Association between Neurodegeneration and Macular Perfusion in the Progression of Diabetic Retinopathy: A 3-Year Longitudinal Study

Inês P. Marques, Sónia Ferreira, Torcato Santos, Maria H. Madeira, Ana Rita Santos, Luís Mendes, Conceição Lobo, José Cunha-Vaz

AIBILI – Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal; Faculty of Medicine, Coimbra Institute for Clinical and Biomedical Research (iCBR), University of Coimbra, Coimbra, Portugal; Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; Department of Orthoptics, School of Health, Polytechnic of Porto, Porto, Portugal; Department of Ophthalmology, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Keywords
Diabetes · Retinopathy · Neurodegeneration · Vessel closure · Progression

Abstract
Objective and Purpose: The aim of this study was to explore the relation between retinal neurodegenerative changes and vessel closure (VC) in individuals with nonproliferative diabetic retinopathy (NPDR) in a follow-up period of 3 years.

Design: This is a 3-year prospective longitudinal study with four annual visits. Participants: This study involved 74 individuals with type 2 diabetes, NPDR, and Early Treatment Diabetic Retinopathy Study grades from 10 to 47, one eye/person. An age-matched healthy control population of 84 eyes was used as control group.

Methods: Participants were annually examined by color fundus photography, spectral domain-optical coherence tomography (SD-OCT) and OCT-angiography (OCTA). VC was assessed by OCTA vessel density maps. SD-OCT segmentations were performed to access central retinal thickness (CRT) and retinal neurodegeneration considered as thinning of the ganglion cell plus inner plexiform layer (GCL + IPL).

Results: Type 2 diabetic individuals presented significantly higher CRT \((p = 0.001)\), GCL + IPL thinning \((p = 0.042)\), and decreased vessel density at the superficial capillary plexus \((p < 0.001)\) and full retina (FR) \((p = 0.001)\). When looking at changes occurring over the 3-year period of follow-up (Table 2), there were statistically significant decreases in GCL + IPL thickness \((-0.438 \mu m/year; p = 0.038)\), foveal avascular zone circularity \((-0.009; p = 0.047)\), and vessel density in superficial capillary plexus \((-0.172 \text{mm}^{-1}/\text{year}; p < 0.001)\), deep capillary plexus (DCP) \((-0.350 \text{mm}^{-1}/\text{year}; p < 0.001)\), and FR \((-0.182 \text{mm}^{-1}/\text{year}; p < 0.001)\). A statistically significant association was identified between GCL + IPL thinning and decrease in DCP vessel density \((\beta = 0.196 [95\% \text{ confidence interval: 0.037, 0.355}], z = 2.410, p = 0.016)\), after controlling for age, gender, diabetes duration, hemoglobin A1c level, and CRT.

Conclusions: Retinal neurodegenerative changes show a steady progression during a 3-year period of follow-up in eyes with NPDR and appear to be directly associated with progression in decreased vessel density including vascular closure through preferential involvement of the DCP. Our findings provide evidence that retinal neuropathy is linked with microvascular changes occurring in diabetic patients.

© 2022 The Author(s). Published by S. Karger AG, Basel
Introduction

Diabetic retinopathy (DR) is one of the leading causes of vision loss and preventable blindness worldwide and the most common complication of diabetes [1, 2], having a major impact in life quality due to the significant disability caused in multiple areas of well-being including independence, mobility, leisure, and self-care [3]. The disease progression presents inter-individual variability, with different progression rates on different individuals, reflected in the development of major vision threatening complications in some patients, namely diabetic macular edema and proliferative DR [4]. Indeed, the appearance of these complications have been shown to be associated with the DR risk phenotypes [5], observed in different individuals [4]. Likewise, these inter-individual differences appear to be of major relevance, supporting the need for specific risk biomarkers and offering the opportunity for different therapeutic strategies depending on the risk profile of each individual [6].

