New ophthalmologic imaging techniques for detection and monitoring of neurodegenerative changes in diabetes: a systematic review

New ophthalmologic imaging techniques for detection and monitoring of neurodegenerative changes in diabetes: a systematic review

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Optical coherence tomography (OCT) of the retina and around the optic nerve head and corneal confocal microscopy (CCM) are non-invasive and repeatable techniques that can quantify ocular neurodegenerative changes in individuals with diabetes. We systematically reviewed studies of ocular neurodegenerative changes in adults with type 1 or type 2 diabetes and noted changes in the retina, the optic nerve head, and the cornea. Of the 30 studies that met our inclusion criteria, 14 used OCT and 16 used CCM to assess ocular neurodegenerative changes. Even in the absence of diabetic retinopathy, several layers in the retina and the mean retinal nerve fibre layer around the optic nerve head were significantly thinner (–5·36 μm [95% CI –7·13 to –3·58]) in individuals with type 2 diabetes compared with individuals without diabetes. In individuals with type 1 diabetes without retinopathy none of the intraretinal layer thicknesses were significantly reduced compared with individuals without diabetes. In the absence of diabetic polyneuropathy, individuals with type 2 diabetes had a lower nerve density (nerve branch density: –1·10/mm² [95% CI –4·22 to 2·02]), nerve fibre density: –5·80/mm² [–8·06 to –3·54], and nerve fibre length: –4·00 mm/mm² [–5·93 to –2·07]) in the subbasal nerve plexus of the cornea than individuals without diabetes. Individuals with type 1 diabetes without polyneuropathy also had a lower nerve density (nerve branch density: –7·74/mm² [95% CI –14·13 to –1·34], nerve fibre density: –2·68/mm² [–5·56 to 0·20]), and nerve fibre length: –2·58 mm/mm² [–3·94 to –1·21]). Ocular neurodegenerative changes are more evident when diabetic retinopathy or polyneuropathy is present. OCT and CCM are potentially useful, in addition to conventional clinical methods, to assess diabetic neurodegenerative changes. Additional research is needed to determine their incremental benefit and to standardise procedures before the application of OCT and CCM in daily practice.

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Introduction

Diabetic retinopathy and diabetic polyneuropathy are major complications of diabetes that can start insidiously, with few or no symptoms.[1] Improved assessments in clinical practice are urgently needed to predict onset and monitor progression of diabetic retinopathy[2] and diabetic polyneuropathy.[3,4]

Diabetic retinopathy is widely thought to be the earliest microvascular complication of diabetes, which is typically detected by ophthalmoscopic examination. However, retinal neurodegenerative changes—notably glial activation and neuronal apoptosis leading to a thinning of the neuronal layers in the retina—also occur in the eyes of individuals with diabetes at an early stage of diabetic retinopathy, even when microvascular lesions cannot be detected by ophthalmologic examination.[5–8] Because early diabetic retinopathy includes a neurodegenerative component, the assessment of diabetic retinopathy should also quantify these structural changes in the retina.

Investigations of diabetic polyneuropathy are commonly based on (semi-)quantitative sensory tests, electrophysiology, or examination of skin biopsy samples. However, the onset of diabetic polyneuropathy is difficult to detect by electrophysiology[9] and vibration perception tests,[10] which only quantify large fibre deficits. The earliest nerve damage occurs at the level of the small nerve fibres.[11] These fibres can be assessed by thermal and pain perception tests, but these measures are extremely variable.[10,12] Skin biopsy and sural nerve biopsy are invasive procedures and might not be suitable for long-term follow-up or clinical trials.

Two new ophthalmological imaging techniques, optical coherence tomography (OCT) and corneal confocal microscopy (CCM), could be of value to identify early neurodegenerative changes in individuals with diabetes, and to monitor changes over time. Both techniques are non-invasive, repeatable, and user-friendly.[13–18] Several software programs exist to measure ocular neurodegenerative changes.[19–26]

OCT provides 2-dimensional high-resolution (5–10 μm) cross-sectional scans of the retina, showing an amount of detail that resembles histological specimens (figure 1).[27,28] OCT can quantify the thickness of separate retinal layers in the macula and around the optic nerve head. Segmentation of intraretinal layers showed thinning in the earliest stages of diabetic retinopathy.[29–31] OCT could help clinically to detect early retinal neurodegeneration to define at-risk patients and to plan preventive therapy before the development of vascular lesions detectable by ophthalmoscopy. Furthermore, OCT can be used to monitor progression of diabetic retinopathy and to assess therapeutic efficacy of existing or new treatment methods.[32–34]

CCM can directly visualise and quantify the nerve fibres of the cornea. The transparency of the cornea enables visualisation of the subbasal nerve plexus by two-dimensional scans (figure 2), real-time mapping of several images, or by 3-dimensional images.[35] These

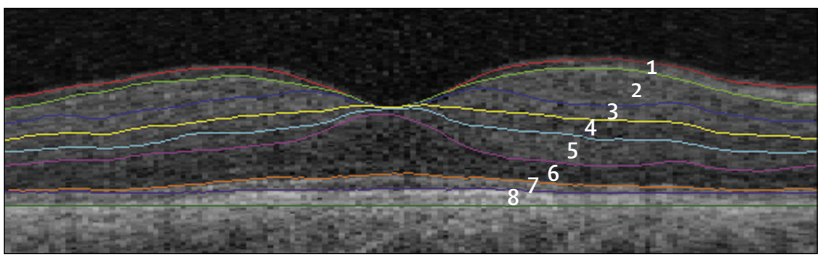

Figure 1: **Optical coherence tomography of the macula showing the distinct retinal layers**
Layer 1=retinal nerve fibre layer. Layer 2=ganglion cell layer. Layer 3=inner plexiform layer. Layer 4=inner nuclear layer. Layer 5=outer plexiform layer. Layer 6=outer nuclear layer + inner segments (photoreceptors). Layer 7=outer segments (photoreceptors). Layer 8=retinal pigment epithelium. Reproduced with permission from van Dijk and colleagues.[27]

