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Retina

Patterns of Progression of Nonproliferative Diabetic Retinopathy Using Non-Invasive Imaging

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Purpose: To identify progression of nonproliferative diabetic retinopathy (NPDR) in patients with type 2 diabetes by combining optical coherence tomography angiography (OCTA) metrics and color fundus photography (CFP) images.

Methods: This study was a post hoc analysis of a prospective longitudinal cohort study (CORDIS, NCT03696810) with 2-year duration. This study enrolled 122 eyes. Ophthalmological examinations included OCTA and CFP. OCTA metrics included skeletonized vessel density (SVD) and perfusion density (PD) at the superficial capillary plexus (SCP) and deep capillary plexus (DCP). Microaneurysm turnover analysis and Early Treatment Diabetic Retinopathy Study (ETDRS) grading for diabetic retinopathy (DR) severity assessment were performed on 7-field CFP.

Results: Eyes graded as ETDRS level 20 showed significant capillary nonperfusion predominantly in the inner ring area in the SCP ($P < 0.001$), whereas eyes graded as ETDRS level 35 and ETDRS levels 43 and 47 showed significant capillary nonperfusion in both the SCP and DCP in both inner and outer rings ($P < 0.001$). When evaluating rates of progression in capillary nonperfusion for the 2-year period of follow-up, changes were found predominantly in the DCP for SVD and PD and were better identified in the outer ring area. Microaneurysm turnover contributes to the characterization of NPDR progression by discriminating ETDRS level 35 from ETDRS levels 43 and 47 ($P < 0.001$), which could not be achieved using only OCTA metrics.

Conclusions: Patterns of progression of NPDR can be identified combining OCTA examinations of the superficial and deep retinal capillary plexi of central retina and determination of microaneurysm turnover from fundus photographs.

Translational Relevance: Our study reports results from a registered clinical trial that advances understanding of disease progression in NPDR.

Introduction

Diabetic retinopathy (DR) is a leading cause of blindness in adults. The International Diabetes Federation estimated that by 2045 there will be 783 million people worldwide with diabetes. Approximately one-third of people with diabetes develop signs of retinopathy, with 10% developing vision-threatening complications.[1,2] The ability to predict DR progression in an

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individual patient is an important unmet clinical need, as early identification of individuals at risk of progression is critical to individualize clinical assessment and timely intervention before vision loss.

Clinical staging based on color fundus photography (CFP), such as the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria,[3] is currently the basis for characterizing stages of DR progression. The ETDRS classification is laborious, time consuming, and difficult to perform in clinical practice. Furthermore, easier to perform classification systems are not sufficiently accurate to detect progression and establish a prognostic.[4]

DR is a microvascular disease of the retina. Fluorescein angiography is an invasive imaging procedure that provides functional information regarding leakage of the dye to provide information on the permeability of vessels, as well as the ability to stain structures such as fibrous tissue or subretinal fluid, which is not available with optical coherence tomography angiography (OCTA). Fluorescein angiography is also the gold standard to evaluate peripheral ischemia; however, OCTA offers the opportunity to perform non-invasive imaging capable of providing information about capillary nonperfusion in both the superficial capillary plexus (SCP) and deep capillary plexus (DCP). Furthermore, the quality of the OCTA image is not influenced by vessel leakage, and it is an objective way to measure retinal ischemia. Indeed, many cross-sectional and longitudinal studies have shown correlations between OCTA vascular metrics and DR severity.[5–7] The methodology used for OCTA analysis, however, varies among different studies, and questions remain regarding the most appropriate OCTA metrics to use as biomarkers of progression.[5,8] Concurrently, microaneurysm formation and disappearance rates have been shown to be relevant indicators of nonproliferative diabetic retinopathy (NPDR) progression.[9,10]

We revisited a 2-year longitudinal follow-up study, Characterization of Retinal Vascular Disease in Eyes With Mild to Moderate NPDR in Diabetes Type 2 (CORDIS, NCT03696810),[11] and looked specifically at the characterization of NPDR progression using OCTA metrics and microaneurysm formation and disappearance rates from fundus photographs images using Retmarker DR software (Retmarker SA – Meteda Group, Rome, Italy).[7,12] A comparative analysis of the different OCTA metrics and their relative value to identify the different ETDRS stages of retinopathy is of major relevance. It is expected that a better understanding of the progression of the microvascular changes occurring in NPDR and in its different ETDRS stages will establish the foundations on which to base new developments in automated replacement of ETDRS staging.