DR is now understood as a complex disease, in which besides microvascular alterations, neurodegeneration also appears as a relevant disease pathway [7–9]. In individuals with type 2 diabetes (T2D), the use of optical coherence tomography (OCT) offered the opportunity of identification of these neurodegenerative changes and showed that the thinning of the ganglion cell plus inner plexiform layers (GCL + IPL) occurs since the initial stages of retinopathy, progressing over time, accompanying microvascular disease progression and retinopathy progression [7]. Our group has recently reported, in a 5-year longitudinal study, that neurodegeneration occurs in all DR risk phenotypes and progresses steadily over time [10]. In this study, a 3-year longitudinal report, taking advantage of OCT-angiography (OCTA) metrics quantifying retinal microvasculature and OCT measurements of retinal neurodegeneration [11, 12], we have paid particular attention to retinopathy progression, performing a more detailed analysis of the correlations between neurodegenerative and microvascular changes in T2D non-proliferative DR patients.

Methods

This study is a subanalysis of the data reported previously in a 3-year prospective longitudinal study designed to analyze eyes of individuals with T2D and with nonproliferative DR (Early Treatment Diabetic Retinopathy Study [ETDRS] severity scale grades 10–47), with full annual ophthalmologic examination, including visual acuity, 7-field color fundus photography (CFP), OCT, and OCTA imaging (ClinicalTrials.gov identifier: NCT04650165) [12]. The tenets of the Declaration of Helsinki were followed, and approval was obtained from the AIBILI’S Ethics Committee for Health with the number CEC/011/20 (PROGRESS 10). All participants signed a written informed consent after all procedures were explained.

A total of 90 eyes of individuals with T2D and ETDRS levels between 10 and 47 were included, with a maximum glycated hemoglobin A1c (HbA1c) value of 10%. Exclusion criteria included the presence of other retinal disease, previous treatment with laser or intravitreal injections, high ametropia, or any other systemic disease that could affect the eye, as previously defined [12]. An age-matched healthy control population of 182 eyes of individuals without diabetes was used as the control group to set normal values and identify abnormal deviations between diabetic and nondiabetic control population (imagined in a single visit within the scope of the screening program).

At the baseline visit, demographic and systemic data, including age, diabetes duration, HbA1c levels, and blood pressure, were registered. All participants underwent full ophthalmologic examination, as stated above, at the baseline (visit 1) and at the 1-year (visit 2), 2-year (visit 3), and 3-year (visit 4) follow-up visits.

CFP and ETDRS Grading

The 7-fields CFP was obtained on the Topcon TRC-50DX camera (Topcon Medical Systems, Tokyo, Japan), as previously described [12]. The DR severity score was determined by 2 independent graders in a context of an experienced reading center (Coimbra Ophthalmology Reading Center [CORC], Coimbra, Portugal) according to the ETDRS classification Protocol [13, 14].

Best-Corrected Visual Acuity Evaluation

Best-corrected visual acuity (BCVA) was assessed and recorded as letters read at 4 m on ETDRS charts. The BCVA letter score for each participant was determined by adding the number of letters read at 4 m plus 30 (or the number of letters read at 1 m). BCVA was evaluated using the Snellen scale and converted into logarithm units of the minimal angle of resolution [15]. The presence of any visual loss was recorded.

Optical Coherence Tomography

OCT was performed using the Cirrus HD-OCT 5000 (Carl Zeiss Meditec, Inc., Dublin, CA, USA) with the acquisition protocol “Macular Cube 512 × 128,” which consists of 128 B-scans with 512 A-scans each and was used to assess the subjects’ central retinal thickness (CRT). Retinal layer segmentation to access ganglion cell + inner plexiform layer (GCL + IPL) thickness was performed using the segmentation algorithm implemented at AIBILI [4, 9] and with the automated segmentation algorithm available on the Zeiss device. The results here reported are from the automated Zeiss “Ganglion Cell Analysis” protocol.