See Online for appendix

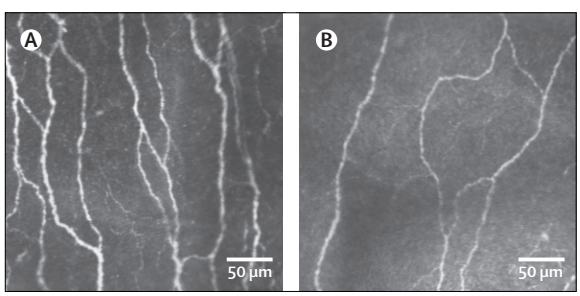

Figure 2: **Confocal microscopy of the cornea**
Single image from the subbasal nerve plexus of the cornea in an individual without diabetes (A) and in an individual with diabetes showing a decrease in the nerve density and nerve length, and an increase in the tortuosity (B).

images can be made with or without making contact with the cornea.[36] All layers can be imaged at a cellular level at a resolution of 1–2 µm.[37] CCM can detect changes in nerve density, nerve length, and tortuosity in individuals with diabetes.[38–40] CCM could help clinically to diagnose diabetic polyneuropathy non-invasively, and could detect biomarkers that predict the onset of polyneuropathy before changes are detected by electrophysiology and vibration perception.[11] Additionally, CCM can be used to monitor progression of diabetic polyneuropathy, to assess therapeutic efficacy of existing or new treatments, and to detect regeneration of corneal nerves after intensive treatment of diabetes.[41–43] However, studies of neurodegenerative changes in the eyes of individuals with diabetes have still not been systematically reviewed to define biomarkers that predict the onset and monitor progression of diabetic retinopathy and polyneuropathy.

The aim of this Review is to systematically investigate to what extent ocular neurodegenerative changes can be shown in individuals with diabetes with these ophthalmologic imaging techniques, and whether these changes can also be detected in the absence of diabetic retinopathy and diabetic polyneuropathy, as defined with classic methods.

Methods

Search strategy and selection criteria

Following the PRISMA statement and checklist,[44,45] we searched Medline, the Cochrane Controlled Trials Register, and Embase and used a broad systematic search strategy according to the Cochrane collaboration (appendix pp 54–59). We searched for articles published up to Jan 20, 2015, with the terms "diabetes", "cornea", "nerve fibers", "macula", "retinal layers", "retinal thickness", "optical coherence tomography", "colour vision", "dark adaptation", "electrooculography", "electrophysiology", "perimetry", "contrast sensitivity", "red-free photography", "optic disk", "optic nerve head", "scanning laser polarimetry", and "heidelberg retinal tomography", in English, French, German, or Dutch. The search was limited to studies in human beings. The bibliographies of included articles were screened until no new articles were found. In addition, information about possible studies was collected through personal communication with other researchers.

We included randomised controlled trials, cohort studies, prospective and retrospective case series, and case-control studies of adults (≥18 years) with type 1 or type 2 diabetes. Included studies had to have one or more of three outcome measures. First, neurodegenerative changes in the layers of the retina—ie, changes located at the central fovea or at the foveal, the pericentral, or the peripheral area of the macula. The layers that were assessed at these locations are the retinal nerve fibre layer, ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, photoreceptor layers (ie, outer nuclear layer, inner segments, and outer segments), retinal pigment epithelium, and the total retinal thickness. Second, neurodegenerative changes around the optic nerve head—ie, changes in retinal nerve fibre layer thickness in the mean, superior, temporal, inferior, and nasal quadrants around the optic nerve head. Third, neurodegenerative changes in the subbasal nerve plexus of the cornea—ie, changes in nerve density, nerve length, and tortuosity.

EEBDC selected the eligible studies and JSAGS checked the selection. Study selection was done in two stages. First, we screened papers by reading the title, abstract, and keywords. We excluded reviews, letters, and comments. Second, we screened the full text of eligible papers and included them if they assessed one or more of the preselected outcome measures for neurodegenerative changes in the retina, the optic nerve head, or subbasal nerve plexus of the cornea. Studies were excluded if they included individuals with clinically significant diabetic macular oedema, if they did not distinguish between type 1 and type 2 diabetes, or if they did not use an OCT device for measurements in the retina and around the optic nerve head.

Data extraction and analysis

EEBDC reviewed the studies for inclusion and quality and extracted the data. The data extraction sheet was based on the Cochrane Consumers and Communication Review Group's data extraction template. JSAGS checked the data. Disagreements were resolved by discussion between the two review authors. Studies were not blinded for the

journal or any other aspect of the article. The data extracted were: author and year of publication, type of study, study design, country, type of diabetes, number of individuals with and without diabetes, subgroups, inclusion and exclusion criteria, method of the measurement, neurodegenerative variables studied, mean value and 95% CI of the variables for individuals with and without diabetes. In addition, the age of the individuals in the study and duration of diabetes (mean [SD]) were extracted.

Methodological quality was assessed according to the Delphi list with two additional items. Table 1 describes the six domains that were assessed. These domains were assessed by a score of ''Yes'' (high quality), ''No'' (low quality), or ''Unclear'' (uncertain quality).

Risk of bias was assessed according to Cochrane guidelines. Figure 3 shows the four domains that were assessed. These domains were assessed by a score of ''Yes'' (low risk of bias), ''No'' (high risk of bias), or ''Unclear'' (uncertain risk of bias).

All pooled analyses were based on random effects models because of the differences between included studies in terms of study population, intervention, and outcomes. Statistical analyses were done with SPSS version 21. Mean neurodegenerative variables of individuals with and without diabetes were compared and the summary point estimate with 95% CIs from meta-analysis was calculated with Stata IC 12.

Subgroup analyses were done in studies quantifying ocular neurodegenerative changes by OCT of the retinal layers and around the optic nerve head making four comparisons (appendix pp 52–53). First, we established whether retinal neuronal layers were thinner in individuals with diabetes compared with individuals without diabetes. Second, we established whether retinal neuronal layers were already thinner in an early stage in individuals with diabetes without retinopathy compared with individuals without diabetes, because early diabetic retinopathy can include a neurodegenerative component. Third, in individuals with diabetes, we established whether retinal neuronal layers were thinner when retinopathy is present. Fourth, we established whether retinal neuronal layers were thinner in individuals with diabetic polyneuropathy compared with individuals without diabetes, because retinal neurodegenerative changes can be related to diabetic polyneuropathy. We did the analyses separately for type 1 and type 2 diabetes. Corneal neurodegenerative changes detected by CCM could be an early biomarker of diabetic polyneuropathy. For studies quantifying corneal neurodegenerative changes by CCM, we did the first three of the four subgroup analyses done for OCT studies.

