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ORIGINAL ARTICLE

Corneal nerve loss in children with type 1 diabetes mellitus without retinopathy or microalbuminuria

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ABSTRACT

Aims/Introduction: Corneal confocal microscopy is a rapid, non-invasive ophthalmic technique to identify subclinical neuropathy. The aim of this study was to quantify corneal nerve morphology in children with type 1 diabetes mellitus compared with age-matched healthy controls using corneal confocal microscopy.

Materials and Methods: A total of 20 participants with type 1 diabetes mellitus (age 14 ± 2 years, diabetes duration 4.08 ± 2.91 years, glycated hemoglobin 9.3 ± 2.1%) without retinopathy or microalbuminuria and 20 healthy controls were recruited from outpatient clinics. Corneal confocal microscopy was undertaken, and corneal nerve fiber density (n/mm^2), corneal nerve branch density (n/mm^2), corneal nerve fiber length (mm/mm^2), corneal nerve fiber tortuosity and inferior whorl length (mm/mm^2) were quantified manually.

Results: Corneal nerve fiber density (22.73 ± 8.84 vs 32.92 ± 8.59; $P < 0.001$), corneal nerve branch density (26.19 ± 14.64 vs 47.34 ± 20.01; $P < 0.001$), corneal nerve fiber length (13.26 ± 4.06 vs 19.52 ± 4.54; $P < 0.001$) and inferior whorl length (15.50 ± 5.48 vs 23.42 ± 3.94; $P < 0.0001$) were significantly lower, whereas corneal nerve fiber tortuosity (14.88 ± 5.28 vs 13.52 ± 3.01; $P = 0.323$) did not differ between children with type 1 diabetes mellitus and controls. Glycated hemoglobin correlated with corneal nerve fiber tortuosity ($P < 0.006$) and aspartate aminotransferase correlated with corneal nerve fiber density ($P = 0.039$), corneal nerve branch density ($P = 0.003$) and corneal nerve fiber length ($P = 0.037$).

Conclusion: Corneal confocal microscopy identifies significant subclinical corneal nerve loss, especially in the inferior whorl of children with type 1 diabetes mellitus without retinopathy or microalbuminuria.

INTRODUCTION

Type 1 diabetes mellitus affects over half a million children worldwide[1,2]. Diabetes is associated with chronic microvascular complications in adults, which increase morbidity and all-cause mortality[3]. Diabetes is the main cause of distal symmetric polyneuropathy (DSPN)[4–6]. Adults with DSPN present with a combination of symptoms, such as numbness, pain and tingling in the feet[7]. The American Diabetes Association endorses screening for DSPN at diagnosis of type 2 diabetes, 5 years after the diagnosis of type 1 diabetes and annually thereafter [8]. Children and adolescents with type 1 diabetes rarely complain of neuropathic symptoms. However, a study of children with type 1 diabetes showed reduced motor and sensory nerve

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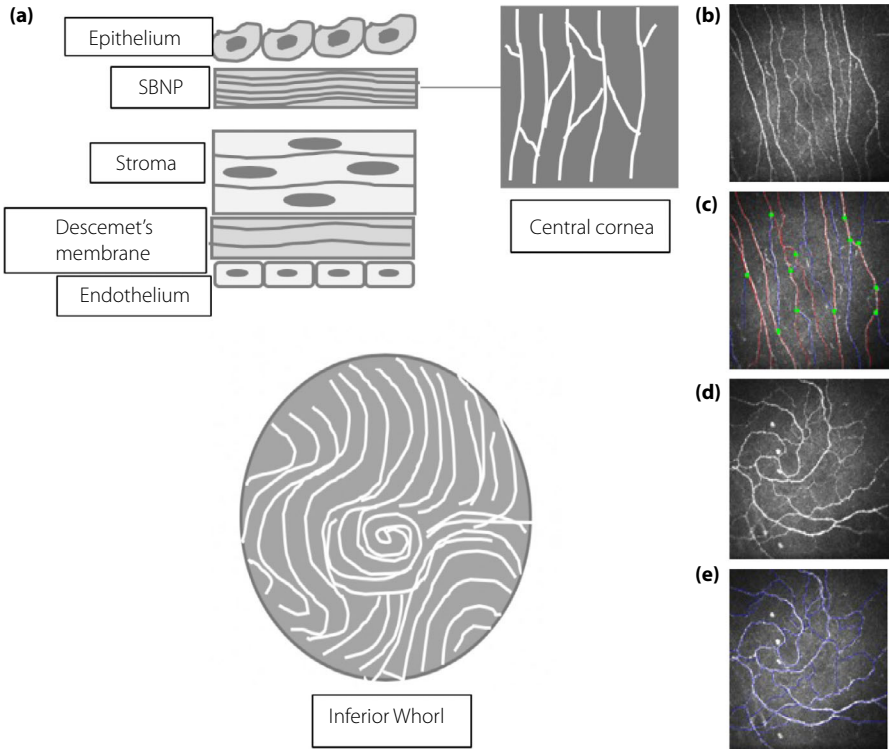

