

Characterization of Retinal Disease Progression in a 1-Year Longitudinal Study of Eyes With Mild Nonproliferative Retinopathy in Diabetes Type 2

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PURPOSE. To identify eyes of patients with diabetes type 2 that show progression of retinal disease within a 1-year period using noninvasive techniques.

METHODS. Three hundred seventy-four type 2 diabetic patients with mild nonproliferative diabetic retinopathy (Early Treatment Diabetic Retinopathy Study [ETDRS] level 20 or 35) were included in a 12-month prospective observational study to identify retinopathy progression. Four visits were scheduled at 0, 3, 6, and 12 months. Microaneurysm (MA) activity using the RetmarkerDR and retinal thickness using spectral-domain optical coherence tomography (SD-OCT) were assessed by a central reading center at all visits and ETDRS severity level in the first and last visits.

RESULTS. Three hundred thirty-one eyes/patients completed the study. Microaneurysm formation rate greater than or equal to 2 was present in 68.1% of the eyes and MA turnover greater than or equal to 6 in 54.0% at month 6. Higher MA turnover values were registered in eyes that showed progression in ETDRS severity level ($P < 0.03$). There were also significant correlations between increased microaneurysm activity and increases in retinal thickness. Spectral-domain OCT identified clinical macular edema in 24 eyes/patients (6.7%) and subclinical macular edema in 104 eyes/patients (28.9%) at baseline. Progression of retinal thickening was registered in eyes that had either subclinical or clinical macular edema at baseline.

CONCLUSIONS. Changes in MA activity measured with RetmarkerDR and in central retinal thickness in eyes with mild nonproliferative diabetic retinopathy and diabetes type 2 are able to identify eyes at risk of progression. These eyes/patients should be selected for inclusion in future clinical trials of drugs targeted to prevent diabetic retinopathy progression to vision-threatening complications. (ClinicalTrials.gov number, NCT01145599.)

Keywords: diabetic retinopathy, biomarkers, microaneurysms, macular edema, diabetes type 2

Diabetic retinopathy (DR) is a common and serious ophthalmic condition. It is the leading cause of blindness among working age adults in the United States.¹ Disability consequent to vision loss related to eye disease among people with diabetes threatens independence and can lead to reduced quality of life.²

However, the rate of progression and risk for vision loss appears to vary widely between patients despite similar metabolic control.³

Therefore, the identification of different DR phenotypes characterized by different dominant retinal pathology and different rates of progression to vision-threatening retinopathy and vision loss including center-involved macular edema, is of major relevance for personalized management of DR and for clinical trial design and patient selection.³ The inclusion of eyes/patients in a clinical trial that do not progress during the period of the trial makes it impractical to enroll the required number of patients to detect differences in efficacy between the drug being tested and the placebo.⁴

It is therefore of great interest to identify and characterize eyes/patients that show disease activity and are at higher risk for developing vision-threatening retinopathy. The aim of this 1-year multicenter observational study was to identify progression in patients with mild nonproliferative DR (NPDR) by evaluating repeated noninvasive routine tests such as color fundus photography (CFP) and spectral-domain optical coherence tomography (SD-OCT).

METHODS

Patients

This was a prospective, multicenter, observational study, designed to follow eyes/patients with mild NPDR (20 and 35 of the Early Treatment Diabetic Retinopathy Study [ETDRS] classification) for a period of 1 year. The study was an investigator-driven observational study sponsored by the European Vision Institute Clinical Research Network (provided in the public domain by EVICR.net).^{5,6}

A total of 374 patients were recruited between September 2010 and June 2012 in 19 European clinical sites and followed over a 1-year period. Men and women with diagnosed adult-onset type 2 diabetes, age 35 to 82 years, mild NPDR (20 and 35 of ETDRS classification), best corrected visual acuity (BCVA) greater than or equal to 75 letters ($\geq 20/32$) and refraction with spherical equivalent less than ± 5 diopters (D) were included in the study. Exclusion criteria included the presence of cataract or other eye disease that may interfere with fundus examination, glaucoma, other retinal disease, any eye surgery within a period of 6 months, dilatation of the pupil less than 5 mm and previous or requiring treatment for DR at screening. All patients gave written consent. The tenets of the Declaration of Helsinki were followed and approval was obtained from each institutional review board (ClinicalTrials.gov Identifier: NCT01145599).