Changes in neurodegenerative variables were summarised for the retina, the optic nerve head, and the cornea, with summary point estimate from random-effects meta-analyses and 95% CIs. The variables were ranked according to the size of the difference between individuals with and without diabetes. Negative values indicate that the neuronal variable is decreased in individuals with diabetes compared with those without diabetes or that the neuronal variable is decreased in individuals with diabetic retinopathy or polyneuropathy compared with those with diabetes without diabetic retinopathy or polyneuropathy. In all analyses, the weighted mean value and weighted SD of the age and duration of diabetes were calculated for both groups. Heterogeneity between studies was addressed with a statistical χ^2 test and I^2 test. If heterogeneity was present (χ^2 test: $p<0{\cdot}05$ or I^2 test ≥30%), we investigated whether all values of the difference between diabetes and no diabetes pointed in the same direction. If not all studies pointed in the same direction, we reported how many did and tried to find an explanation for the heterogeneity.

	Quality criteria	Number of publications scored "Yes"
Added by authors	Consecutive or randomly selected patients?	12 [30,31,60–69]
Delphi list*	Were inclusion criteria specified?	30 [17,29–31,42,43,46–69]
Delphi list*	Were exclusion criteria specified?	28 [17,29–31,43,46–48,50–69]
Added by authors	Were groups similar with respect to age and sex?	13 [29,30,43,48,50,55,57,62,63,64,66,68,69]
Delphi list	Were point estimates and measures of variability presented for the primary outcome measures?	30 [17,29–31,42,43,46–69]
Considered for Delphi list	Was calculation of statistical power reported?	5 [47,62,65–67]

*Item split into inclusion and exclusion criteria.

Table 1: **Quality items and number of publications by source[70]**

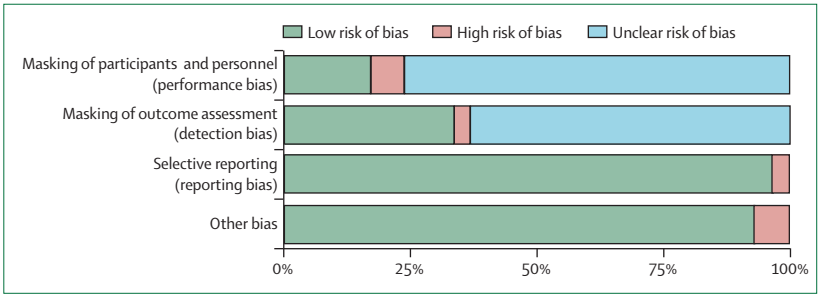

Figure 3: **Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies**

The following domains were assessed. (1) Were data collectors masked with respect to the identity of the patient and medical and neurological results of subjects (performance bias)? (2) Were outcome assessors masked with respect to the identity of patients and medical and neurological results of patients (detection bias)? (3) Were reports of the study free of selective outcome reporting (reporting bias)? (4) Was the study apparently free of other factors that could put it at risk of bias (selection bias, attrition bias, or other bias)?

Findings

Selected studies

4546 articles were identified through database searching. After removing duplicates, 3434 articles were screened. Only 73 unique articles assessed ocular neurodegenerative changes in individuals with diabetes. Two additional records were identified through other sources. Finally, 30 articles met our inclusion criteria (figure 4). 13 articles only reported on individuals with type 1 diabetes (n=871), 16 only reported on individuals with type 2 diabetes

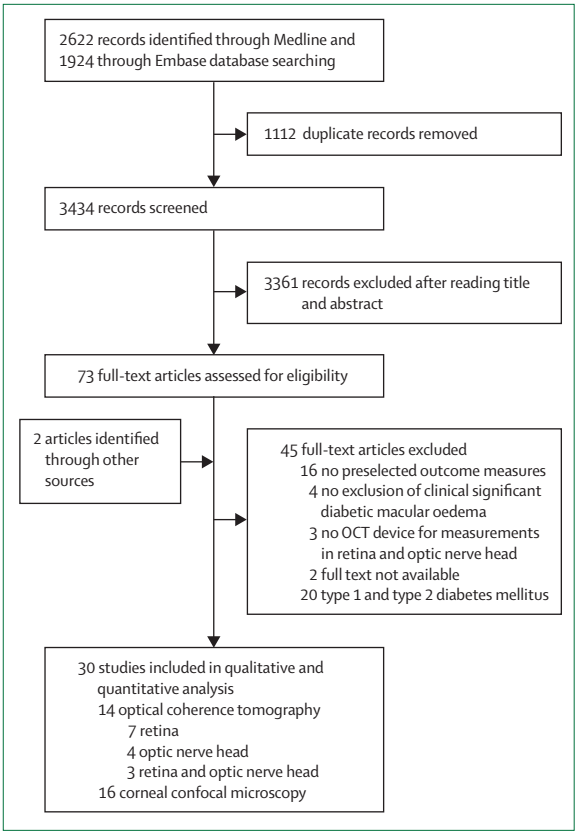

Figure 4: **Flow diagram of study selection**

(n=1359), and one study reported on type 1 and type 2 diabetes separately (n=25 and n=18, respectively). 31 neurodegenerative variables were analysed. Characteristics of the included studies are shown in appendix pp 22–51. 14 studies used OCT and 16 studies used CCM to assess ocular neurodegenerative changes. 28 studies were prospective case series and two were prospective longitudinal studies.

Quality assessment

Information about consecutiveness of the sample was insufficient in 18 of 30 studies.[17,29,42,43,46–59] One study selected participants randomly.[60] In two of 30 studies, reasons for exclusion were not reported.[42,49] The selection criteria were heterogeneous among studies. Ten studies excluded significant media opacities,[29–31,52–54,59,61–63] ten studies excluded glaucoma,[29–31,52–55,59,63,64] 11 studies excluded corneal disease,[36,47,48,54,56–58,60,62,65,66] and 11 studies excluded for neuropathy attributable to causes other than diabetes.[17,43,47,48,50,51,56,58,65–67] 13 of 30 studies included healthy age-matched and sex-matched control participants[29,30,43,48,50,55,57,62,63,64,66,68,69] and ten of 30 studies included only age-matched control participants.[31,42,46,49,51,53,54,56,59,65] In some studies, other prognostic indicators also did not differ significantly between both groups: HDL cholesterol (n=1)[43] and triglyceride concentrations[43,51,55,63] (n=4), vision[46,53] (n=2), refractive error[61] (n=1), mean intraocular pressure[46,60,64] (n=3), axial length[61] (n=1), central corneal thickness[64] (n=1), urinary albumin:creatinine ratio[51,63] (n=2), serum creatinine[63] (n=1), waist circumference[67] (n=1), BMI[51,67] (n=2), weight[67] (n=1), height[67] (n=1), systolic and diastolic blood pressure[51,55,63,67,68] (n=5), smoking[67,68] (n=2), and alcohol[67] (n=1). All studies reported point estimates for the outcome measures. In 22 of 30 studies, the SD was mentioned. Three studies reported the mean difference with 95% CIs,[29–31] four studies reported the SE of the mean value,[50–52,56] and one study reported the median with IQR.[68] Calculation of statistical power was reported in five studies.[47,62,65–67] Quality assessment of the included articles is shown in appendix pp 22–51. Table 1 shows the quality assessment across studies.