Figure 1 | Central corneal sub-basal nerve plexus and inferior whorl. (a) Schematic presentation of the sub-basal nerve plexus (SBNP) at the central and inferior whorl. (b) Nerve fibers at the central cornea, (c) tracing of the nerves using CCMetrics, (d) nerve fibers at the inferior whorl and (e) tracing of the inferior whorl using CCMetrics.

Table 1 | Clinical and laboratory measures in patients with type 1 diabetes and controls

	Healthy (n = 20)	T1DM (n = 20)	P-value
Age (years)	12.83 ± 1.91	14.47 ± 2.43	**0.02**
Duration of T1DM	-	4.08 ± 2.91	NA
Height (m)	1.45 ± 0.13	1.54 ± 0.09	**0.02**
Weight (kg)	47.87 ± 18.63	51.65 ± 13.46	0.467
BMI (kg/m^2)	22.26 ± 5.47	21.68 ± 5.09	0.733
HbA_{1c} (%)	-	9.3 ± 2.1	NA
Bilirubin (μmol/L)	10.54 ± 5.4	13.22 ± 5.92	0.206
AST (IU/L)	24.83 ± 5.45	20.44 ± 4.23	**0.02**
ALT (IU/L)	15.08 ± 4.03	16.44 ± 3.74	0.339
25(OH)D (ng/mL)	23.88 ± 8.96	18.16 ± 8.56	0.085
Microalbuminuria, n (%)			
Yes	-	0	NA
No	-	11 (55.0%)	
Diabetic retinopathy, n (%)			
Yes	-	0	NA
No	-	8 (40.0%)	

Bold signifies the statistically significant comparisons. Data are presented as mean ± SD. 25(OH)D, 250hydroxy vitamin D; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HbA_{1c}, glycated hemoglobin; NA, not available; T1DM, type 1 diabetes.

conduction velocities (24%), and at least one neuropathic symptom (60%) or sign (58%)[9]. In another study, symptomatic neuropathy was present in 13.5% of patients, whereas 22.5% of patients had neurophysiological evidence of neuropathy[10] and 18% had impaired vibrotactile sense[11]. Furthermore, in one study, 36% of patients had more than two abnormal autonomic function tests, and 18.8% had severe autonomic neuropathy[12]. In a prospective study, abnormal nerve conduction velocity was found in 31.6% at baseline, which increased to 63.2% after 5 years[13]. In another study, over a period of 10 years, the prevalence of clinical neuropathy increased from 6.5% to 16.1%, whereas nerve conduction velocity abnormalities increased from 17.7% to 46.8%[14]. Although neurophysiological assessments are highly sensitive, they are not easily carried out in children[15]. Vibration perception threshold and tactile perception threshold tests are easy to carry out, but lack sensitivity for the early detection of DSPN[16]. There is a need for non-invasive sensitive screening tools for the early detection of neuropathy in children with diabetes.