Methods

CORDIS, a prospective longitudinal cohort study (NCT03696810, clinicaltrials.gov) was conducted during 2 years of follow-up in patients with type 2 diabetes, with mild or moderate NPDR (ETDRS levels 20, 35, 43, and 47).[3] The study clinical protocol was reviewed and approved by the Association for Innovation and Biomedical Research on Light and Image Ethics Committee for Health and followed the tenets of the Declaration of Helsinki. After receiving a thorough explanation, each participant signed a written informed consent form.

The study included 122 patients diagnosed with type 2 diabetes and ETDRS levels between 20 and 47. Patients were excluded from participation if significant cataract was present or if they had confirmed glaucoma, eye surgery within a period of 6 months before the baseline visit, other retinal vascular disease, previous laser treatment or intravitreal injections, or dilation of pupil < 5 mm. Other exclusion criteria included glycated hemoglobin A1C (HbA1c) level $>$ 10% (85.8 mmol/mol) or other systemic disease that could affect the eye, particularly uncontrolled systemic hypertension or history of heart disease. Examinations were performed at baseline and after 12 and 24 months (visits 0, 1, and 2). All individuals underwent ophthalmological examinations at their annual visits, including seven-field ETDRS CFP and OCTA. An age-matched healthy control population of 65 individuals was used as reference for demographic and ocular characteristics.

CFP and ETDRS Classification

Seven-field CFP images were obtained at a 35° field of view using a TRC-50DX mydriatic retinal camera (Topcon Medical Systems, Tokyo, Japan) with a resolution of 3596×2448 pixels. DR severity grading was performed according to the ETDRS protocol based on the identification of lesions, such as microaneurysms, hemorrhages, intraretinal microvascular abnormalities (IRMAs), soft or hard exudates, venous beading, or presence of neovascularization. The DR severity scores were classified at the Coimbra Ophthalmology Reading Centre (CORC) according to the Diabetic Retinopathy Severity Scoring System. CORC reports 93.8% agreement among independent graders on their regular discrepancy assessments. ETDRS classification was performed at baseline and at every annual visit to evaluate the progression of DR severity.

Microaneurysm Turnover Assessment

Microaneurysm (MA) classification was automatically performed on 50° two-field images using Retmarker DR software, which is computer-aided diagnostic software that performs MA earmarking and identification of macular red dot-like vascular lesions.[9,12] It allows the comparison of lesions within the same retinal location between different visits. Likewise, this algorithm computes the number and localization of MAs in each visit, allowing calculation of the MA disappearance and formation rates between visits. Microaneurysm turnover (MAT) is determined as the sum of the MA formation and disappearance rates. Retmarker DR has been used daily since 2011, and Retmarker DR Biomarker is currently certified as a class IIA medical device in Europe. The Retmarker DR software is highly conservative in order to reduce variability.

Optical Coherence Tomography Angiography

At each visit, all participants underwent OCTA examinations with the ZEISS CIRRUS 5000 AngioPlex (Carl Zeiss Meditec, Dublin, CA). OCTA metrics were collected for the inner ring, with the annulus centered at the fovea with a radius of 500 to 1500 µm, and for the outer ring, with the annulus centered at the fovea with a radius of 1500 to 3000 µm, using the angiography 3 × 3 mm and angiography 6 × 6 mm acquisition protocols, respectively. The angiography 6 × 6 mm acquisition protocol, consisting of 350 clusters of two B-scan repetitions with 350 A-scans, provides a wider field of view but lower digital sampling when compared to the angiography 3 × 3 mm acquisition protocol, which consists of 245 clusters of four B-scan repetitions with 245 A-scans.

The acquired 3 × 3 mm and 6 × 6 mm OCTA examinations were processed using the Carl Zeiss Meditec Density Exerciser (version 10.0.12787) to compute tabulated and image data for the skeletonized vessel density (SVD) and perfusion density (PD) metrics at the superficial capillary plexus (SCP) and deep capillary plexus (DCP). SVD was defined as the mean of the skeletonized slab within a desired region of interest, scaled by the distance between pixels (245 pixels per 3 mm and 350 pixels per 6 mm for 3 × 3 mm and 6 × 6 mm angiography, respectively). It represents the number of individual capillaries that are carry-