OCT-Angiography

OCTA was performed on Cirrus HD-OCT 5000 AngioPlex device using the Anglexography 3 x 3-mm² acquisition protocol, which of a set of 245 clusters of 4 B-scans repetitions, where each B-scan consists of 245 A-scans, over a 3 x 3 x 2 mm³ volume in the central macula [12, 16]. Microvascular data were collected using the Carl Zeiss Meditec Density Exerciser (version: 10.0.12787). This software segments the structural OCTA data to identify the boundaries for the superficial capillary plexus (SCP), the deep capillary plexus...
Table 1. Comparison of the baseline (visit 1–0 years) variables between all patients and healthy participants

| Variable                        | DR (n = 90) | Healthy (n = 182) | Test value | p value |
|---------------------------------|-------------|-------------------|------------|---------|
| Age, years                      | 67.15 (9.2) | 66.00 (20.42)     | z = −2.843<sup>b</sup> | 0.004*  |
| Gender                          |             |                   |            |         |
| Male                            | 68 (75.56)  | 83 (45.60)        | χ² (1) = 21.875<sup>c</sup> | <0.001<sup>*</sup> |
| Female                          | 22 (24.44)  | 99 (54.40)        |            |         |
| Diabetes duration, years        | 17.51±6.53  | –                  | –          | –       |
| HbA1c, %                        | 7.40±1.19   | –                  | –          | –       |
| Visual acuity (logMAR)          | 0.06±0.11   | –                  | –          | –       |
| CRT thickness, μm               | 268.81±25.91| 261.45±19.20      | t (258) = −2.595<sup>d</sup> | 0.010*  |
| GCL + IPL thickness, μm         | 79.33±8.12  | 81.09±5.41        | t (250) = 2.043<sup>d</sup> | 0.042*  |
| FAZ area, mm<sup>2</sup>        | 0.240 (0.15)| 0.24 (0.15)       | z = −0.883<sup>b</sup> | 0.379   |
| FAZ perimeter, mm<sup>e</sup>   | 2.23 (0.55) | 2.11 (0.73)       | z = −1.708<sup>b</sup> | 0.088   |
| FAZ circularity<sup>f</sup>     | 0.62±0.10   | 0.65±0.08         | t (165) = 2.371<sup>d</sup> | 0.019*  |
| Vessel density SCP, mm<sup>−1f</sup> | 21.37 (1.76)| 22.13 (1.14)    | z = 5.397<sup>b</sup> | <0.001<sup>*</sup> |
| Vessel density DCP, mm<sup>−1f</sup> | 16.59±2.14 | 17.04±2.14        | t (160) = 1.348<sup>d</sup> | 0.180   |
| Vessel density FR, mm<sup>−1f</sup> | 23.21 (1.53)| 23.61 (0.95)     | z = 3.426<sup>b</sup> | 0.001*  |

Data are expressed as mean±standard deviation, median (interquartile range), or n (%). logMAR, logarithm units of the minimal angle of resolution. *Statistical significance for p < 0.050. ①Not all healthy participants had data available for retinal thickness and microvascular-related variables. ②Mann-Whitney test results for continuous nonparametric variables. ③χ² test results for categorical variables. *t test results for continuous parametric variables. ④Data available for 83 patients. ⑤Data available for 74 patients.

(DCP) and the full retina (FR) of the angiography data. Microangiography en face images for each segmented layer are calculated using a maximum projection algorithm on the OCTA data. Each of these en face images is then binarized using a threshold algorithm, followed by a centerline algorithm to generate skeletonized images of the retinal vasculature [16]. The skeletonized vascular densities for the SCP, DCP, and FR were considered at the inner ring. Area, perimeter, and circularity index of the foveal avascular zone (FAZ) detected on the SCP was also considered. The Density Exerciser is a closed automated software from Carl Zeiss Meditec for OCTA data processing that replicates the algorithms available on the device. As previously described [12], OCTA images underwent a quality check and normalization of signal strength [17, 18]. Likewise, from 90 eyes included in this study, 13.3% were excluded as they did not meet the set of quality criteria, resulting in a final number of 74 eyes that allowed analysis of the 3-year progression.

Statistical Analysis

Statistical significance was considered for α = 0.05. Statistical analyses were conducted in Stata (version 16.1; StataCorp LLC, College Station, TX, USA).

Data normality was assessed with the Shapiro-Wilk test and visually verified with histogram plots. Normally distributed variables were described as mean ± standard deviation, and variables not following normal distribution were described as median (interquartile range). Categorical variables were described as frequency (percentage).