Risk of bias in included studies

Risk of bias among the included articles is presented in detail in appendix pp 22–51. Figure 3 shows the risk of bias graph. In five of 30 included studies the data collectors were masked[42,56,62,65,67] and in ten studies the outcome assessors were masked.[17,29,31,42,43,57,58,62,65,67] One report did not include all expected outcomes.[52] 28 studies seemed to be free of other sources of bias. Two studies had few female participants[17,55]—in one of these all participants were aged between 60 and 75 years.[55]

Outcome analyses and investigation of heterogeneity

Table 2 shows the number of studies, number of individuals with and without diabetes, mean age, and duration of diabetes and tables 3 and 4 show numerical data for the neuronal variables. In the results presented in the following sections, we mainly report the presence (not the absence) of significant differences only; we found no heterogeneity in estimates reported unless stated otherwise. Graphical data, and χ^2 and I^2 values for heterogeneity, are shown in full in appendix pp 4–21. Table 5 summarises the results of our analyses and their clinical implications.

Retinal neurodegenerative changes assessed by OCT

Compared with individuals without diabetes, individuals with type 1 diabetes had a significantly thinner retinal nerve fibre layer and photoreceptor layers (table 3, appendix p 4).[30,31,63,68,69] Compared with individuals without diabetes, individuals with type 2 diabetes showed a significantly thinner retinal nerve fibre layer, ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, and photoreceptor layers (table 3). For these estimates, heterogeneity was moderate for the ganglion cell layer and substantial for the inner plexiform layer. Despite this heterogeneity, all studies showed a lower value in individuals with type 2 diabetes compared with those without diabetes (appendix p 5).[29,46,53,55,59,61]

In individuals with type 1 diabetes without retinopathy, none of the intraretinal layer thicknesses were signi-

	Type 1 diabetes *vs* controls							Type 2 diabetes *vs* controls						
	Type 1 diabetes				Controls			Type 2 diabetes				Controls		
	Studies (n)	Individuals (n)	Age (years)	Duration of diabetes (years)	Individuals (n)	Age (years)	Duration of diabetes (years)	Studies (n)	Individuals (n)	Age (years)	Duration of diabetes (years)	Individuals (n)	Age (years)	Duration of diabetes (years)
Retinal neurodegenerative changes assessed by OCT														
Diabetes *vs* no diabetes	5	329	31·7 (10·8)	13·7 (7·7)	200	34·0 (11·7)	NA	6	761	60·7 (8·3)	10·4 (6·2)	282	59·5 (10·9)	NA
Diabetes without DR *vs* no diabetes	5	183	30·1 (11·1)	10·6 (7·2)	200	34·0 (11·7)	NA	5	278	59·1 (8·8)	9·3 (7·1)	254	60·2 (11·1)	NA
Diabetes with DR *vs* diabetes without DR	5	145	33·6 (8·9)	17·6 (7·6)	183	30·1 (11·1)	10·6 (7·2)	3	73	63·8 (6·8)	13·2 (7·1)	176	63·2 (7·9)	7·7 (6·7)
Neurodegenerative changes around the optic nerve head assessed by OCT														
Diabetes *vs* no diabetes	1	77	35 (29–42)*	10 (9–14)*	31	46 (25–50)*	NA	6	333	56·5 (8·5)	11·2 (7·7)	193	54·1 (11·3)	NA
Diabetes without DR *vs* no diabetes	1	54	35 (29–42)*	10 (9–14)*	31	46 (25–50)*	NA	4	157	54·8 (10·3)	7 (6·6)	119	53 (13·5)	NA
Diabetes with DR *vs* diabetes without DR	1	23	35 (29–42)*	10 (9–14)*	54	35 (29–42)*	10 (9–14)*	2	56	56·4 (7·2)	10·7 (6·1)	55	59·7 (10·6)	4·8 (4·4)
Diabetes with DPN *vs* no diabetes	NA	NA	NA	NA	NA	NA	NA	1	82	60·5 (6·4)	13·6 (9·1)	24	59·1 (7·2)	NA
Corneal neurodegenerative changes assessed by CCM														
Diabetes *vs* no diabetes	9	698	44·5 (14·5)	22·1 (13·7)	434	44 (15·0)	NA	8	580	59·8 (8·2)	11·4 (6·8)	252	57·9 (9·4)	NA
Diabetes without DPN *vs* no diabetes	7	456	41·6 (15·1)	18·8 (13·8)	409	43·8 (15·4)	NA	1	23	48·1 (10·6)	5·8 (5·8)	28	50·2 (7·4)	NA
Diabetes with DPN *vs* diabetes without DPN	6	315	49·8 (14·0)	25·6 (14·5)	431	41·7 (14·9)	19·1 (14·0)	1	55	55·8 (1·9)	9·3 (1·8)	23	48·1 (10·6)	5·8 (5·8)

Data are mean (SD), except for one study giving median (IQR)* for the total group of individuals with type 1 diabetes. CCM=corneal confocal microscopy. DR=diabetic retinopathy. DPN=diabetic polyneuropathy. OCT=optical coherence tomography. NA=not applicable.