Table 1. Baseline Characteristics by ETDRS Severity Groups

	Healthy ($n = 65$)	ETDRS 20 ($n = 12$)	P (Healthy vs. ETDRS 20)	ETDRS 35 ($n = 74$)	P (Healthy vs. ETDRS 35)	ETDRS 43 and 47 ($n = 36$)	P (Healthy vs. ETDRS 43 and 47)	P Value: ETDRS 20 vs. ETDRS 35	P Value: ETDRS 35 vs. ETDRS 43 and 47
Demographic Characteristics									
Male/female gender, n (%)	35/30 (53.8/46.2)	8/4 (66.7/33.3)	0.533	54/20 (73.0/27.0)	0.022	31/5 (86.1/13.9)	0.001	0.731	0.150
Age (yr), mean ± SD	67.26 ± 2.72	68.00 ± 4.69	0.697	67.07 ± 6.76	0.480	66.94 ± 7.14	0.232	0.870	0.880
Diabetes duration (yr), mean ± SD	—	19.33 ± 9.06	—	19.31 ± 7.41	—	20.31 ± 6.94	—	0.958	0.696
OCTA Characteristics, Mean ± SD									
SVD SCP (mm^{-1}), 3 × 3 mm inner ring	22.35 ± 0.87	21.29 ± 0.95	**0.001**	20.54 ± 1.38	**<0.001**	20.56 ± 1.25	**<0.001**	0.099	0.871
SVD DCP (mm^{-1}), 3 × 3 mm inner ring	17.26 ± 2.16	15.82 ± 2.31	0.096	15.86 ± 2.27	**0.001**	15.68 ± 1.94	**<0.001**	0.939	0.625
SVD SCP (mm^{-1}), 6 × 6 mm outer ring	18.54 ± 0.63	18.85 ± 0.91	0.511	18.12 ± 0.95	**0.012**	18.25 ± 0.83	0.060	**0.023**	0.447
SVD DCP (mm^{-1}), 6 × 6 mm outer ring	15.69 ± 1.57	15.26 ± 1.82	0.450	13.33 ± 2.26	**<0.001**	12.99 ± 1.99	**<0.001**	**0.009**	0.366
PD SCP (a.u.), 3 × 3 mm inner ring	0.40 ± 0.02	0.39 ± 0.01	**0.029**	0.39 ± 0.02	**<0.001**	0.39 ± 0.02	**0.030**	0.796	0.347
PD DCP (a.u.), 3 × 3 mm inner ring	0.33 ± 0.04	0.30 ± 0.04	0.093	0.31 ± 0.04	**0.002**	0.31 ± 0.03	**0.006**	0.972	0.986
PD SCP (a.u.), 6 × 6 mm outer ring	0.46 ± 0.01	0.47 ± 0.01	0.181	0.45 ± 0.02	**0.017**	0.46 ± 0.02	0.373	**0.017**	0.109
PD DCP (a.u.), 6 × 6 mm outer ring	0.34 ± 0.04	0.33 ± 0.05	0.331	0.28 ± 0.06	**<0.001**	0.28 ± 0.05	**<0.001**	**0.013**	0.481

a.u., arbitrary units; DCP, deep capillary plexus; ETDRS, Early Treatment Diabetic Retinopathy Study; n, number of participants; SD, standard deviation; SCP, superficial capillary plexus.

Bold values represent statistically significant differences with $P < 0.05$, using χ^2 test for categorical variables and Mann–Whitney U test for continuous variables between healthy group versus DR severity groups and between DR severity groups.

ing red blood cells, and its decrease indicates capillary closure. PD, a different metric, was defined as the total area of perfused vasculature per unit area in a region of measurement, calculated by taking the mean of the binary slab within a desired region of interest. It represents changes in vessel perfusion—namely, capillary vasodilation or vasoconstriction.[5,6,13] Areas of abnormal intercapillary spaces[13] were not reported because they did not give reliable results when identifying closure in the DCP.

OCTA quality checks were performed by a masked grader to ensure signal strength of ≥7 and that areas with motion artifacts, defocus, or blur were not present in more than 25% of the image area. Eight of the 122 OCTA images (6%) were discarded for inner ring areas (3 × 3 mm acquisition protocol), whereas 17 of the 122 OCTA images (14%) were discarded for outer ring areas (6 × 6 mm acquisition protocol). Normalization of the signal strength was performed in all OCTA examinations as previously described.[5]

Statistical Analysis

Statistical analyses of the data were performed using Stata 16.1 (StataCorp, College Station, TX). $P < 0.05$ was considered statistically significant. Percentages were reported for categorical variables, and the χ^2 test was used to compare differences between the groups.

Distribution of normality was assessed using the Kolmogorov–Smirnov test. Demographic and ocular characteristics were presented as the means and corresponding standard deviations for continuous variables. The Mann–Whitney *U* test (variables not distributed normality) was used to compare characteristics between the healthy group and the DR severity groups (healthy vs. ETDRS level 20, healthy vs. ETDRS level 35, and healthy vs. ETDRS levels 43 and 47) and among the DR severity groups (ETDRS level 20 vs. ETDRS level 35 vs. ETDRS levels 43 and 47).