The comparison of baseline characteristics (visit 1–0 years) between patients and healthy participants was performed with a t test (t value) for normally distributed continuous variables or the Mann-Whitney test (z value) for continuous variables not following a normal distribution (age; CRT; GCL + IPL thickness; FAZ area, perimeter, and circularity; SCP, DCP, and FR vessel densities) and the χ² test (χ² value) for categorical variables (gender).

Since some patients had missing data for some of visits, linear mixed models were applied to study longitudinal changes in retinal thicknesses (CRT and GCL + IPL) and microvascular-related variables (FAZ area, perimeter, circularity; DCP, SCP, and FR vessel and densities), using a restricted maximum likelihood approach. Visit (0–3 years) was used as a continuous fixed variable. The participant was used for random intercepts and slopes in the model.

Linear mixed models were also used to analyze the longitudinal correlation between GCL + IPL thickness and microvascular-related variables, using a restricted maximum likelihood approach. Visit and the microvascular-related variable were used as continuous fixed parameters for each model. The participant was used for random intercepts and slopes in the model.

Models with statistically significant associations with GCL + IPL thickness changes were repeated using baseline CRT, age, diabetes duration, HbA1c, and gender as fixed covariates to control for these effects. Models showing a statistically significant longitudinal correlation between GCL + IPL thickness and the microvascular-related variable were repeated three times using different groups of participants as an additional fixed variable: phenotype (A; B; C); ETDRS level at the baseline (10–20; 35; 43–47); and ETDRS level change between the baseline and the last visit (improve, equal, worse). The main effect of the group, the interaction between the group and visit, and the interaction between the group and the microvascular-related variable were tested.

To verify the linear mixed models’ assumptions, homoscedasticity and normality of residuals in the models were visually inspected with residuals versus predicted and Q-Q plots, respectively. The effects of the predicting variables were described with beta coefficients with 95% confidence intervals (CIs).
**Results**

Baseline demographic and systemic data of the complete study population are depicted on Table 1. A more detailed characterization of the study population with complete OCTA examination, after quality check, was previously described [12]. When compared with the healthy control population (Table 1), T2D individuals presented significantly higher CRT ($p = 0.010$), GCL + IPL thinning ($p = 0.042$), and decreased vessel density at the SCP ($p < 0.001$) and FR ($p = 0.001$).

When looking at changes occurring over the 3-year period of follow-up (Table 2), statistically significant decreases occur in GCL + IPL thickness ($−0.438 \mu m/\text{year}; p = 0.038$), FAZ circularity ($−0.009; p = 0.047$), and vessel density, with significant decreases in SCP vessel density ($−0.172 \text{mm}^{-1}/\text{year}; p < 0.001$), DCP vessel density ($−0.350 \text{mm}^{-1}/\text{year}; p < 0.001$), and FR vessel density ($−0.182 \text{mm}^{-1}/\text{year}; p < 0.001$). Of note, the rate of vessel density decrease in the 3-year period was particularly pronounced in the DCP.

When examining the longitudinal associations between changes occurring in time over the 3-year period of follow-up, considering GCL + IPL thinning, indicative of neurodegeneration, and microvascular-related variables identified by OCTA metrics of vessel density (Table 3), a statistically significant association was identified between GCL + IPL thinning and decrease in DCP vessel density ($\beta$ value $0.188 [0.033–0.343 \text{ 95\% CI}]; p = 0.018$). This association remains statistically significant ($\beta = 0.196 [95\% \text{ CI 0.037, 0.355}], z = 2.410, p = 0.016$) after controlling for age, gender, diabetes duration, HbA1c level, and CRT thickness (Fig. 1, 2). The association between GCL + IPL thickness and DCP vessel density is present in all ETDRS levels and remains present when comparing different ETDRS levels (ETDRS 10–20 VS 35: $\beta = −0.196 [95\% \text{ CI 0.037, 0.355}], z = 2.410, p = 0.016$) after controlling for age, gender, diabetes duration, HbA1c level, and CRT thickness (Fig. 1, 2). The association between GCL + IPL thickness and DCP vessel density is present in all ETDRS levels and remains present when comparing different ETDRS levels (ETDRS 10–20 VS 35: $\beta = −0.196 [95\% \text{ CI 0.037, 0.355}], z = 2.410, p = 0.016$) after controlling for age, gender, diabetes duration, HbA1c level, and CRT thickness (Fig. 1, 2).