Table 2: **Number of studies reviewed, with the number of individuals with or without diabetes, mean age, and duration of diabetes therein**

ficantly different than in individuals without diabetes (table 3, appendix p 6).[30,31,63,68,69] By contrast, individuals with type 2 diabetes without diabetic retinopathy showed a significant reduction in retinal nerve fibre layer thickness compared with individuals without diabetes, although these results were derived from only one study (table 3, appendix p 7).[29,46,53,55,61]

Compared with individuals with type 1 diabetes without retinopathy, individuals with type 1 diabetes and retinopathy showed a significant reduction in the thickness of the fovea, the retinal nerve fibre layer, ganglion cell layer, mean ganglion cell layer and inner plexiform layer, and inner nuclear layer (table 3, appendix p 8).[30,31,63,68,69] Compared with individuals with type 2 diabetes without retinopathy, individuals with type 2 diabetes and retinopathy showed a significant reduction of the inner plexiform layer in one study (table 3, appendix p 9).[29,46,55]

Neurodegenerative changes around the optic nerve head assessed by OCT

Only one study compared retinal nerve fibre layer thicknesses in individuals with type 1 diabetes to those in individuals without diabetes. In this study, the inferior and mean retinal nerve fibre layer thicknesses were significantly increased (table 3, appendix p 10).[68] Compared with individuals without diabetes, individuals with type 2 diabetes showed a significant decrease in the thickness of the superior and the inferior retinal nerve fibre layers (table 3). Moderate heterogeneity was noted for both estimates (appendix p 11).[46,52–54,61,64]

In individuals with type 1 diabetes without retinopathy, the inferior, temporal, and mean retinal nerve fibre layer thicknesses were significantly increased compared with individuals without diabetes in one study (table 3, appendix p 12).[68] Compared with individuals without diabetes, individuals with type 2 diabetes without retinopathy showed a significant reduction in mean retinal nerve fibre layer thickness (table 3, appendix p 13).[46,53,54,61]

Compared with individuals with type 1 diabetes without retinopathy, individuals with type 1 diabetes and retinopathy showed a significant decrease of the thickness of the inferior, nasal, temporal, and mean retinal nerve fibre layer in one study (table 3, appendix p 14).[68] Compared with individuals with type 2 diabetes without retinopathy, individuals with type 2 diabetes and

	Diabetes *vs* no diabetes		Diabetes without DR *vs* no diabetes		Diabetes with DR *vs* diabetes without DR	
	Type 1 diabetes	Type 2 diabetes	Type 1 diabetes	Type 2 diabetes	Type 1 diabetes	Type 2 diabetes
Retinal neurodegenerative changes assessed by OCT						
Central fovea, µm	7·31 (–1·59 to 16·20)	3·09 (–5·44 to 11·63)	9·00 (–4·48 to 22·48)	–1·36 (–6·28 to 3·56)	–3·00 (–16·74 to 10·74)	3·00 (–9·22 to 15·22)
Fovea, µm	–1·12 (–6·00 to 3·77)	1·14 (–8·94 to 11·22)	0·91 (–1·92 to 3·73)	–7·49 (–20·15 to 5·18)	–7·64 (–12·41 to –2·88)*	12·36 (–10·66 to 35·38)
Pericentral RNFL, µm	–0·63 (–1·39 to 0·13)	–1·29 (–2·24 to –0·33)*	–0·06 (–0·93 to 0·80)	–0·90 (–1·96 to 0·16)	–1·36 (–2·44 to –0·28)*	–1·00 (–2·38 to 0·38)
Pericentral GCL, µm	–1·78 (–8·15 to 4·59)	–3·43 (–6·43 to –0·42)*	1·40 (–2·30 to 5·10)	–2·10 (–4·80 to 0·60)	–6·50 (–10·97 to –2·03)*	–3·10 (–6·76 to 0·56)
Pericentral IPL, µm	–1·11 (–3·17 to 0·94)	–2·99 (–5·93 to –0·05)*	0·00 (–2·18 to 2·18)	–1·50 (–3·14 to 0·14)	–2·10 (–4·37 to 0·17)	–3·00 (–4·78 to –1·22)*
Pericentral GCL+IPL, µm	–2·57 (–7·67 to 2·52)	NA	–0·20 (–3·29 to 2·89)	NA	–5·22 (–9·83 to –0·61)*	NA
Pericentral INL, µm	–0·60 (–1·82 to 0·62)	–1·24 (–2·34 to –0·14)*	0·32 (–0·85 to 1·50)	–1·40 (–2·83 to 0·03)	–2·04 (–3·43 to –0·64)*	0·40 (–1·35 to 2·15)
Pericentral OPL, µm	0·78 (0·01 to 1·54)	–0·79 (–1·76 to 0·19)	0·63 (–1·03 to 2·29)	–0·70 (–1·99 to 0·59)	0·24 (–1·91 to 2·39)	–0·20 (–1·69 to 1·29)
Pericentral ONL+IS, µm	–2·44 (–4·60 to –0·28)*	–3·66 (–6·38 to –0·95)*	–1·99 (–5·02 to 1·03)	–3·10 (–6·71 to 0·51)	–0·89 (–4·56 to 2·79)	–1·30 (–5·88 to 3·28)
Pericentral OS, µm	0·53 (–0·19 to 1·24)	–0·56 (–1·25 to 0·12)	0·14 (–0·59 to 0·88)	–0·80 (–1·68 to 0·08)	0·92 (–0·00 to 1·85)	0·60 (–0·57 to 1·77)
Pericentral RPE, µm	1·11 (0·13 to 2·08)	0·10 (–0·61 to 0·81)	1·60 (0·36 to 2·84)	0·10 (–0·83 to 1·03)	–1·00 (–2·41 to 0·41)	0·00 (–1·14 to 1·14)
Pericentral retinal thickness, µm	–2·20 (–6·95 to 2·56)	2·01 (–8·56 to 12·57)	–0·67 (–8·19 to 6·85)	2·01 (–8·56 to 12·57)	–3·91 (–10·73 to 2·91)	NA
Peripheral RNFL, µm	–1·42 (–2·67 to –0·17)*	–2·55 (–4·13 to –0·96)*	–0·85 (–2·44 to 0·75)	–2·20 (–4·16 to –0·24)*	–1·52 (–3·93 to 0·90)	–1·00 (–3·63 to 1·63)
Peripheral GCL, µm	–0·09 (–1·80 to 1·63)	–0·46 (–2·28 to 1·37)	0·60 (–1·07 to 2·27)	0·30 (–0·98 to 1·58)	–1·80 (–4·23 to 0·63)	–1·90 (–3·96 to 0·16)
Peripheral IPL, µm	–1·23 (–3·19 to 0·73)	–1·74 (–4·77 to 1·30)	–0·20 (–2·13 to 1·73)	–0·20 (–1·64 to 1·24)	–2·00 (–4·11 to 0·11)	–3·10 (–4·77 to –1·43)*
Peripheral GCL+IPL, µm	–1·12 (–2·85 to 0·60)	NA	–0·36 (–2·08 to 1·36)	NA	–1·78 (–4·10 to 0·54)	NA
Peripheral INL, µm	–0·29 (–1·05 to 0·47)	–0·82 (–1·79 to 0·15)	0·27 (–0·50 to 1·04)	–0·40 (–1·36 to 0·56)	–1·30 (–2·20 to –0·40)*	–1·00 (–2·15 to 0·15)
Peripheral OPL, µm	0·08 (–0·38 to 0·53)	–0·62 (–1·19 to –0·04)*	0·05 (–0·54 to 0·63)	–0·40 (–1·12 to 0·32)	0·13 (–0·91 to 1·16)	–0·60 (–1·51 to 0·31)
Peripheral ONL+IS, µm	–1·14 (–2·69 to 0·40)	–1·99 (–3·95 to –0·03)*	–1·32 (–3·62 to 0·98)	–1·30 (–4·05 to 1·45)	0·36 (–2·23 to 2·95)	–1·40 (–4·58 to 1·78)
Peripheral OS, µm	0·47 (–0·11 to 1·05)	–0·22 (–0·85 to 0·40)	0·78 (0·09 to 1·47)	–0·30 (–1·09 to 0·49)	–0·80 (–2·18 to 0·58)	0·20 (–0·85 to 1·25)
Peripheral RPE, µm	1·12 (0·41 to 1·83)	0·08 (–0·50 to 0·65)	0·80 (–0·16 to 1·76)	0·00 (–0·73 to 0·73)	0·70 (–0·46 to 1·86)	0·20 (–0·75 to 1·15)
Peripheral retinal thickness, µm	2·23 (–0·69 to 5·16)	NA	1·43 (–2·56 to 5·42)	NA	1·63 (–4·15 to 7·41)	NA
Neurodegenerative changes around the optic nerve head assessed by OCT						
Nasal RNFL thickness, µm	–2·06 (–9·12 to 4·99)	–3·50 (–10·32 to 3·32)	1·50 (–0·04 to 3·04)	–3·50 (–10·32 to 3·32)	–7·20 (–9·24 to –5·16)†	NA
Superior RNFL thickness, µm	0·79 (–1·38 to 2·96)	–7·69 (–14·30 to –1·08)†	1·70 (–1·13 to 4·53)	–4·99 (–11·16 to 1·18)	–2·20 (–5·13 to 0·73)	–12·30 (–24·21 to –0·39)†
Temporal RNFL thickness, µm	3·28 (–0·84 to 7·39)	–0·50 (–8·00 to 7·00)	5·30 (3·70 to 6·90)	–0·50 (–8·00 to 7·00)	–4·20 (–6·16 to –2·24)†	NA
Inferior RNFL thickness, µm	19·69 (5·67 to 33·70)	–6·23 (–11·28 to –0·63)†	26·80 (24·67 to 28·93)	–3·99 (–11·59 to 3·61)	–14·30 (–17·01 to –11·59)†	–12·00 (–23·61 to –0·39)†
Mean RNFL thickness, µm	6·43 (0·75 to 12·11)	–2·96 (–6·35 to 0·44)	9·30 (7·69 to 10·91)	–5·36 (–7·13 to –3·58)†	–5·80 (–7·47 to –4·13)†	–5·30 (–11·67 to 1·08)