Rates of progression based on mean longitudinal changes for OCTA metrics (inner and outer rings: 3 × 3 mm and 6 × 6 mm, respectively) were evaluated using linear mixed models with restricted maximum likelihood (REML) estimation, where visit (baseline, 12 months, and 24 months) was used as a continuous fixed variable. The patients were used as a random effect (intercept only). Linear mixed models were applied to compare ETDRS severity groups or ETDRS severity changes using an additional fixed variable: for ETDRS severity group, mild NPDR versus moderate NPDR versus moderately severe NPDR; for ETDRS severity change, worsening versus maintained/improved ETDRS severity.

Linear mixed models were also used to evaluate the correlation between MAT and microvascular-related variables. Visits, ETDRS severity, and microvascular-related variables served as continuous fixed variables. Linear mixed model assumptions such as homoscedasticity (also known as homogeneity of error variance) and normality of residuals in the models were visually inspected with residuals versus predicted and Q-Q plots, respectively. The interactions between groups and visits were tested. The estimated effects of the predicting variables were described by beta coefficients with 95% confidence intervals (CIs).

Results

In the CORDIS study, 122 patients with type 2 diabetes (one eye for each patient, $n = 122$ eyes with NPDR) completed the 2-year follow-up period of

Table 2. Rates of Changes per Year for OCTA Metrics

	β	Standard Error	95% CI		z	P
SVD SCP (mm^{-1}), 3 × 3 mm inner ring	−0.024/yr	0.034	−0.091	0.044	−0.680	0.494
SVD DCP (mm^{-1}), 3 × 3 mm inner ring	−0.163/yr	0.069	−0.297	−0.028	−2.370	**0.018**
SVD SCP (mm^{-1}), 6 × 6 mm outer ring	0.000/yr	0.035	−0.068	0.068	<0.001	0.999
SVD DCP (mm^{-1}), 6 × 6 mm outer ring	−0.199/yr	0.070	−0.337	−0.061	−2.830	**0.005**
PD SCP (a.u.), 3 × 3 mm inner ring	−0.003/yr	0.001	−0.004	−0.001	−3.450	**0.001**
PD DCP (a.u.), 3 × 3 mm inner ring	−0.003/yr	0.001	−0.005	<0.001	−2.220	**0.027**
PD SCP (a.u.), 6 × 6 mm outer ring	−0.002/yr	0.001	−0.004	<0.001	−2.420	**0.015**
PD DCP (a.u.), 6 × 6 mm outer ring	−0.005/yr	0.002	−0.008	−0.002	−2.880	**0.004**

a.u., arbitrary units; CI, confidence interval; DCP, deep capillary plexus; PD, perfusion density; SCP, superficial capillary plexus; SVD, skeletonized vessel density.

Bold values represent statistically significant differences with $P < 0.05$, using linear mixed models to evaluate longitudinal changes of all patients ($n = 122$) where visit (baseline, 12 months, and 24 months) was used as a continuous fixed variable.

the study. The eye with more severe ETDRS severity grading was chosen and included in the study. When both eyes had the same ETDRS level, the right eye was chosen. Twelve eyes (10%) were graded at baseline as ETDRS level 20, 74 eyes (61%) as ETDRS level 35, and 36 eyes (29%) as ETDRS level 43 or 47 (25 eyes graded as ETDRS 43 and 11 eyes graded as ETDRS 47). Baseline demographic and systemic parameters have been previously reported[7,11] and are briefly presented in Table 1. The mean age of patients included was 67.12 ± 6.66 years; 76% were male. An age-matched healthy control population of 65 individuals (67.26 ± 2.72 years; 54% male) was used as reference for demographic and ocular characteristics.

Capillary nonperfusion values at baseline measured by decreased SVD and PD for both retinal inner and outer rings (3 × 3 mm and 6 × 6 mm) are presented in Table 1. The eyes graded as ETDRS level 20 showed significant capillary nonperfusion compared to healthy controls in the inner ring of the SCP, detected as decreases in both SVD (P = 0.001) and PD (P = 0.029). Eyes classified as ETDRS level 35 showed increased capillary nonperfusion in both retinal capillary plexi (SCP and DCP) and in both inner and outer rings. Similar results were observed in eyes classified as ETDRS level 43 and level 47, showing a significant capillary nonperfusion in both retinal capillary plexi and both retinal areas of the inner and outer rings (3 × 3 mm and 6 × 6 mm).

When comparing eyes with ETDRS levels 20 and 35, a statistically significant increase in capillary nonperfusion (i.e., decrease in both SVD and PD) was identified in both retinal capillary plexi only on the outer ring (6 × 6 mm). When comparing eyes with ETDRS level 35 and those with ETDRS level 43 and level 47, no differences were identified in capillary nonperfusion values (Table 1).