| Variable                  | Association strength | $\beta$ 95% CI | $z$ value | $p$ value |
|---------------------------|----------------------|----------------|-----------|-----------|
| FAZ area (mm$^2$)         | −1.144               | −6.656 4.368   | −0.410    | 0.684     |
| FAZ perimeter (mm)        | −0.108               | −1.027 0.810   | −0.230    | 0.817     |
| FAZ circularity           | 0.450                | −4.219 5.119   | 0.190     | 0.850     |
| Vessel density SCP (mm$^{-1}$) | 0.100            | −0.153 0.353   | 0.770     | 0.440     |
| Vessel density DCP (mm$^{-1}$) | **0.188**          | **0.033 0.343** | **2.370** | **0.018*** |
| Vessel density FR (mm$^{-1}$) | 0.009               | −0.257 0.275   | 0.070     | 0.946     |

* Statistical significance for $p < 0.050$.  

| Variable                  | Mean change | Change 95% CI | $z$ value | $p$ value |
|---------------------------|-------------|---------------|-----------|-----------|
| CRT thickness (μm/year)   | 1.100       | −0.090 2.290   | 1.810     | 0.070     |
| GCL + IPL thickness (μm/year) | −0.438     | −0.851 −0.024 | −2.080    | 0.038*    |
| FAZ area (mm$^2$/year)    | −0.002      | −0.009 0.006  | −0.410    | 0.684     |
| FAZ perimeter (mm/year)   | 0.007       | −0.040 0.055  | 0.310     | 0.758     |
| FAZ circularity           | −0.009      | −0.019 −0.001 | −1.990    | 0.047*    |
| Vessel density SCP (mm$^{-1}$/year) | −0.172      | −0.259 −0.084 | −3.860    | <0.001*   |
| Vessel density DCP (mm$^{-1}$/year) | −0.350      | −0.492 −0.209 | −4.850    | <0.001*   |
| Vessel density FR (mm$^{-1}$/year) | −0.182      | −0.266 −0.098 | −4.250    | <0.001*   |

* Statistical significance for $p < 0.050$.  

Table 2. Changes with time (3 years) for retinal thickness and microvascular-related variables  

Table 3. Longitudinal associations between changes with time (3 years) in GCL + IPL thickness and microvascular-related variables  

**Table 2.** Changes with time (3 years) for retinal thickness and microvascular-related variables  

**Table 3.** Longitudinal associations between changes with time (3 years) in GCL + IPL thickness and microvascular-related variables
Discussion

In this study, we have followed for a period of 3 years; eyes categorized with no or minimal, mild, and moderate retinopathy; and the results here reported show that eyes in the initial stages of retinopathy in T2D individuals have both progressive vessel closure (VC) decrease in vessel density and neurodegeneration and that these changes are associated.

When examining the 3-year progression of the retinopathy, the neurodegenerative changes, evidenced by GCL + IPL thinning, show a steady progression, at a rate of −0.438 μm/year, which appears to be directly associated with progression in vascular closure through preferential involvement of the DCP. This is in agreement with the work of Aschauer and colleagues [19], who have previously presented data from a 2-year longitudinal study, suggesting that these pathways appear to run in parallel.

As previously described [12], a decrease in vessel density represented by skeletonized vessel density metrics (mean of the skeletonized slab within a desired region of interest), was the metric that better reflected the retinal VC in the initial stages DR [16]. The reduced skeletonized vessel density is first detected in the SCP. We believe that the earliest changes associated with DR involve the decrease in the number of capillaries that carry blood cells,
indicating a decrease in retinal blood flow in selected capillaries and representing by vessel density metrics, instead of changes in capillary diameter that would be better reflected by binarized vessel density (mean of the binary slab within a desired region of interest) and perfusion density metrics.