Data are mean effect size (95% CI). See appendix (pp 4–21) for graphical presentation of these data, p values, details of heterogeneity for all estimates, and relevant references. DR=diabetic retinopathy. OCT=optical coherence tomography. NA=not applicable. RNFL=retinal nerve fibre layer. GCL=ganglion cell layer. IPL=inner plexiform layer. INL=inner nuclear layer. OPL=outer plexiform layer. ONL=outer nuclear layer. IS=inner segments. OS=outer segments. RPE=retinal pigment epithelium. *Significant thinning of intraretinal layer. †Significant thinning of RNFL.

Table 3: **Neurodegenerative changes assessed by OCT in the retina and around the optic nerve head for detection and monitoring of diabetic retinopathy**

retinopathy showed a significant decrease in the thickness of the superior and inferior retinal nerve fibre layers in one study (table 3, appendix p 15).[46,54]

One study noted an association between diabetic polyneuropathy and inferior retinal nerve fibre layer thinning in individuals with type 2 diabetes (p=0·03), particularly in individuals with moderate and severe polyneuropathy (p<0·005).[52]

Corneal neurodegenerative changes assessed by CCM

Compared with individuals without diabetes, individuals with type 1 diabetes showed a significant reduction of nerve density and nerve length in the subbasal nerve plexus of the cornea (table 4). Substantial heterogeneity was present for all variables. All but two studies showed a lower value for the variables measured in individuals with diabetes (appendix p 16).[17,42,43,47,50,58,65–67] Compared with individuals

	Diabetes *vs* no diabetes		Diabetes without DPN *vs* no diabetes		Diabetes with DPN *vs* diabetes without DPN	
	Type 1 diabetes	Type 2 diabetes	Type 1 diabetes	Type 2 diabetes	Type 1 diabetes	Type 2 diabetes
Nerve branch density, /mm²	−19·38 (−27·52 to −11·24)*	−14·32 (−18·86 to −9·79)*	−7·74 (−14·13 to −1·34)*	−1·10 (−4·22 to 2·02)	−9·61 (−15·60 to −3·62)*	−2·83 (−6·16 to 0·50)
Nerve fibre density, /mm²	−9·33 (−15·28 to −3·38)*	−10·36 (−13·09 to −7·64)*	−2·68 (−5·56 to 0·20)	−5·80 (−8·06 to −3·54)*	−8·72 (−13·54 to −3·90)*	−4·17 (−7·87 to −0·47)*
Nerve fibre length, mm/mm²	−5·05 (−7·27 to −2·84)*	−5·09 (−6·55 to −3·62)*	−2·58 (−3·94 to −1·21)*	−4·00 (−5·93 to −2·07)*	−4·12 (−5·43 to −2·82)*	−1·23 (−2·19 to −0·27)*
Nerve fibre tortuosity	−1·55 (−3·24 to 0·15)	0·48 (0·30 to 0·65)*	−1·86 (−3·95 to 0·24)	0·63 (0·43 to 0·83)*	0·74 (−1·21 to 2·69)	0·00 (−0·15 to 0·15)

Data are the mean effect size (95% CI). See appendix (pp 4–21) for graphical presentation of these data, p values, details of heterogeneity for all estimates, and relevant references. CCM=corneal confocal microscopy. DPN=diabetic polyneuropathy. *Significant decrease or increase of corneal neuronal variable.