When evaluating the rates of progression of capillary nonperfusion per year for the 2-year period of follow-up in the 122 eyes (Table 2), statistically significant decreases in SVD were observed only in the DCP in both inner and outer ring areas (3 × 3 mm and 6 × 6 mm). In contrast, changes in PD were observed in both retinal plexi (SCP and DCP) in both retinal areas (3 × 3 mm and 6 × 6 mm).

When analyzing the rates of progression per year in capillary nonperfusion according to DR severity levels (Table 3), eyes with ETDRS level 20 showed an increased progression in capillary nonperfusion detected mainly in the outer ring (6 × 6 mm). In eyes with ETDRS level 35 and ETDRS levels 43 and 47, the progression of capillary nonperfusion also involved mainly the DCP and was better identified in the inner and outer rings. In summary, when the rates of change

Table 3. Rates of Change per Year for Each ETDRS Severity Level

	ETDRS 20 (n = 12)		ETDRS 35 (n = 74)		ETDRS 43 and 47 (n = 36)		Post Hoc Analysis			
							ETDRS 20 vs. ETDRS 35		ETDRS 35 vs. ETDRS 43 and 47	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	Mean Change (95% CI)	P	Mean Change (95% CI)	P
SVD SCP (mm^{-1}), 3 × 3 mm inner ring	−0.100 (−0.291 to 0.090)	0.303	0.009 (−0.081 to 0.098)	0.851	−0.063 (−0.186 to 0.060)	0.315	0.112 (−0.125 to 0.349)	0.354	−0.070 (−0.221 to 0.081)	0.365
SVD DCP (mm^{-1}), 3 × 3 mm inner ring	0.184 (−0.240 to 0.608)	0.394	−0.124 (−0.286 to 0.039)	0.135	−0.354 (−0.628 to −0.080)	**0.011**	−0.311 (−0.774 to 0.152)	0.188	−0.225 (−0.523 to 0.074)	0.140
SVD SCP (mm^{-1}), 6 × 6 mm outer ring	−0.127 (−0.301 to 0.048)	0.155	0.026 (−0.061 to 0.113)	0.553	−0.027 (−0.166 to 0.112)	0.707	0.152 (−0.084 to 0.388)	0.206	−0.045 (−0.202 to 0.112)	0.577
SVD DCP (mm^{-1}), 6 × 6 mm outer ring	−0.679 (−1.037 to −0.321)	**<0.001**	−0.131 (−0.306 to 0.045)	0.144	−0.181 (−0.456 to 0.094)	0.197	0.563 (0.089 to 1.036)	**0.020**	−0.054 (−0.370 to 0.262)	0.737
OD SCP (a.u.), 3 × 3 mm inner ring	−0.001 (−0.005 to 0.002)	0.501	−0.002 (−0.004 to −0.000)	**0.027**	−0.004 (−0.006 to −0.001)	**0.006**	−0.001 (−0.006 to 0.004)	0.803	−0.002 (−0.005 to 0.001)	0.281
PD DCP (a.u.), 3 × 3 mm inner ring	0.003 (−0.004 to 0.011)	0.375	−0.002 (−0.005 to 0.001)	0.203	−0.006 (−0.011 to −0.002)	**0.010**	−0.005 (−0.013 to 0.003)	0.202	−0.004 (−0.01 to 0.001)	0.095
PD SCP (a.u.), 6 × 6 mm outer ring	−0.007 (−0.012 to −0.001)	**0.012**	−0.001 (−0.003 to 0.001)	0.215	−0.003 (−0.006 to 0.001)	0.116	0.004 (−0.001 to 0.010)	0.129	−0.001 (−0.005 to 0.003)	0.601
PD DCP (a.u.), 6 × 6 mm outer ring	−0.018 (−0.026 to −0.009)	**<0.001**	−0.003 (−0.008 to 0.001)	0.137	−0.004 (−0.011 to 0.002)	0.201	0.015 (0.003 to 0.026)	**0.014**	−0.001 (−0.009 to 0.007)	0.768

a.u., arbitrary unit; CI, confidence interval; ETDRS, Early Treatment Diabetic Retinopathy Study; DCP, deep capillary plexus; n, number of participants; PD, perfusion density; SCP, superficial capillary plexus; SVD, skeletonized vessel density.

Bold values represent statistically significant differences with $P < 0.05$, using linear mixed models to evaluate longitudinal changes for each ETDRS severity level and post hoc analysis, where visit (baseline, 12 months, and 24 months) and ETDRS severity levels were used as fixed variables.