At baseline visit, patients' body weight, height, blood pressure, and concomitant medication were recorded. Based on the inclusion/exclusion criteria, one eye per patient was selected by the physician as the study eye. When both eyes fulfilled the same criteria, one of the eyes was selected by choosing sequentially the left or the right eye.

Patients performed four study visits: V0 (month 0), V3 (month 3), V6 (month 6), and V12 (month 12). At each visit, the study eyes underwent a complete ophthalmic examination, which included BCVA, slit-lamp examination, IOP measurements, digital fundus photography (CFP), and SD-OCT. Data on diabetes systemic medication, blood pressure measurements,

HbA1c levels, and body mass index (BMI) values were collected.

A central reading center (CORC-Coimbra Ophthalmology Reading Center, Portugal) classified the CFP for ETDRS grading and microaneurysms (MA) activity and the SD-OCT images.

During the period of the study and outside of the study visits, patients were followed in the clinical centers according to usual clinical practice. Identification of clinically significant macular edema (CSME) was based on ETDRS criteria.^{7,8}

Color Fundus Photography

Color fundus photography was performed according to the ETDRS protocol at the first and last visits. The seven-field photographs were obtained at 30°/35° for DR classification according to ETDRS grading scale.⁷ The grading of ETDRS levels was performed by double grading, with an intergrader reproducibility of 87% ($\kappa = 0.84$) for sublevels of ETDRS and 93% ($\kappa = 0.91$) for one step ETDRS level. Considering MAs and hard exudates, the more frequent alterations present in the eyes graded in this study, the intergrader reproducibility is 90% ($\kappa = 0.75$) for MA and 83% ($\kappa = 0.71$) for hard exudates. Additionally, 45°/50° field-2 color fundus images (macula) were taken at all visits for automated MA analysis, using an automated computer-aided diagnostic system (RetmarkerDR, Retmarker SA, Coimbra, Portugal) at the reading center.

RetmarkerDR is a patented computer software certified as a CE mark Class IIa medical device (European Union Certification of Conformity). The software was trained and tested prior to the CE medical device classification, that is, the algorithm (including the classifier) is objective and reproducible, it is not modified or retrained to any individual dataset.

This automated computer-aided diagnostic system consists of software earmarking MAs; it includes a coregistration algorithm that allows comparison within the same retinal location between different visits for the same eye.

RetmarkerDR calculates for each eye/patient the number of MA in each visit and the number of MAs that appear and/or disappear from one visit to the other, allowing calculation of the number of MA appearing and/or disappearing per time interval (i.e., the MA formation rate and the MA disappearance rate, respectively). The MA turnover is computed as the sum of the MA formation and MA disappearance rates.⁹⁻¹¹

Previous work from our group^{9,12} showed a good intergrader agreement for the total number of MAs earmarked and the MA turnover for three independent human graders.

The RetmarkerDR shows a similar intergrader agreement for the total number of MAs, MA formation rate, and MA turnover (when compared with a human grader, intraclass correlation coefficients were 0.857 and 0.806, respectively) while showing no intragrader variability as opposed to human graders, being, therefore, a reliable tool for MA assessment.

In this study, the MA formation rate and MA turnover were computed for all the eyes/patients at months 3, 6, and 12 (V3, V6, and V12, respectively). Patients were thereafter classified based on the presence of MA formation rate greater than or equal to 2, according to Nunes et al.¹² and on the presence of a MA turnover greater than or equal to 6³, according to Nunes et al.³ An independent verification was also achieved by revisiting the CALDIRET data.¹¹

These cut-off values have been proposed as predictive of progression of diabetic retinopathy.^{3,11,12}

Optical Coherence Tomography

Spectral-domain OCT was performed using Cirrus ($n = 194$; Carl Zeiss Meditec, Inc., Dublin, CA, USA), Spectralis ($n = 166$; Heidelberg Engineering, Heidelberg, Germany), Copernicus (n

= 4; Optopol, Zawiercie, Poland), or three-dimensional (3D) OCT-1000 ($n = 10$; Topcon Corporation, Tokyo, Japan).

Retinal thickness (RT) in the central subfield (central area with 1 mm in diameter), and the four areas of the inner and outer rings (within 1–3 mm and 3–6 mm, respectively) were collected. Only Cirrus and Spectralis SD-OCT data are reported ($n = 360$), because of availability of normative data and the number of patients examined. The data from these instruments were analyzed independently.