Corneal confocal microscopy (CCM) is a rapid, non-invasive and well-tolerated technique to detect and quantify neuropathy in adults with type 1 diabetes[17–24]. An early study found no significant changes in CCM parameters among children with

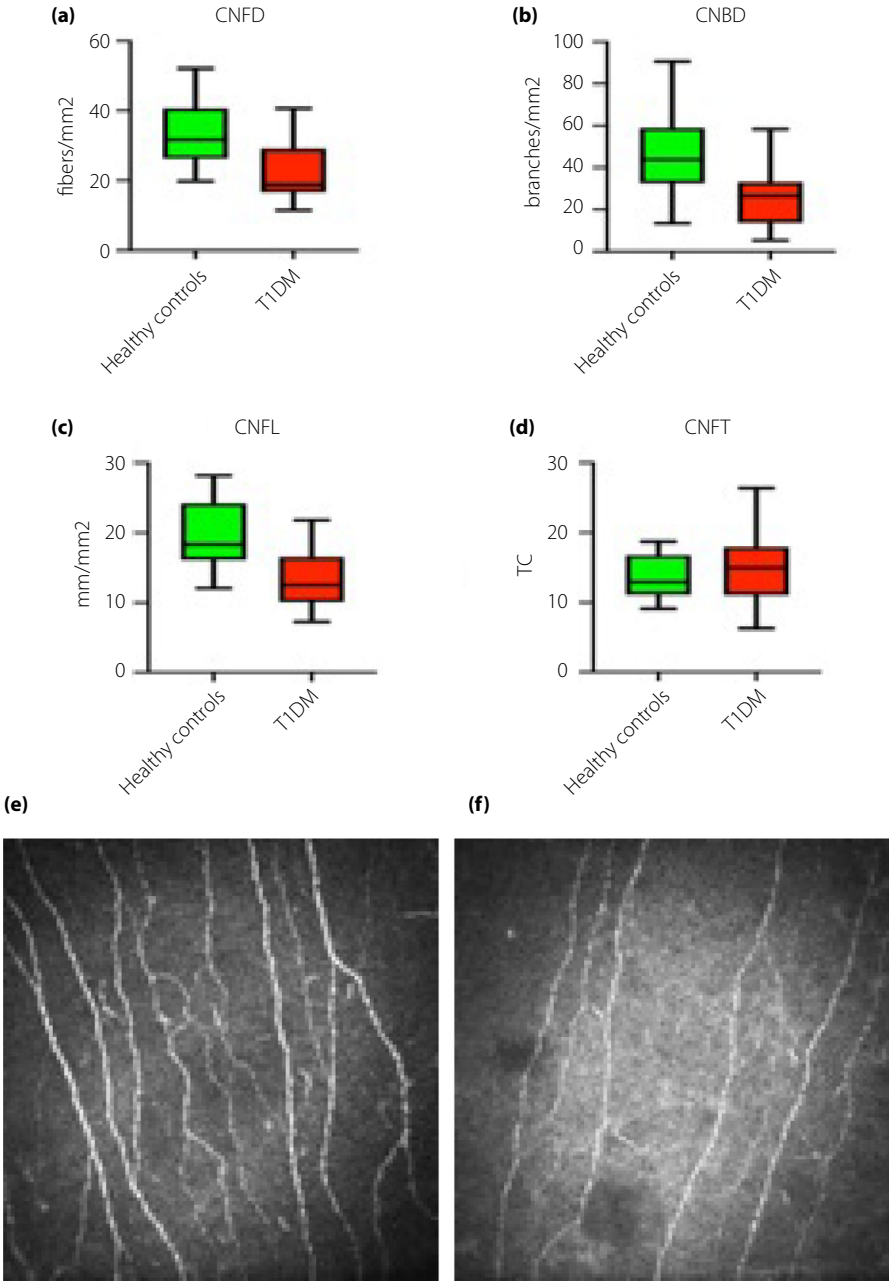

Figure 2 | Corneal confocal microscopy parameters and images of the sub-basal plexus in children with type 1 diabetes (T1DM) and healthy controls. (a) Corneal nerve fiber density (CNFD), (b) corneal nerve branch density (CNBD), (c) corneal nerve fiber length (CNFL), (d) corneal nerve fiber tortuosity (CNFT) and (e) corneal confocal microscopy image of corneal nerves in a healthy control. (f) Corneal confocal microscopy image of reduced corneal nerves in a child with type 1 diabetes.

type 1 diabetes[15]. However, a more recent study has shown a significant reduction in corneal nerve fiber measures in young children with type 1 diabetes with and without diabetic retinopathy[25]. The aim of the present study was to quantify corneal nerve morphology in the central cornea and inferior whorl of children with type 1 diabetes compared with age-matched healthy controls using CCM.