Table 4. Microaneurysm Progression Rates Between ETDRS Severity Levels at 1 Year

	ETDRS 20 ($n = 12$)	ETDRS 35 ($n = 74$)	ETDRS 43 and 47 ($n = 36$)	*P* Value	
				ETDRS 20 vs. ETDRS 35	ETDRS 35 vs. ETDRS 43 and 47
Microaneurysm formation rate	0.34 ± 0.67	0.95 ± 1.50	4.20 ± 3.84	0.158	**<0.001**
Microaneurysm disappearance rate	0.60 ± 0.83	0.58 ± 0.87	2.18 ± 2.46	0.757	**<0.001**
Microaneurysm turnover	0.94 ± 1.03	1.48 ± 2.01	6.20 ± 5.46	0.818	**<0.001**

Bold values represent statistically significant differences with $P < 0.05$, using Mann–Whitney *U* test.

per year by ETDRS level were analyzed, the progression of capillary nonperfusion was better identified in the DCP and when examining the outer ring (6 × 6 mm). Of particular interest was the observation that MAT values and microaneurysm formation and disappearance rates are capable of discriminating ETDRS levels 35 from ETDRS levels 43 and 47 (Table 4), which could not be achieved using only OCTA metrics (Table 1).

When combining OCTA microvascular metrics with MAT for a better understanding of the DR progression, the analysis revealed statistically significant associations in different stages of the disease (Table 5). Capillary nonperfusion in the DCP (3 × 3 mm) associated with MAT is capable of discriminating ETDRS level 20 from ETDRS level 35: SVD = 1.321 (95% CI, 0.176–2.466; $P = 0.024$) and PD = 0.022 (95% CI, 0.001–0.042; $P = 0.039$). Capillary nonperfusion in SCP (3 × 3 mm and 6 × 6 mm) associated with MAT is capable of discriminating ETDRS level 35 from ETDRS levels 43 and 47: inner ring SVD = 0.189 (95% CI, 0.038–0.339; $P = 0.014$) and outer ring SVD = 0.115 (95% CI, 0.010–0.221; $P = 0.032$).

Since only 17 eyes worsened over a period of 2 years (considering all ETDRS levels), the study was not powered to define the potential of OCTA metrics to characterize the progression of the microvascular changes occurring in NPDR. However, when considering only ETDRS level 35, which represented 10 of the 17 eyes that worsened, and when comparing the eyes that showed ETDRS grade worsening with the eyes that did not, significant differences in SVD and PD progression were observed in both retinal capillary plexi when examining the outer ring (6 × 6 mm), with more positive β values: SCP SVD = 0.323 (95% CI, 0.101–0.545; $P = 0.004$); DCP SVD = 0.495 (95% CI, 0.125–0.865; $P = 0.009$); SCP PD = 0.007 (95% CI, 0.002–0.012; $P = 0.006$); DCP PD = 0.012 (95% CI, 0.003–0.020; $P = 0.008$). The Figure demonstrates an example of retinal vascular changes for an heathy individual and two patients with different DR severity (ETDRS level 35 and ETDRS level 43).

Discussion

Capillary NPDR, which occurs progressively and increases with the time of disease, can be detected by such OCTA metrics as SVD and PD. Our data show that capillary nonperfusion initially involves the SCP, progressing later to the DCP. The capillary nonperfusion appears to begin centrally and spreads peripherally.

Previous studies have shown that capillary closure is present in the preclinical stage of diabetic retinal disease in the central macula,[14] increasing in subsequent ETDRS severity levels.[11,15,16] In ETDRS level 20, the earliest stage of NPDR, the capillary closure remains mainly located in the SCP and the central area of the retina, the inner ring (3 × 3 mm). In ETDRS level 35 (mild NPDR), the capillary closure is present in both the SCP and DCP and begin extending to the outer ring of the central retina (6 × 6 mm). Finally, in ETDRS levels 43 and 47 (moderate and moderately severe NPDR), the capillary nonperfusion is also present in both plexuses and extends progressively to more peripheral areas of the retina, appearing to reach a plateau in the central retina with similar central ischemia in ETDRS level 35 and ETDRS levels 43 and 47. These findings demonstrate progressive decentralization of the ischemia and involvement of more peripheral regions of the retina confirming in a previous report by our group.[17]

As the retinopathy progresses, the changes in capillary closure are better understood when associated with the progressive increase in microaneurysm turnover and microaneurysm formation and disappearance rates, particularly in ETDRS severity levels 43 and 47. Microaneurysms are preferentially located in abnormally dilated shunt vessels as previously shown in a number of histological studies and considered to be directly associated with the progressive capillary nonperfusion.[18–20] The number of microaneurysms and their formation and disappearance rates increase with capillary nonperfusion and appear to be good indicators of the development of abnor-