To identify eyes/patients with increased RT in the central subfield (clinical and subclinical macular edema) and in the inner and outer rings, the reference values established by DRCR.net were used:

For clinical macular edema (ClinicalTrials.gov Identifier: NCT01909791)¹³:

1. Retinal thickness greater than or equal to 290 μm in women and greater than or equal to 305 μm in men for Cirrus SD-OCT; and
2. Retinal thickness greater than or equal to 305 μm in women and greater than or equal to 320 μm in men for Spectralis SD-OCT.

For subclinical macular edema^{14–16}:

1. Retinal thickness greater than 260 μm and less than 290 μm in women and greater than 275 μm and less than 305 μm in men, for Cirrus SD-OCT; and
2. Retinal thickness greater than 275 μm and less than 305 μm in women and greater than 290 μm and less than 320 μm in men for Spectralis SD-OCT.

For the inner and outer rings (ClinicalTrials.gov Identifier: NCT01331005):

1. Normal RT, if there was no more than one area above normal range (normal mean + 2 SD) and no area 15 μm above normal range; and
2. Increased RT, if there was at least two areas above normal range and/or one area 15 μm above normal range.

The nine SD-OCT areas were classified as decreased, normal, or increased relatively to available Cirrus normative data. Because no normative database is available for Spectralis SD-OCT, a scaling factor was used based on analysis of 113 eyes/patients that performed both Cirrus and Spectralis SD-OCT in the study. The RT values were higher with Spectralis SD-OCT: +16.6 μm for the central subfield, +18.1 μm for the inner ring, and +17.7 μm for the outer ring. Based on these differences, RT values of Spectralis SD-OCT were scaled to Cirrus SD-OCT considering $-18 \mu\text{m}$ in inner and outer ring. For the central subfield the mean difference between Spectralis and Cirrus SD-OCT of $-15 \mu\text{m}$ reported by the DRCR.net was taken into account.

Sample Size

To characterize retinal disease progression in type 2 diabetic patients with early NPDR a sample size minimum of 356 eyes/patients was estimated based on the MA turnover results published by Nunes et al.¹² for a statistical power of 90%, an α level of 0.05 and a drop-out of 10% (i.e., considering that 60% of the patients with a MA formation rate ≥ 2 will develop CSME in a 2 years' time interval and that only 20% of the patients with mild NPDR will have a MA formation rate ≥ 2).

Data Analysis

Categorical variables are summarized with frequencies and percentages and numerical variables with mean and SD.

To test statistically significance differences at baseline between eyes/patients included and dropped out of the study a univariate analysis was performed with Bonferroni correction of the P value. The χ^2 test was used for categorical variables, and the Student's t -test or the nonparametric Mann-Whitney U tests were used for continuous variables.

To compare the average evolution between visits of MA parameters (formation rate and turnover) and RT parameters the repeated measures ANOVA was performed.

To analyze associations between continuous variables the Pearson correlation was computed.

All statistical analyses were performed with STATA version 12.1 (StataCorp LP, College Station, TX, USA), and P values less or equal than 0.05 were considered statistically significant results.

RESULTS

From the 374 patients/eyes enrolled in the study (174 left eyes and 200 right eyes), 331 completed the study, that is, completed the 1-year follow-up period or developed CSME (ETDRS criteria) needing treatment (three eyes/patients). Two patients deceased between baseline and the last visit and 41 withdrew from the study (Fig.).

Baseline characteristics of patients enrolled in the study are presented in Table 1.

No statistically significant differences were found at baseline between the eyes/patients included in the study and the 43 eyes/patients that dropped out of the study.

Three eyes/patients were diagnosed with CSME during the 1-year follow-up period, all males, age ranging from 61 to 72 years and with HbA1c levels ranging from 5.9% to 8.5%. The ETDRS level was 35C for one eye/patient and 35D for the other 2. All three eyes had a MA formation rate at month 3 greater than or equal to 2 and a MA turnover greater than or equal to 6. One of three patients had at baseline the OCT criteria for macular edema (central subfield $> 305 \mu\text{m}$; Cirrus SD-OCT).

Twenty three eyes/patients showed an increase in ETDRS level from screening visit to V12 and 24 patients showed a decrease in ETDRS level (Table 2).

