1c (HbA1c), an established measure of long-term glycemic control. Indeed, each 10% increase in TIR was associated with a 28.2% (95% CI: 0.595–0.866, $P = 0.001$) decrease in the risk of abnormal corneal nerve fiber length (CNFL). This supports recent studies showing that TIR is associated with other long-term complications of diabetes, including symptomatic diabetic neuropathy in patients with nephropathy[2] and diabetic retinopathy.[3]

In 2003, we pioneered the use of CCM to objectively quantify neurodegeneration in sub-clinical and more advanced diabetic neuropathy[4] and dared to suggest that CCM could act as an objective surrogate marker for diabetic neuropathy in longitudinal studies, especially as an end-point in clinical trials.[5] This was based on our initial study, which showed a significant reduction in all three corneal nerve parameters in patients with moderate and severe neuropathy, and especially a significant reduction in corneal nerve branch density in those with mild neuropathy.[4] This was followed by the demonstration of corneal nerve regeneration within 6 months of simultaneous pancreas and kidney transplantation in patients with type 1 diabetes and severe baseline diabetic neuropathy.[6] Twenty years later, with over 500 published studies from Europe, Canada, Australia, and latterly Japan and China, CCM is a firmly established measure of nerve fiber damage and repair in diabetic neuropathy,[7] and shows promise in other peripheral neuropathies and central neurodegenerative diseases.[8] A Web of Science search on 1 March 2022, with "corneal confocal microscopy" and "nerves" as the primary search terms, returned 1382 publications which have been cited 35,489 times and have a H-index of 90.

Indeed, we have shown that corneal nerve loss is comparable to intraepidermal nerve fiber (IENF) loss,[9,10] the gold standard for assessing small fiber damage. It has excellent diagnostic[11,12] and prognostic[13] value in patients with diabetic neuropathy. Stem *et al*[14] observed corneal nerve loss in patients with type 2 diabetes and diabetic peripheral neuropathy (DPN), as well as patients with type 1 diabetes without DPN, based on symptoms/signs and nerve conduction velocity (NCV), and they suggested that the type of diabetes may influence the extent of corneal nerve loss. However, evidence of corneal nerve loss in children with type 1 diabetes[15] and subjects with impaired glucose tolerance[16] and recently diagnosed type 2 diabetes[17] suggests that CCM can identify early sub-clinical neuropathy in both types of diabetes, and that minor glycemic perturbations and other factors such as obesity, hypertension, and hyperlipidemia may drive early neurodegeneration. Interestingly, in a Canadian study of 64 healthy volunteers, there was a strong independent association between CNFL and HbA1c in the normal range, suggesting that even minimal glycemic exposure may lead to sub-clinical nerve injury.[18] Additionally, we have published normative values from 343 healthy volunteers and showed a small age dependent decrease

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in corneal nerve fiber density (CNFD) and CNFL, but no impact of height, weight, or body mass index.[19] In our recent study of a cohort of 490 participants, corneal nerve loss was associated with low-density lipoprotein (LDL)-cholesterol and triglycerides, as opposed to hyperglycemia, in type 1 diabetes, and with age, weight, and HbA1c in type 2 diabetes.[20] In a study from Norway, 144 participants with screen detected type 2 diabetes with and without diabetic neuropathy showed a lower CNFD, which was associated with age, height, and total and LDL-cholesterol.[21] Furthermore, reduced CNFL predicts 4-year incident DPN,[22] and a more rapid decline in CNFL is associated with the development of clinical diabetic neuropathy.[23] Indeed, in a large longitudinal study of 261 patients without DPN, we have recently shown that a CNFL of $<14.1\,mm/mm^2$ was associated with 67% sensitivity, 71% specificity, and a hazard ratio of 2.95 (95% CI 1.70–5.11; $P < 0.001$) for new-onset DPN over a mean follow up of 5.8 years.[13] In a Japanese study of patients with type 1 diabetes, the mean annual HbA1c level 7 to 10 years prior to CCM was an independent predictor of reduced CNFL and CNFD.[24] In a study from Australia of 231 individuals with type 1 and type 2 diabetes and mild neuropathy, HbA1c showed a significant correlation with CNFL.[25]

To further understand the relationship between glucose perturbation and corneal nerve loss and repair, it is important to consider whether improved glycemia is associated with corneal nerve regeneration. We initially showed that simultaneous pancreas and kidney (SPK) transplantation and normalization of HbA1c in patients with type 1 diabetes was associated with corneal nerve regeneration after 6 months.[6] We extended this study and showed continued corneal nerve fiber repair 12 months after SPK, but with no impact on conventional neuropathy end points, for example, symptoms, nerve conduction, and IENF repair.[26] Furthermore, SPK was associated with continued regeneration of corneal nerve fibers, followed by an improvement in neuropathic symptoms after 24 months, and nerve conduction after 36 months.[27] Previously we showed that an improvement in HbA1c, blood pressure, and total cholesterol over 24 months was associated with corneal nerve regeneration.[28] In relation to the link between CNFL and glucose variability observed by Zhao *et al*[1], we have previously shown that continuous subcutaneous insulin infusion with lower glucose variability, as compared to basal bolus insulin, was associated with corneal nerve regeneration, despite a comparable HbA1c.[29] In a recent longitudinal study of patients with type 1 diabetes over 6.5 years, those with the highest HbA1c (68.1–86.7 mmol/mol) showed corneal nerve loss, while those in the optimally controlled tertile (HbA1c, 35.0–54.0 mmol/mol) showed corneal nerve regeneration.[30] Recently, however, we have shown progressive corneal nerve fiber degeneration despite an improvement in HbA1c and total cholesterol.[31] Corneal nerve regeneration has been demonstrated after bariatric surgery in obese subjects with[32] and without[33] diabetes, and was independently associated with an improvement in triglycerides, but not HbA1c. In a randomized clinical trial of once weekly glucagon-like peptide-1 or basal bolus insulin over 12 months, a marked improvement in HbA1c by ~3% was associated with corneal nerve regeneration, but with no change in vibration perception or sudomotor function.[34] Two separate trials with omega-3 fatty acid in patients with type 1 diabetes have independently shown corneal nerve regeneration, with no change in NCV and sensory and autonomic nerve function.[35,36]

In conclusion, Zhao *et al*[1] confirm that CCM identifies sub-clinical and established neuropathy, and shows the dynamic and responsive nature of corneal nerves in relation to even minor glucose perturbations detected using CGM. This further emphasizes the key role of CCM as an end-point in clinical trials of diabetic neuropathy, and perhaps other neurodegenerative diseases.

### Conflicts of interest

None.

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