Interestingly, the results here reported suggest that the vascular component of the retinopathy appears to initiate in the SCP with subsequent preferential involvement of the DCP with retinopathy progression. It is of particular relevance that we have identified a significant association between neurodegenerative changes and microvascular capillary closure underlying retinopathy progression. Our observations suggest that DR in its nonproliferative stage is initiated in the central macula with signs of damage of the neuronal tissue, followed by failure of the microvascular response, resulting in retinal capillary closure, better identified in alterations occurring in the FAZ and involving predominantly the SCP. Afterward, progression of the disease occurs by progressive neurodegenerative changes and involvement of the DCP, which by itself may latter contribute to increased ischemic neurodegeneration and thinning of the GCL + IPL.

Previous work by our group has already suggested that neurodegenerative changes precede microvascular alterations in DR, playing a relevant role in the disease development and progression [7]. Apparently retinal neurodegenerative changes trigger a microvascular response which varies between individuals with T2D [10]. The retinal microvasculature may fail to respond to the neuroglial damage by compensatory vasodilation and initiate a process of microvascular closure and thrombosis [20].

These microvascular changes appears to progress mainly through preferential capillary closure involvement of the DCP, which by itself contribute to further neurodegenerative changes. Two important conclusions can be drawn from this study. First, the longitudinal results obtained over a period of 3 years show that there were signs of neurodegeneration and capillary closure since the initial stages of DR and that these alterations are closely associated. Second, these alterations progress over time since the initial stages of the diabetic retinal disease. A limitation of this study is the number of eyes included. Other limitations include the lack of use with OCTA analyses of an effective projection removal algorithm and the limited field examined (3 × 3 mm), which prevents an evaluation of more peripheral regions of the retina. Of special value, a strict quality check was performed by a masked grader and normalization of OCTA metrics based on signal strength was performed.

In conclusion, information on retinal neurodegeneration demonstrated by thinning of the GCL + IPL layers of the retina and OCTA metrics of retinal VC, more specifically vessel density measurements based on skeletonized images, obtained in a noninvasive manner that allow repeated examinations and close follow-up, offer relevant information on DR progression that is expected to impact disease management by allowing personalized and timely intervention.

**Statement of Ethics**

The tenets of the Declaration of Helsinki were followed, and the study was reviewed and approved by the AIBILI’s Ethics Committee for Health with the number CEC/011/20 (PROGRESS 10), NCT04650165 (ClinicalTrials.gov identifier). A written informed consent was signed by each participant signed, agreeing to participate in the study, after all procedures were explained.

**Conflict of Interest Statement**

I.P.M., S.F., T.S. M.H.M., A.R.S., L.M., and C.L. declare no conflicts of interest. José Cunha-Vaz reports grants from Carl Zeiss Meditec and is a consultant for Alimera Sciences, AbbVie, Bayer, Gene Signal, Novartis, Pfizer, Precision Ocular Ltd., Roche, Sanofi-Aventis, Vifor Pharma, and Carl Zeiss Meditec. The funders had no role in the design or writing of the manuscript.

**Funding Sources**

This work was supported by AIBILI, COMPETE Portugal2020, and the Fundo de Inovação Tecnologia e Economia Circular (FITEC) – Programa Interface (FITEC/CIT/2018/2).

**Author Contributions**

I.P.M., S.F., T.S. M.H.M., A.R.S., and L.M. collected data, analyzed, wrote, and reviewed and edited the manuscript. C.L. assisted in the analysis and interpretation of the data. J.C.-V. is the guarantor of this work and, as such, had full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. All the authors have read and agreed to the published version of the manuscript.

**Data Availability Statement**

Data will be available upon request to correspondent author.
References

1 Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, et al. Causes of vision loss worldwide, 1990–2010: a systematic analysis. Lancet Glob Heal. 2013;1(6):e339–49.

2 International Diabetes Federation. IDF Diabetes Atlas. 9th ed. International Diabetes Federation; 2019.

3 Milne A, Johnson JA, Tennant M, Rudinsky C, Dryden DM. Measuring health-related quality of life for patients with diabetic retinopathy. Rockville, MD: Agency for Healthcare Research and Quality; 2012.