Microaneurysm Parameters

Microaneurysm formation rate greater than or equal to 2 was present in 68.1% [95% CI: 63.0%–73.1%] of the eyes and MA turnover greater than or equal to 6 in 54.0% [95% CI: 48.5%–59.4%] at V6.

The MA formation rate and MA turnover at V6 showed a strong correlation with the values at V3 and V12. For the MA formation rate the correlation between V12 and V3 was $r = 0.950$ ($P < 0.001$), and between V12 and V6 $r = 0.912$ ($P < 0.001$). For the MA turnover the correlation between V12 and V3 was $r = 0.957$ ($P < 0.001$), and between V12 and V6 $r = 0.937$ ($P < 0.001$).

The results show that there is a correlation between MA turnover values obtained at 6 and 12 months and changes in ETDRS level.

For the 23 eyes/patients that showed an increase in ETDRS level from baseline to V12, 39.1% had a MA turnover greater than or equal to 6 at V6, whereas only 8.3% of the eyes/patients that showed a decrease in ETDRS level had a MA turnover greater than or equal to 6 ($P = 0.013$). At V12, 34.8% of the 23 eyes/patients that showed an increase in ETDRS level from baseline to V12, had a MA turnover greater than or equal to 6, whereas only 8.3% of the eyes/patients that showed a decrease in ETDRS level had a MA turnover greater than or equal to 6 ($P = 0.027$; Table 3).

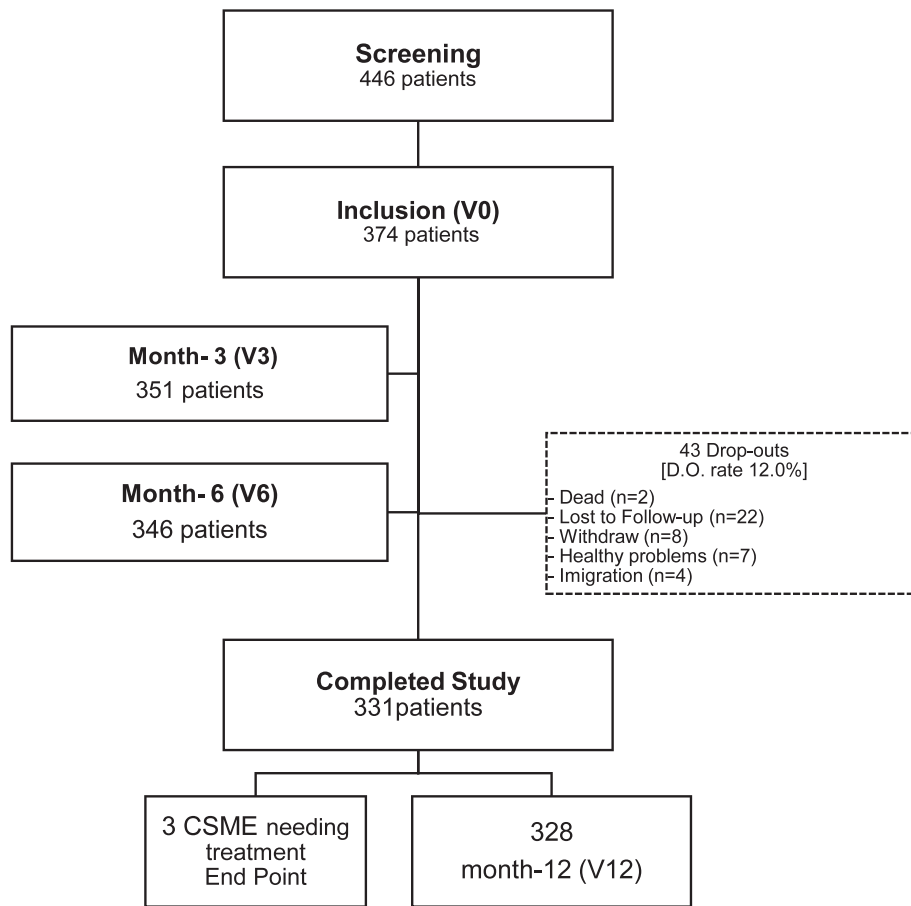


FIGURE. Consort flow chart.