Table 4: **Corneal neurodegenerative changes assessed by CCM for detection and monitoring of diabetic polyneuropathy**

	Number of studies (type 1 diabetes: type 2 diabetes)	Image location in the eye	Comparison	Results	Clinical implications
OCT	5:6	Retinal layers	Diabetes *vs* no diabetes	Type 1 diabetes: thinner RNFL and photoreceptor layer; type 2 diabetes: thinner RNFL, GCL, and photoreceptor layer	OCT can detect retinal neurodegenerative changes in the diabetic eye
OCT	5:5	Retinal layers	Diabetes without DR *vs* no diabetes	Type 1 diabetes: no significant changes; type 2 diabetes: thinner RNFL	Thinning of retinal layers might be an early sign of diabetic retinopathy in type 2 diabetes
OCT	5:3	Retinal layers	Diabetes with DR *vs* diabetes without DR	Type 1 diabetes: thinner RNFL and GCL; type 2 diabetes: thinner GCL	Thinning of retinal layers is more evident when diabetic retinopathy is present
OCT	1:6	RNFL around the optic nerve head	Diabetes *vs* no diabetes	Type 1 diabetes: thicker RNFL; type 2 diabetes: thinner superior and inferior RNFL	OCT can detect neurodegenerative changes around the optic nerve head in type 2 diabetes
OCT	1:4	RNFL around the optic nerve head	Diabetes without DR *vs* no diabetes	Type 1 diabetes: thicker RNFL; type 2 diabetes: thinner mean RNFL	RNFL thinning around the optic nerve head could be an early sign of diabetic retinopathy in type 2 diabetes
OCT	1:2	RNFL around the optic nerve head	Diabetes with DR *vs* diabetes without DR	Type 1 diabetes: thinner inferior, nasal, temporal, and mean RNFL; type 2 diabetes: thinner superior and inferior RNFL	RNFL thinning around the optic nerve head is more evident when diabetic retinopathy is present
OCT	0:1	RNFL around the optic nerve head	Diabetes with DPN *vs* no diabetes	Type 1 diabetes: no data; type 2 diabetes: inferior RNFL thinner with increasing severity of DPN	RNFL thinning around the optic nerve head is related to the severity of diabetic polyneuropathy in type 2 diabetes
CCM	9:8	Subbasal nerve plexus of the cornea	Diabetes *vs* no diabetes	Type 1 diabetes: reduced nerve density and length; type 2 diabetes: reduced nerve density and length, and increased tortuosity	CCM can detect corneal neurodegenerative changes in the diabetic eye
CCM	7:1	Subbasal nerve plexus of the cornea	Diabetes without DPN *vs* no diabetes	Type 1 diabetes: reduced nerve density and length; type 2 diabetes: reduced nerve density and length, and increased tortuosity	Corneal neurodegenerative changes could be an early sign of diabetic polyneuropathy
CCM	6:1	Subbasal nerve plexus of the cornea	Diabetes with DPN *vs* diabetes without DPN	Type 1 diabetes: reduced nerve density and length; type 2 diabetes: reduced nerve density and length	Corneal neurodegenerative changes are more evident when diabetic polyneuropathy is present

OCT=optical coherence tomography. RNFL=retinal nerve fibre layer. GCL=ganglion cell layer. DR=diabetic retinopathy. DPN=diabetic polyneuropathy. CCM=corneal confocal microscopy.

Table 5: **Summary of the results by studies by image technique and clinical implications**

without diabetes, individuals with type 2 diabetes showed a significant reduction in nerve density and nerve length. The tortuosity showed a small increase in individuals with type 2 diabetes (table 4). Substantial heterogeneity was present for all variables. For nerve density and nerve length all studies showed a lower value in individuals with type 2 diabetes, and for tortuosity all studies showed a higher value in individuals with diabetes (appendix p 17).[48,49,51,56–58,60,62]

Compared with individuals without diabetes, individuals with type 1 diabetes without polyneuropathy showed significant reduction in nerve branch density and nerve length. There was substantial heterogeneity for both variables. However, there was a reduction of the nerve branch density in all but one study,[50] which had a point estimate of 1·7, whereas the other point estimates ranged from −5·1 to −21·8. There was also a reduction of the nerve fibre length in all but one study, which had a point estimate of 0·97, whereas the other point estimates ranged from −1·7 to −5·6 (appendix p 18).[17,47,50,58,65–67] Compared with individuals without diabetes, individuals with type 2 diabetes without polyneuropathy showed a significant reduction in nerve density and nerve length in one study. The tortuosity showed a small increase (appendix p 19).[51]

Compared with individuals with type 1 diabetes without polyneuropathy, individuals with type 1 diabetes and polyneuropathy showed a significant reduction of nerve

density and nerve length. Substantial heterogeneity was present for all variables. However, all studies showed a lower value in individuals with type 1 diabetes and polyneuropathy (appendix p 20).[17,47,50,65–67] Compared with individuals with type 2 diabetes without polyneuropathy, individuals with type 2 diabetes and polyneuropathy showed a significant reduction in nerve fibre density and nerve length. Moderate heterogeneity was present for nerve length and substantial heterogeneity was present for the nerve fibre density (appendix p 21).[51]

Discussion

In this paper, we have summarised the features of OCT and CCM, and systematically reviewed studies of ocular neurodegenerative changes as assessed with these imaging techniques in adults with type 1 or type 2 diabetes. To our knowledge, this is the first systematic review to evaluate these changes in the retina, the optic nerve head, and the cornea. We noted that ocular neurodegenerative changes occur in individuals with diabetes, even at an early stage when diabetic retinopathy or polyneuropathy as assessed with conventional methods are not present. These findings suggest that thinning of retinal layers is an early sign of diabetic retinopathy, and that changes in corneal nerve fibres are an early sign of diabetic polyneuropathy. Ocular neurodegenerative changes are more evident when diabetic retinopathy or polyneuropathy is present.