METHODS

A total of 20 participants with type 1 diabetes and 20 age-matched healthy controls underwent CCM. Patients with a history of any other cause of neuropathy, malignancy, deficiency of vitamin B_{12} or folate, chronic renal failure, liver failure, connective tissue or systemic disease (rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, systemic scleroderma,

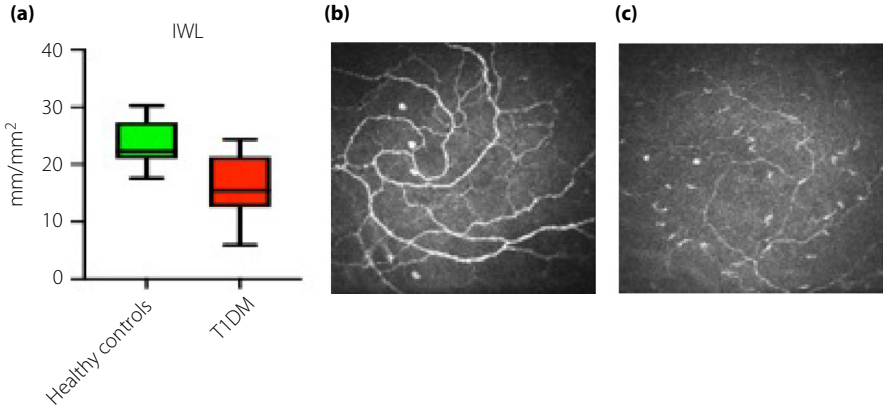

Figure 3 | Inferior whorl length and corneal confocal microscopy images of the inferior whorl in children with type 1 diabetes (T1DM) and healthy controls. (a) Inferior whorl length (IWL) in healthy controls and children with type 1 diabetes, (b) corneal confocal microscopy image of IWL in a healthy control and (c) corneal confocal microscopy image of IWL in a child with type 1 diabetes.

Raynaud's phenomenon), previous corneal trauma or systemic disease that affects the cornea, surgery and a history of or current contact lens wear were excluded from the study. All participants provided assent and parental informed consent. The research adhered to the tenets of the Declaration of Helsinki, and was approved by Sidra Medicine and the Weill Cornell Medicine Research Ethics Committee.

Image selection and quantification

Six central sub-basal nerve plexus images were selected from the central cornea and corneal nerve fiber density (CNFD; n/mm^2), corneal nerve branch density (CNBD; n/mm^2), corneal nerve fiber length (CNFL; mm/mm^2) and corneal nerve fiber tortuosity (CNFT) were quantified using manual CCMetrics (The University of Manchester, Manchester, UK). Six images centered on the inferior whorl and adjacent areas (upper right/left corner and lower right/left corners) were selected, and the inferior whorl length (IWL) (mm/mm^2) was quantified utilizing the manual CNFL mode in CCMetrics (Figure 1)[26]. The investigator was blind to the study group when carrying out CCM and analyzing CCM images.

Statistical analysis

All statistical analyses were carried out using IBM SPSS Statistics software version 26 (IBM Corporation, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant. Normally distributed data were expressed as the mean ± standard deviation, and the means were compared using an independent sample *t*-test. Pearson's correlation was undertaken to investigate the association between clinical parameters and corneal nerve fiber parameters. GraphPad Prism version 8 (La Jolla, CA, USA) was used to build the plots.

RESULTS

A total of 20 participants with type 1 diabetes and 20 healthy controls underwent CCM. Participants with type 1 diabetes were slightly older ($P < 0.02$) and taller ($P < 0.02$), but had comparable weight and body mass index (BMI). They also had a lower aspartate aminotransferase (AST; $P < 0.02$), but comparable bilirubin and alanine aminotransferase (Table 1).

Just four (20%) of the patients met the American Diabetes Association criteria (aged >10 years and >5 years of diabetes) to undergo screening for microvascular complications. Eight (40.0%) underwent assessment for retinopathy, and 11 (55.0%) underwent assessment for microalbuminuria, of whom none had retinopathy or microalbuminuria.