The MA parameters showed very weak, positive statistically significant correlations with HbA1c levels and BMI ($r < 0.181$).

There was a very weak, positive statistically significant correlation at V6 between MA parameters (number, formation rate, and turnover) and the RT in the central subfield ($r = 0.141$, $r = 0.170$, and $r = 0.156$, respectively). For the inner and outer ring areas statistically significant weak correlations were also found for most of the respective areas and visits (for the inner ring $r < 0.193$ and for the outer ring $r < 0.328$).

Associations were found between the MA formation rate and MA turnover cut-off values (≥ 2 and ≥ 6 , respectively) and

the presence of increased RT in the central subfield, inner and/or outer ring:

1. Of the eyes/patients with an increased RT in the central subfield, inner and/or outer ring, 61.8% (95% CI: 44.6%–79.0%) to 76.7% (95% CI: 65.6%–87.7%) showed an MA formation rate greater than or equal to 2 and/or an MA turnover greater than or equal to 6; and
2. Of the eyes/patients with a MA formation rate less than 2 and/or a MA turnover less than 6, 81.3% (95% CI: 74.8%–87.8%) to 87.7% (95% CI: 82.3%–90.1%) showed a normal RT in the central subfield, inner and/or outer ring.

TABLE 1. Baseline Characteristics of the Eyes/Patients That Were Included and Dropped Out of the Study

	Patients Included in the Study, <i>n</i> = 374	Drop-Out Patients, <i>n</i> = 43	<i>P</i>
Sex, female/male, frequency (%)	131 (35.0)/243 (65.0)	19 (44.2)/24 (55.8)	0.24
ETDRS level			
20, frequency (%)	70 (18.7)	12 (27.9)	0.15
35, frequency (%)	304 (81.3)	31 (72.1)	
Age, mean (SD), y	60.3 (9.0)	58.3 (9.89)	0.17
HbA1c, mean (SD), %	7.8 (1.5)	7.8 (1.5)	0.83
Systolic blood pressure, mean (SD), mm Hg	137.6 (16.6)	135.6 (17.8)	0.46
Diastolic blood pressure, mean (SD), mm Hg	77.4 (10.0)	81.1 (8.0)	0.02*
BMI, mean (SD), kg/m ²	29.9 (5.4)	30.6 (5.4)	0.40
BCVA, mean (SD), letters	85.3 (4.6)	84.1 (5.0)	0.11
No. of MAs, mean (SD)	3.7 (5.4)	2.5 (3.5)	0.16

* Bonferroni corrected *P* value 0.006.

TABLE 2. Number of Eyes/Patients With ETDRS Changes From V0 to V12

ETDRS Level at V0	ETDRS Level at V12						Total
	10	15	20	35	43	47	
20	1	1	0	19	0	0	21
35	2	0	20	0	3	1	26
Total	3	1	20	19	3	1	47

Finally, MA parameters obtained at V6 were the most informative, indicating that the time interval of 6 months is necessary to evaluate MA activity.

Retinal Thickness

Considering results obtained at baseline with the Cirrus and the Spectralis and based on their respective normal database, an increased RT in the central subfield was found in 9.8% (95% CI: 5.6%-13.9%) when using the Cirrus and 10.8% (95% CI: 6.1%-15.6%) using Spectralis for a total of 10.3% of the eyes examined, while a decreased RT was found in 1.5% (95% CI: 0%-3.3%) using the Cirrus and 9.6% (95% CI: 5.1%-14.1%) using Spectralis for a total of 5.3% of the eyes (Table 4). In the inner ring areas, this proportion was maintained. The highest number of eyes showing increased RT was found in the outer ring areas (13.3%-23.1%).

In the central subfield 24 eyes/patients (6.7%, corresponding to 6.2% [95% CI: 2.8%-9.6%] with the Cirrus and 7.2% [95% CI: 3.3%-11.2%] with the Spectralis) had a OCT diagnosis of clinical macular edema, according to the DRCR.net standards (Table 4).¹³ Subclinical macular edema was identified in 104 eyes/patients (28.9%, corresponding to 32.0% [95% CI: 25.4%-38.5%] with the Cirrus and 25.3% [95% CI: 18.7%-31.9%] with the Spectralis; Table 4).

Eyes/patients classified as subclinical and clinical macular at baseline occurred in eyes/patients with ETDRS level 20 and 35, respectively 8.3% and 25.9%.