4 Marques IP, Madeira MH, Messias AL, Santos T, Martinho AC, Figueira J, et al. Retinopathy phenotypes in type 2 diabetes with different risks for macular edema and proliferative retinopathy. J Clin Med. 2020;9(5):1433.

5 Nunes S, Ribeiro L, Lobo C, Cunha-Vaz J. Three different phenotypes of mild nonproliferative diabetic retinopathy with different risks for development of clinically significant macular edema. Invest Ophthalmol Vis Sci. 2013;54(7):4595–604.

6 Cunha-Vaz J, Mendes L. Characterization of risk profiles for diabetic retinopathy progression. J Pers Med. 2021;11(8):826.

7 Sohn EH, van Dijk HW, Jiao C, Kok PH, Jeong W, Daalderop K, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci U S A. 2016 May;113(19):E2655–64.

8 Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia. 2018 Sep;61(9):1902–12.

9 Marques IP, Alves D, Santos T, Mendes L, Lobo C, Santos AR, et al. Characterization of disease progression in the initial stages of retinopathy in type 2 diabetes: a 2-year longitudinal study. Invest Ophthalmol Vis Sci. 2020 Mar;61(3):20.

10 Madeira MH, Marques IP, Ferreira S, Tavares D, Santos T, Santos AR, et al. Retinal neurodegeneration in different risk phenotypes of diabetic retinal disease. Front Neurosci. 2021 Dec;15. Epub ahead of print.

11 Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Stanga G. Optical coherence tomography angiography angiography. Prog Retin Eye Res. 2018 May;64:1–55.

12 Marques IP, Kubach S, Santos T, Mendes L, Madeira MH, de Sisternes L, et al. Optical coherence tomography angiography metrics monitor severity progression of diabetic retinopathy – 3-year Longitudinal Study. J Clin Med. 2021 May;10(11):2296.

13 Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs – an extension of the modified Airlie House classification: ETDRS report number 10. Ophthalmology. 2020 Apr;127(4S):599–119.

14 Soares M, Neves C, Marques IP, Pires J, Schwartz C, Costa MA, et al. Comparison of diabetic retinopathy classification using fluorescein angiography and optical coherence tomography angiography. Br J Ophthalmol. 2017 Jan;101(1):62–8.

15 Khoshnood B, Mesbah M, Jeanbat V, Lafuma A, Berdeaux G. Transforming scales of measurement of visual acuity at the group level. Ophthalmic Physiol Opt. 2010 Nov;30(6):816–23.

16 Durbin MK, An L, Shemonski ND, Soares M, Santos T, Lopes M, et al. Quantification of retinal microvascular density in optical coherence tomographic angiography images in diabetic retinopathy. JAMA Ophthalmol. 2017 Apr;135(4):370–6.

17 Lei J, Durbin MK, Shi Y, Uji A, Balasubramanian S, Bagdasaryan E, et al. Repeatability and reproducibility of superficial macular retinal vessel density measurements using optical coherence tomography angiography en face images. JAMA Ophthalmol. 2017 Oct;135(10):1092–9.

18 Santos T, Warren LH, Santos AR, Marques IP, Kubach S, Mendes LG, et al. S swept-source OCTA quantification of capillary closure predicts ETDRS severity staging of NPDR. Br J Ophthalmol. 2020 Dec;104(12):17890. Epub ahead of print.

19 Aschauer J, Pollreisz A, Karst S, Hülsmann M, Hajdu D, Daalderop K, et al. Longitudinal analysis of microvascular perfusion and neurodegenerative changes in early type 2 diabetic retinal disease. Br J Ophthalmol. 2022 Apr;106(4):528–33.

20 Ludovico J, Bernardes R, Pires I, Figueira J, Lobo C, Cunha-Vaz J. Alterations of retinal capillary blood flow in preclinical retinopathy in subjects with type 2 diabetes. Graefes Arch Clin Exp Ophthalmol. 2003 Mar;241(3):181–6.