CNFD (22.73 ± 8.84 vs 32.92 ± 8.59; $P = 0.001$), CNBD (26.19 ± 14.64 vs 47.34 ± 20.01; $P < 0.001$) and CNFL (13.26 ± 4.06 vs 19.52 ± 4.54; $P < 0.001$) were lower in patients with type 1 diabetes compared with healthy controls (Figure 2a–c). CNFT did not differ between groups (14.88 ± 5.28 vs 13.52 ± 3.01; $P = 0.323$; Figure 3d). IWL was significantly lower in patients with type 1 diabetes ($n = 19$) compared with controls ($n = 19$; 15.50 ± 5.48 vs 23.42 ± 3.94; $P < 0.0001$; Figure 3a–c). CNFD, CNBD, CNFL and IWL were more than two standard deviations lower than the mean of controls in 15, 10, 30 and 50% of patients with type 1 diabetes.

Correlation between CCM parameters and clinical/laboratory measures

Age, height, BMI, 25-hydroxy vitamin D, bilirubin and alanine aminotransferase did not correlate with any CCM parameter ($P > 0.05$). There was no correlation between duration of diabetes and CCM parameters ($P > 0.05$), and only glycated hemoglobin correlated significantly with CNFT ($P < 0.006$). AST correlated with BMI ($P < 0.01$), CNFD ($P = 0.039$), CNBD ($P = 0.003$) and CNFL ($P = 0.037$; Table 2).

DISCUSSION

In the present study, there was evidence of significant corneal nerve loss in children with type 1 diabetes without retinopathy

Table 2 | Correlations between corneal confocal microscopy parameters and clinical and metabolic parameters

	Age (years)	Duration of disease (years)	HbA_{1c} (%)	Height (m)	BMI (kg/m^2)	25(OH)D (ng/mL)	Bilirubin (μmol/L)	AST (IU/L)	ALT (IU/L)
CNFD (n/mm^2)	−0.278 (0.235)	0.027 (0.915)	−0.300 (0.226)	−0.028 (0.907)	−0.396 (0.084)	0.072 (0.783)	−0.207 (0.41)	0.489 (**0.039**)	−0.121 (0.633)
CNBD (n/mm^2)	−0.144 (0.544)	0.108 (0.670)	0.221 (0.377)	0.040 (0.876)	−0.329 (0.157)	−0.002 (0.995)	0.033 (0.897)	0.666 (**0.003**)	0.129 (0.611)
CNFL (mm/mm^2)	−0.188 (0.428)	0.067 (0.791)	−0.036 (0.887)	0.053 (0.824)	−0.257 (0.275)	−0.099 (0.706)	−0.127 (0.615)	0.495 (**0.037**)	−0.013 (0.959)
CNFT (TC)	0.28 (0.231)	0.032 (0.899)	0.619 (**0.006**)	0.202 (0.392)	0.203 (0.39)	0.244 (0.344)	0.267 (0.284)	−0.165 (0.505)	−0.282 (0.256)
IWL (mm/mm^2)	0.195 (0.423)	0.029 (0.911)	−0.009 (0.974)	0.063 (0.798)	0.358 (0.133)	−0.380 (0.132)	−0.456 (0.066)	0.014 (0.957)	−0.001 (0.995)

Bold signifies the statistically significant comparisons. 25(OH)D, 25-hydroxy vitamin D; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CNFT, corneal nerve fiber tortuosity; HbA_{1c}, glycated hemoglobin; IWL, inferior whorl length; TC, tortuosity coefficient.

or microalbuminuria. It is critical to detect and prevent nerve damage at the earliest stage of diabetic neuropathy, as improvement in glycemic control and other risk factors, such as obesity, hypertension and dyslipidemia, might prevent nerve degeneration and promote nerve regeneration[27,28].

Previous studies of adults with type 1 diabetes have found a significant reduction in central CNFD, CNBD and CNFL compared with healthy controls[18,29–35] and in type 1 diabetes patients without retinopathy or microalbuminuria[34]. Corneal nerve loss has good diagnostic utility for both diabetic somatic and autonomic neuropathy[22]. Furthermore, a lower CNFL is associated with the development of clinical diabetic neuropathy[31,36,37], and a more rapid reduction in CNFL predicts the development and progression of diabetic neuropathy[38]. Significant improvements in CNFD, CNBD and CNFL have been observed in type 1 diabetes patients after simultaneous pancreas and kidney transplantation[39,40], omega-3 supplementation[41], and an improvement in multiple risk factors for diabetic neuropathy[42].