No correlation was found between the eyes/patients that showed an increase in ETDRS level at the end of 1 year of follow-up and the eyes/patients that presented higher retinal thickness values in the central subfield (i.e., subclinical and clinical macular edema; $P = 0.61$).

The eyes that showed increases in RT were in general the same eyes during the entire follow-up period. Eighty-two percent (82.9%) of the eyes with increased RT in the central subfield at baseline had increased RT at V12. Increase in RT occurred also in the inner ring (73.2%) and in the outer ring (72.8%).

Increase in central subfield RT over the 1-year follow-up was registered in eyes that had subclinical and clinical macular edema at baseline. In the eyes with clinical macular edema at baseline the mean RT increase was 4.8 μm (95% CI: -3.3 μm to 12.9 μm) for Cirrus SD-OCT and 7.63 μm (95% CI: -25.5 μm to 40.8 μm) for Spectralis SD-OCT.

DISCUSSION

This 1-year, observational, longitudinal study of 374 patients with diabetes type 2, mild NPDR (ETDRS levels 20 and 35) and good visual acuity, examined at baseline and at 3, 6, and 12 months, by 19 clinical sites across Europe, showed that changes in RT and MA activity vary widely between patients with similar metabolic control, duration of disease and ETDRS retinopathy severity level, confirming previous reports.^{3,12}

TABLE 3. Microaneurysm Formation Rate and MA Turnover at V3, V6, and V12 Correlated With Changes in ETDRS Level

	V3		V6		V12	
	MA Formation Rate	MA Turnover	MA Formation Rate	MA Turnover	MA Formation Rate	MA Turnover
ETDRS Level	<2	≥ 6	<2	≥ 6	<2	≥ 6
Decrease, %	17 (70.8)	3 (12.5)	14 (58.3)	2 (8.3)	17 (70.8)	2 (8.3)
Increase, %	12 (52.2)	8 (34.8)	10 (43.5)	9 (39.1)	11 (47.8)	8 (34.8)
	$P = 0.071$		$P = 0.308$		$P = 0.108$	
	$P = 0.188$		$P = 0.013^*$		$P = 0.027^*$	

* P value is statistically significant.

TABLE 4. Frequency (Number and Percentage) of the Eyes/Patients With Decreased/Normal/Increased RT at Baseline

	Cirrus, n = 194	Spectralis, n = 166
Central subfield		
Decreased (mean - 2 SD)	3 (1.5)	16 (9.6)
Normal	172 (88.7)	132 (79.5)
Increased (mean + 2 SD)	19 (9.8)	18 (10.8)
Inner ring		
Nasal		
Decreased (mean - 2 SD)	7 (3.6)	28 (16.9)
Normal	168 (86.6)	124 (74.7)
Increased (mean + 2 SD)	19 (9.8)	14 (8.4)
→Increased (mean + 2 SD + 15 μm)	→5 (2.6)	→2 (1.2)
Superior		
Decreased (mean - 2 SD)	5 (2.6)	26 (15.7)
Normal	168 (86.6)	123 (74.1)
Increased (mean + 2 SD)	21 (10.8)	17 (10.2)
→Increased (mean + 2 SD + 15 μm)	→4 (2.1)	→1 (0.6)
Temporal		
Decreased (mean - 2 SD)	7 (3.6)	26 (15.7)
Normal	167 (86.1)	118 (71.1)
Increased (mean + 2 SD)	20 (10.3)	22 (13.3)
→Increased (mean + 2 SD + 15 μm)	→5 (2.6)	→4 (2.4)
Inferior		
Decreased (mean - 2 SD)	10 (5.2)	28 (16.9)
Normal	163 (84)	126 (75.9)
Increased (mean + 2 SD)	21 (10.8)	12 (7.2)
→Increased (mean + 2 SD + 15 μm)	→5 (2.6)	→2 (1.2)
Outer ring		
Nasal		
Decreased (mean - 2 SD)	6 (3.1)	13 (7.8)
Normal	158 (81.4)	135 (81.3)
Increased (mean + 2 SD)	30 (15.5)	18 (10.8)
→Increased (mean + 2 SD + 15 μm)	→10 (5.2)	→0 (0.0)
Superior		
Decreased (mean - 2 SD)	9 (4.6)	9 (5.5)
Normal	158 (81.4)	120 (72.7)
Increased (mean + 2 SD)	27 (13.9)	36 (21.8)
→Increased (mean + 2 SD + 15 μm)	→7 (3.6)	→0 (0.0)
Temporal		
Decreased (mean - 2 SD)	4 (2.1)	7 (4.2)
Normal	160 (82.5)	106 (63.9)
Increased (mean + 2 SD)	30 (15.5)	53 (31.9)
→Increased (mean + 2 SD + 15 μm)	→10 (5.2)	→2 (1.2)
Inferior		
Decreased (mean - 2 SD)	8 (4.1)	9 (5.4)
Normal	162 (83.5)	118 (71.1)
Increased (mean + 2 SD)	24 (12.4)	39 (23.5)
→Increased (mean + 2 SD + 15 μm)	→6 (3.1)	→0 (0.0)