Table 5. Combination of MAT and Microvascular Changes

	ETDRS 20 ($n = 12$)		ETDRS 35 ($n = 74$)		ETDRS 43 and 47 ($n = 36$)		Post Hoc Analysis: ETDRS 20 vs. ETDRS 35		Post Hoc Analysis: ETDRS 35 vs. ETDRS 43 and 47	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	Mean Change (95% CI)	*P*	Mean Change (95% CI)	*P*
SVD SCP (3 × 3 mm inner ring) + MA turnover	0.346 (0.853 to 0.162)	0.182	0.246 (0.385 to 0.107)	**0.001**	0.052 (0.120 to 0.017)	0.142	0.057 (−0.630 to 0.744)	0.870	0.189 (0.038 to 0.339)	**0.014**
SVD DCP (3 × 3 mm inner ring) + MA turnover	1.460 (2.220 to 0.699)	**<0.001**	0.180 (0.416 to 0.055)	0.134	0.111 (0.221 to 0.000)	**0.049**	1.321 (0.176 to 2.466)	**0.024**	0.093 (−0.157 to 0.343)	0.465
SVD SCP (6 × 6 mm outer ring) + MA turnover	0.325 (0.942 to 0.291)	0.301	0.146 (0.236 to 0.056)	**0.001**	0.029 (0.078 to 0.021)	0.255	0.136 (−0.356 to 0.628)	0.589	0.115 (0.010 to 0.221)	**0.032**
SVD DCP (6 × 6 mm outer ring) + MA turnover	1.300 (2.348 to 0.253)	**0.015**	0.184 (0.389 to 0.022)	0.081	0.100 (0.212 to 0.013)	0.082	1.027 (−0.062 to 2.115)	0.065	0.093 (−0.142 to 0.329)	0.436
PD SCP (3 × 3 mm inner ring) + MA turnover	0.003 (0.009 to 0.004)	0.474	0.003 (0.005 to 0.001)	**0.017**	0.001 (0.002 to 0.001)	0.291	0.000 (−0.012 to 0.011)	0.947	0.002 (0.000 to 0.005)	0.070
PD DCP (3 × 3 mm inner ring) + MA turnover	0.024 (0.037 to 0.010)	**0.001**	0.003 (0.008 to 0.001)	0.124	0.002 (0.004 to 0.000)	0.065	0.022 (0.001 to 0.042)	**0.039**	0.002 (−0.003 to 0.006)	0.439
PD SCP (6 × 6 mm outer ring) + MA turnover	0.005 (0.015 to 0.005)	0.358	0.003 (0.005 to 0.000)	**0.026**	0.001 (0.002 to 0.001)	0.302	0.003 (−0.009 to 0.015)	0.608	0.002 (0.000 to 0.005)	0.082
PD DCP (6 × 6 mm outer ring) + MA turnover	0.030 (0.057 to 0.003)	**0.030**	0.004 (0.009 to 0.001)	0.134	0.002 (0.005 to 0.001)	0.135	0.024 (−0.004 to 0.051)	0.087	0.002 (−0.004 to 0.008)	0.516

CI, confidence interval; DCP, deep capillary plexus; ETDRS, Early Treatment Diabetic Retinopathy Study; MA, microaneurysms; n, number of participants; PD, perfusion density; SCP, superficial capillary plexus; SVD, skeletonized vessel density.

Bold values represent statistically significant differences with $P < 0.05$, using linear mixed models to evaluate longitudinal changes (12-month) combining MAT with OCTA for each ETDRS severity level and the post hoc analysis, where visit (baseline, 12 months, and 24 months), ETDRS severity levels, and OCTA microvascular variables were used as fixed variables.