In the present study, a significant reduction in central corneal nerve fiber parameters in young children with type 1 diabetes has been shown, which is comparable to a previous study of children and young adolescents with type 1 diabetes [25]. Established risk factors for diabetic neuropathy, such as age, height, glycated hemoglobin and BMI, were not associated with the reduction in corneal nerve parameters, consistent with previous findings in adults with type 1 diabetes[35,43]. CNFT was not altered, in contrast with a study of adults with diabetes where nerve tortuosity was higher[44]. A reduction in corneal nerves occurs, regardless of diabetes duration, in young patients with type 1 diabetes [35] and adults with type 2 diabetes[45].

The American Diabetes Association has recommended initial screening for albuminuria and retinopathy in patients with type 1 diabetes aged >10 years, after 3–5 years of diabetes[46]. Although 20% of this cohort fulfilled the criteria for screening, none had microalbuminuria or retinopathy. Indeed, the significant corneal nerve loss in these children with type 1 diabetes without retinopathy or microalbuminuria agrees with previous findings in adults with type 1 diabetes[25,34], and supports the thesis that neuropathy might precede retinopathy[47]. It also argues for earlier screening of diabetic neuropathy in children with type 1 diabetes using CCM. AST was lower in the present cohort with type 1 diabetes, and correlated with CNFD, CNBD and CNFL. No relationship between AST and CCM has been observed in studies in adults with diabetes[21,22,30,48]. Although the association between BMI and elevated AST is well established as a marker for liver injury in obese adults[49–52], in the present study, AST was inversely correlated with BMI.

The inferior whorl is distal to the central nerves, and might allow the identification of earlier nerve damage[26,53]. Studies of adults with type 1 diabetes and type 2 diabetes have shown a greater reduction in IWL[48,54], especially in those with painful diabetic neuropathy[55,56]. This is the first study of children with type 1 diabetes showing a marked reduction in IWL, with 50%

having a reduction greater than two standard deviations lower than the mean in controls.

A limitation of the current study was the cross-sectional design, relatively small number of participants studied and the lack of additional measures of diabetic neuropathy. Prospective studies are required to assess progression of corneal nerve abnormalities in relation to other complications and risk factors for diabetic neuropathy.