TABLE 4. Continued

	Cirrus, n = 194	Spectralis, n = 166
Central subfield		
Subclinical macular edema	62 (32.0)	42 (25.3)
Clinical macular edema	12 (6.2)	12 (7.2)
Inner ring increased	23 (11.9)	19 (11.5)
Outer ring increased	36 (18.6)	40 (24.1)
1-year change (eyes/patients that completed the study)		
Subclinical to clinical macular edema	5 of 52	7 of 38
Clinical to subclinical macular edema	2 of 10	4 of 11

Higher MA turnover values at V6 were found to be associated with increase in ETDRS level at the end of the 1 year of follow-up, which supports previous reports that MA turnover can identify eyes likely to progress.^{3,12}

Regarding RT changes, it is of relevance that of the 360 eyes/patients (considering only those examined with Cirrus or Spectralis SD-OCT) included in the study, center-involving macular edema with indication for treatment based on OCT values,¹³ was found to be present in 24 eyes (6.7%). These eyes were not diagnosed on slit-lamp examination. There were also 104 eyes (28.9%) that met the OCT criteria for subclinical macular edema.¹⁴⁻¹⁶

Microaneurysms parameters (number, formation rate, and turnover) correlated with RT increases, with the strongest correlations found with MA formation rate and MA turnover. Automated image analysis of MA turnover is shown, in this study, to identify retinal vascular disease activity at this retinopathy stage, predicting ETDRS level changes and showing also, correlations with increased RT (i.e., macular edema).

It is also of note that when considering all the patients included in the study, there was little progression in RT values over the 1-year follow-up period. Progression was only found in the eyes that already had thickness increases at baseline and mainly in the central subfield. It is also noteworthy that the changes identified in RT at baseline remained generally in the same location during the entire period of follow-up. These findings show that 1 year is a relatively short time interval for a clinical trial testing therapies against increases in RT (i.e., retinal edema) unless only patients with increased RT in the central subfield are selected for the trial. However, our observations showed that RT measurements performed with SD-OCT are reliable and may be followed accurately, and that eyes with subclinical macular edema at baseline are good candidates for clinical trials.

It is of interest that no significant correlation was found between the presence of increased RT in the central subfield and ETDRS levels 20 or 35.

There have been a number of reports in the literature, suggesting that a decrease in RT attributed to a neurodegenerative process is a frequent finding in the early stages of diabetic retinal disease.^{17,18} This observation is not confirmed in our study. The number of eyes with abnormally increased RT almost doubled the number of eyes with decreased RT.

This multicenter, multinational study demonstrated that noninvasive procedures such as fundus photography and SD-OCT are useful tools to monitor microvascular disease activity and development of edema in the early stages of diabetes (mild NPDR, ETDRS levels 20 and 35). The findings here described

contribute to improve our understanding of DR progression and to improve the design of clinical trials aiming to test the effect of drugs to prevent DR progression to vision-threatening complications.

In conclusion, changes in MA activity measured with RetmarkerDR on fundus photographs and in central RT measured by SD-OCT show wide variations between different patients with mild NPDR and diabetes type 2. It is possible, using these noninvasive procedures, SD-OCT and fundus photography, to identify the eyes that are at higher risk of progression. These eyes/patients should be the ones selected for inclusion in future clinical trials of drugs targeted to prevent DR progression.

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APPENDIX

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See EVICR.net Study Group (in the public domain) at http://www.evicr.net/downloads/file270_gb.pdf.