OCT shows thinning of the photoreceptor layers and could reflect the loss of photoreceptors, the only neurons that are directly sensitive to light. The intermediate cells transmit the neural signs from the photoreceptors to the ganglion cells. Loss of these intermediate cells could induce thinning of the outer plexiform layer, the inner nuclear layer, and the inner plexiform layer. In the ganglion cells, neural signs take the form of action potentials. Loss of these ganglion cells could explain thinning of the ganglion cell layer measured by OCT. The axons of ganglion cells form the retinal nerve fibre layer and travel towards the optic nerve head. Loss of these nerve fibres that connect the retina with the brain could explain thinning of the retinal nerve fibre layer in the retina and around the optic nerve head, as detected by OCT. All these neurons are involved in the transmission of neural signs to the brain.[71] Loss or disconnection of retinal neuronal cells might cause functional deficits.[27]

The presence of ocular neurodegeneration in diabetes could change our view on diabetic retinopathy, which is traditionally deemed a microvascular complication of diabetes. Loss of neuronal and glial cells has been suggested to be caused by chronic hyperglycaemia, oxidative stress, and accumulation of advanced glycation end products that directly affect metabolism in the retina.[72,73] Animal and human studies have indicated that these insults can result in neuronal apoptosis (including of neurons and photoreceptors), loss of ganglion cell bodies, glial reactivity, and thinning of the neuronal layers in the retina.[74–79]

Neuronal and glial cells interact with vascular cells in the retina forming a neurovascular unit. Molecular changes in diabetes affect all these cells in the retina, leading to a disruption of the neurovascular unit.[80] It has been hypothesised that damage to neuronal and glial cells precedes vascular changes.[74] An increase of the concentration of glutamate and the loss of neuroprotective factors result in an increase of vascular endothelial growth factor leading to a breakdown of the blood–retinal barrier. Neuronal death and glial dysfunction might also cause microvascular changes. Furthermore, a decreased number or dysfunction of endothelial progenitor cells reduces the remodelling capacity of the microvessels, thus further increasing microvascular and neurodegenerative changes. Finally, inflammatory parameters might also contribute to microvascular and neurodegenerative changes.[74]

Structural changes as shown by OCT could explain functional changes of the retina in individuals with diabetes. These functional changes can be assessed by perimetry,[81,82] contrast sensitivity test,[83] colour vision test,[84] dark adaptation test,[85] multifocal electroretinography,[86] electrophysiology,[87] and microperimetry.[88] Some of these functional changes are already present before microvascular abnormalities are visible. In particular, multifocal electroretinographic abnormalities can predict which retinal locations will develop microvascular changes in the near future.[89–92]

OCT and CCM are non-invasive techniques with a high reproducibility.[14–18,93,94] OCT is already used to assess thinning of the retinal neuronal layers in glaucoma and other neurodegenerative diseases, such as multiple sclerosis. OCT is also widely used in diabetes care to assess the presence of diabetic macular oedema and is therefore readily available to assess neurodegenerative changes in individuals with diabetes. OCT could therefore be used more widely in the clinic to help in the detection of retinal neurodegeneration, to define at-risk patients and to plan preventive therapy before overt vascular lesions develop. This technique could also be of use to monitor progression of diabetic retinopathy and to assess therapeutic efficacy of existing or new treatment methods.[32–34] It also seems of relevance in clinical practice in the detection of diabetic polyneuropathy. However, repeated OCT measurements could be needed to reduce measurement error.

CCM is mostly used by ophthalmologists who specialise in corneal or refractive surgery. Generalised use will mean more training and increased costs. However, such investment could be worthwhile: CCM could clinically help in the diagnosis of diabetic polyneuropathy in a non-invasive way[47,95] and might predict future polyneuropathy before changes are detected by electrophysiology and vibratory perception,[11] even in individuals with impaired glucose tolerance.[96] In two longitudinal studies, the onset of diabetic polyneuropathy was predicted by CCM.[65,97] Additionally, CCM can be used to monitor progression of diabetic polyneuropathy,[65,97] to assess therapeutic efficacy

of existing or new treatment methods, and to detect regeneration of corneal nerves after intensive treatment of diabetes.[41–43] CCM can therefore be used in all four clinical domains—diagnosis, prognosis, monitoring, and assessment of treatment effects.

Some methodological issues deserve discussion. Methods of the analysis and inclusion criteria were specified and documented in a protocol, but this protocol was not prospectively registered. In addition, our results could be affected by publication bias, but this could not be assessed because of the small number of included studies. Finally, we did not adjust our analysis for multiple testing. However, our results are in line with the biological and clinical plausibility that diabetes causes ocular neurodegenerative changes.

Some issues at study level also need to be addressed. We detected heterogeneity for some outcome variables, which was mostly due to variation in the effect sizes between studies, rather than a difference in the direction of the effect. This variation can partly be explained by a dose-effect relation between severity of diabetic retinopathy or polyneuropathy in the different studies, and the associated ocular neurodegenerative changes. Only a few of the studies we reviewed mentioned statistical power, and the number of individuals in several studies was small. Most of the studies included were prospective case series, and a few did not mention exclusion of significant media opacities. Finally, we included studies that used different types of OCT devices. In view of the small number of studies, it is not possible to conduct a meta-regression analysis to assess whether the type of OCT device affected our results. However, only differences in retinal layer thickness were reported, implying that the same type of OCT was used for comparison between individuals with and without diabetes in every study (in view of the fact that different OCT devices cannot be used interchangeably).

Before incorporation of OCT and CCM to routine screening programmes for diabetic retinopathy and diabetic polyneuropathy, their incremental benefit needs to be determined in addition to conventional clinical investigations. CCM could detect ocular neurodegenerative changes earlier than does OCT, but a head-to-head study is needed to compare these approaches. Large prospective studies are in progress to determine which neurodegenerative parameters should be used in clinical screening for diabetic retinopathy and diabetic polyneuropathy, to develop standard protocols for image analysis, and to establish reference values for clinical use.[33,67,98] Possible causes and risk factors for the occurrence of ocular neurodegenerative changes—eg, duration of diabetes, glycaemic control, hypertension, age, and sex—need to be studied in depth. Moreover, future studies should address the relation of ocular neurodegenerative changes with diabetic autonomic neuropathy,[99] cognitive decline, and white matter loss. Finally, cost-effective studies for more widespread use of OCT and CCM in the clinical setting are needed.

Contributors

All authors participated in the structure of the review. EEBDC did the study selection, data extraction, and risk of bias assessment. In case of doubt, JSAGS was consulted. EEBDC and JSAGS prepared the first draft and the subsequent versions. All authors commented on the drafts, contributed to writing, and approved the final version.

Declaration of interests

We declare no competing interests.

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