Significant corneal nerve loss has been shown in the central cornea and inferior whorl indicative of neuropathy in children with type 1 diabetes without microalbuminuria or retinopathy. This suggests that CCM could be used to screen for early subclinical neuropathy and to assess disease progression in children with type 1 diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Fox DA, Islam N, Sutherland J, *et al*. Type 1 diabetes incidence and prevalence trends in a cohort of Canadian children and youth. *Pediatr Diabetes* 2018; 19: 501–505.
2. Dabelea D, Stafford JM, Mayer-Davis EJ, *et al*. Association of type 1 diabetes vs type 2 diabetes diagnosed during childhood and adolescence with complications during teenage years and young adulthood. *JAMA* 2017; 317: 825–835.
3. Xu G, Liu B, Sun Y, *et al*. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ* 2018; 362: k1497.
4. Johannsen L, Smith T, Havsager AM, *et al*. Evaluation of patients with symptoms suggestive of chronic polyneuropathy. *J Clin Neuromusc Dis* 2001; 3: 47–52.
5. Lubec D, Mullbacher W, Finsterer J, *et al*. Diagnostic work-up in peripheral neuropathy: an analysis of 171 cases. *Postgrad Med J* 1999; 75: 723–726.
6. Callaghan BC, Kerber KA, Lisabeth LL, *et al*. Role of neurologists and diagnostic tests on the management of distal symmetric polyneuropathy. *JAMA Neurol* 2014; 71: 1143–1149.
7. Callaghan BC, Price RS, Feldman EL. Distal symmetric polyneuropathy: a review. *JAMA* 2015; 314: 2172–2181.
8. Pop-Busui R, Boulton AJ, Feldman EL, *et al*. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes Care* 2017; 40: 136–154.
9. Ghaemi N, Hasanabadi H, Ashrafzadeh F, *et al*. Peripheral neuropathy in children and adolescents with insulin-dependent diabetes mellitus. *Iran J Child Neurol* 2018; 12: 83–90.
10. Turkyilmaz H, Guzel O, Edizer S, *et al*. Evaluation of polyneuropathy and associated risk factors in children with type 1 diabetes mellitus. *Turk J Med Sci* 2017; 47: 942–946.
11. Ising E, Dahlin LB, Elding Larsson H. Impaired vibrotactile sense in children and adolescents with type 1 diabetes - Signs of peripheral neuropathy. *PLoS One* 2018; 13: e0196243.
12. Metwalley KA, Hamed SA, Farghaly HS. Cardiac autonomic function in children with type 1 diabetes. *Eur J Pediatr* 2018; 177: 805–813.
13. Walter-Holiner I, Barbarini DS, Lutschg J, *et al*. High prevalence and incidence of diabetic peripheral neuropathy in children and adolescents with type 1 diabetes mellitus: results from a five-year prospective cohort study. *Pediatr Neurol* 2018; 80: 51–60.
14. Hajas G, Kissova V, Tirpakova A. A 10-yr follow-up study for the detection of peripheral neuropathy in young patients with type 1 diabetes. *Pediatr Diabetes* 2016; 17: 632–641.
15. Sellers EA, Clark I, Tavakoli M, *et al*. The acceptability and feasibility of corneal confocal microscopy to detect early diabetic neuropathy in children: a pilot study. *Diabet Med* 2013; 30: 630–631.
16. Nelson D, Mah JK, Adams C, *et al*. Comparison of conventional and non-invasive techniques for the early identification of diabetic neuropathy in children and adolescents with type 1 diabetes. *Pediatr Diabetes* 2006; 7: 305–310.
17. Xiong Q, Lu B, Ye HY, *et al*. Corneal confocal microscopy as a non-invasive test to assess diabetic peripheral neuropathy. *Diabetes Res Clin Pract* 2018; 136: 85–92.
18. Alam U, Jeziorska M, Petropoulos IN, *et al*. Diagnostic utility of corneal confocal microscopy and intra-epidermal nerve fibre density in diabetic neuropathy. *PLoS One* 2017; 12: e0180175.
19. Tavakoli M, Begum P, McLaughlin J, *et al*. Corneal confocal microscopy for the diagnosis of diabetic autonomic neuropathy. *Muscle Nerve* 2015; 52: 363–370.
20. Jiang MS, Yuan Y, Gu ZX, *et al*. Corneal confocal microscopy for assessment of diabetic peripheral neuropathy: a meta-analysis. *Br J Ophthalmol* 2016; 100: 9–14.
21. Khan A, Petropoulos IN, Ponirakis G, *et al*. Corneal confocal microscopy detects severe small fiber neuropathy in diabetic patients with Charcot neuroarthropathy. *J Diab Investig* 2018; 9: 1167–1172.
22. 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: 1856–1861.
23. Papanas N, Ziegler D. Corneal confocal microscopy: recent progress in the evaluation of diabetic neuropathy. *J Diab Investig* 2015; 6: 381–389.