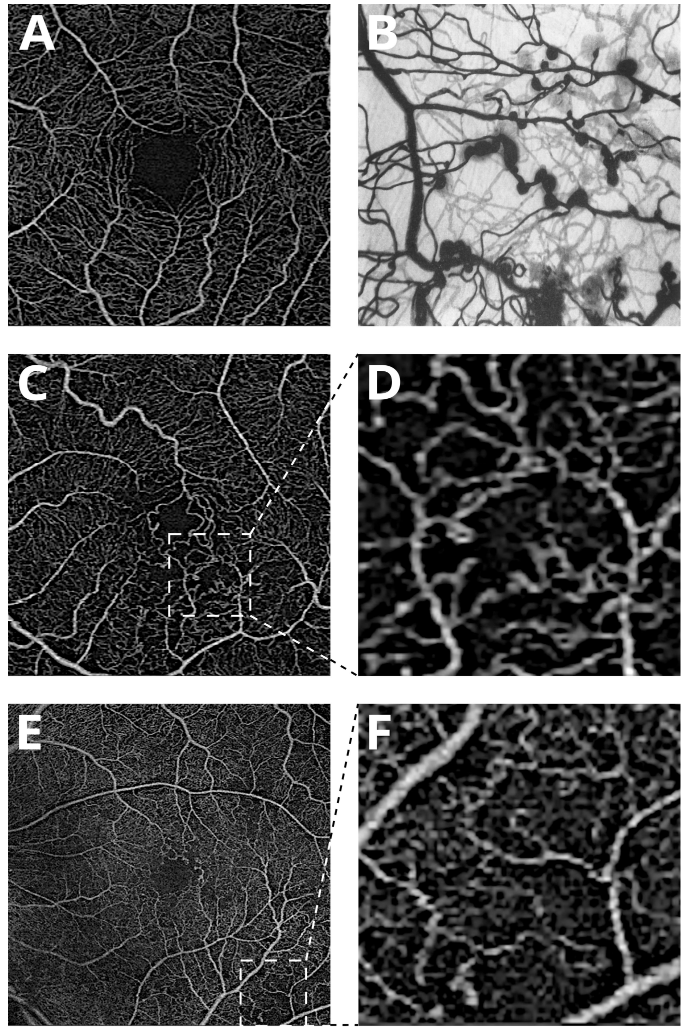

Figure. Example of retinal vascular patterns in a healthy volunteer and in diabetic retinopathy patients. (**A**) OCTA 3 × 3 mm acquisition of the left eye of an healthy volunteer. (**B**) As reference, a postmortem digested retina, injected with India ink, from a diabetic individual shows vascular patterns characterized by capillary nonperfusion and enlarged preferential vessels (shunts). (**C**) OCTA 3 × 3 mm acquisition of the left eye of a diabetic retinopathy patient (ETDRS level 35). (**D**) Highlighted area. (**E**) OCTA 6 × 6 mm acquisition of the left eye of a diabetic retinopathy patient (ETDRS level 43). (**F**) Highlighted area. (**D**) and (**F**) show similar vascular patterns of capillary nonperfusion and enlarged preferential vessels, as seen in (**B**).

mally dilated arteriovenous shunts. These shunt vessels, dilated preferential channels first described by Cogan and Kuwabara,[18] have been proposed as the main sites of formation of microaneurysms and appear to be particularly relevant to NPDR progression as a response to the increasing capillary nonperfusion and ischemia.[21–24]

The evaluation of the progression of severity in ETDRS level 35 indicated that the eyes that showed worsening presented increased vessel perfusion (in 6 × 6 mm area) which may be explained by the development of dilated preferential shunts with increased blood flow. The dilated preferential shunt vessels shown in the Figure appear to be responsible for stabilization of the capillary nonperfusion in the more advanced ETDRS levels 43 and 47 and for the increasing number of microaneurysms. Ultimately, they may be the precursors of IRMAs, which may, in turn, be preferred sites for the development of new vessels and proliferative diabetic retinopathy.[25]

Finally, our study shows the relevance of combining full information on capillary closure data obtained with spectral-domain OCTA, as well as information on microaneurysm turnover as an indicator of the development of shunt vessels. The goal of automated identification and discrimination of the different severity levels of NPDR appears to be achievable by combining OCTA metrics for both areas of the central retina (3 × 3 mm and 6 × 6 mm), both the SCP and DCP, with calculation of microaneurysm turnover.

Another perspective offered by this study is the potential value of determining rates of progression per year in NPDR: capillary closure representing ischemia and microaneurysm formation representing retinal vascular remodeling and formation of dilated preferential shunts and intraretinal vascular abnormalities. Determination of individual rates of progression in 1 year may open the door for closer monitoring of disease progression and personalized intervention on both local and systemic factors.

This study is a post hoc analysis of a clinical trial research study (NCT03696810). The inclusion of eyes with mild to moderate – severe NPDR stages (ETDRS levels 20, 35, 43, and 47) from patients with type 2 diabetes in unbalanced groups with a higher percentage of men and a lack of a comparative longitudinal follow-up of a control group are major limitations of this study. The rather restrictive inclusion criteria, focusing on a relatively well metabolically controlled population is another limitation of the study. However, this last limitation is an added value, as it restricts the influence of extreme systemic factors on the results obtained. Another advantage is the categorization of the different ETDRS levels performed by an independent reading Centre, CORC.

In conclusion, this study offers data that support a coherent interpretation of the progression of the microvascular disease component of NPDR. It indicates that OCTA metrics performed in both central 3 × 3 mm and 6 × 6 mm regions of the retina are not sufficiently discriminative of ETDRS levels, and the additional calculation of microaneurysm turnover offers necessary and complementary information that appears to accurately identify the different severity grades of NPDR in the clinical setting.

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