24. Tavakoli M, Petropoulos IN, Malik RA. Corneal confocal microscopy to assess diabetic neuropathy: an eye on the foot. *J Diab Sci Technol* 2013; 7: 1179–1189.
25. Deak EA, Szalai E, Toth N, *et al*. Longitudinal changes in corneal cell and nerve fiber morphology in young patients with type 1 diabetes with and without diabetic retinopathy: a 2-year follow-up study. *Invest Ophthalmol Vis Sci* 2019; 60: 830–837.
26. 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: 2498–2504.
27. 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: 1261–1267.
28. Tesfaye S, Chaturvedi N, Eaton SE, *et al*. Vascular risk factors and diabetic neuropathy. *N Engl J Med* 2005; 352: 341–350.
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: 5060–5065.
30. Ferdousi M, Petropoulos IN, Kalteniece A, *et al*. No relation between the severity of corneal nerve, epithelial, and keratocyte cell morphology with measures of dry eye disease in type 1 diabetes. *Invest Ophthalmol Vis Sci* 2018; 59: 5525–5530.
31. 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: 390–397.
32. Ahmed A, Bril V, Orszag A, *et al*. Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. *Diabetes Care* 2012; 35: 821–828.
33. Chen X, Graham J, Dabbah MA, *et al*. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. *Diabetes Care* 2015; 38: 1138–1144.
34. 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: e0123517.
35. Szalai E, Deak E, Modis L Jr, *et al*. Early corneal cellular and nerve fiber pathology in young patients with type 1 diabetes mellitus identified using corneal confocal microscopy. *Invest Ophthalmol Vis Sci* 2016; 57: 853–858.
36. Edwards K, Pritchard N, Dehghani C, *et al*. Corneal confocal microscopy best identifies the development and progression of neuropathy in patients with type 1 diabetes. *J Diabetes Complications* 2017; 31: 1325–1327.
37. Pritchard N, Edwards K, Russell AW, *et al*. Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care* 2015; 38: 671–675.
38. Lewis EJH, Lovblom LE, Ferdousi M, *et al*. Rapid corneal nerve fiber loss: a marker of diabetic neuropathy onset and progression. *Diabetes Care* 2020. https://doi.org/10.2337/dc19-0951
39. 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: 254–260.
40. 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: 2608–2612.
41. Lewis EJH, Perkins BA, Lovblom LE, *et al*. Effect of omega-3 supplementation on neuropathy in type 1 diabetes: a 12-month pilot trial. *Neurology* 2017; 88: 2294–2301.
42. Ishibashi F, Taniguchi M, Kosaka A, *et al*. Improvement in neuropathy outcomes with normalizing HbA1c in patients with type 2 diabetes. *Diabetes Care* 2019; 42: 110–118.
43. Liu L, Ding J, Leng X, *et al*. Guidelines for evaluation and management of cerebral collateral circulation in ischaemic stroke 2017. *Stroke Vasc Neurol* 2018; 3: 117–130.
44. Kallinikos P, Berhanu M, O'Donnell C, *et al*. Corneal nerve tortuosity in diabetic patients with neuropathy. *Invest Ophthalmol Vis Sci* 2004; 45: 418–422.
45. Yorek M, Malik RA, Calcutt NA, *et al*. Diabetic neuropathy: new insights to early diagnosis and treatments. *J Diabetes Res* 2018; 2018: 5378439.
46. American Diabetes Association. 13. Children and adolescents: standards of medical care in diabetes-2019. *Diabetes Care* 2019; 42(Suppl 1): S148–S164.
47. Jampol LM, Glassman AR, Sun J. Evaluation and care of patients with diabetic retinopathy. *N Engl J Med.* 2020; 382: 1629–1637.
48. Yan A, Issar T, Tummanapalli SS, *et al*. Relationship between corneal confocal microscopy and markers of peripheral nerve structure and function in Type 2 diabetes. *Diabet Med* 2020; 37: 326–234.
49. Robinson D, Whitehead TP. Effect of body mass and other factors on serum liver enzyme levels in men attending for well population screening. *Ann Clin Biochem* 1989; 26: 393–400.
50. Sull JW, Yun JE, Lee SY, *et al*. Body mass index and serum aminotransferase levels in Korean men and women. *J Clin Gastroenterol* 2009; 43: 869–875.
51. Salvaggio A, Periti M, Miano L, *et al*. Body mass index and liver enzyme activity in serum. *Clin Chem* 1991; 37: 720–723.
52. Loomba R, Bettencourt R, Barrett-Connor E. Synergistic association between alcohol intake and body mass index with serum alanine and aspartate aminotransferase levels in older adults: the Rancho Bernardo Study. *Aliment Pharmacol Ther* 2009; 30: 1137–1149.

53. Utsunomiya T, Nagaoka T, Hanada K, *et al*. Imaging of the corneal subbasal whorl-like nerve plexus: more accurate depiction of the extent of corneal nerve damage in patients with diabetes. *Invest Ophthalmol Vis Sci* 2015; 56: 5417–5423.
54. Tummanapalli SS, Issar T, Kwai N, *et al*. A comparative study on the diagnostic utility of corneal confocal microscopy and tear neuromediator levels in diabetic peripheral neuropathy. *Curr Eye Res* 2019. https://doi.org/10.1080/02713683.2019.1705984
55. Kalteniece A, Ferdousi M, Petropoulos I, *et al*. Greater corneal nerve loss at the inferior whorl is related to the presence of diabetic neuropathy and painful diabetic neuropathy. *Sci Rep* 2018; 8: 3283.
56. 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: 3371.