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One-Year Progression of Diabetic Subclinical Macular Edema in Eyes with Mild Nonproliferative Diabetic Retinopathy: Location of the Increase in Retinal Thickness

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Key Words
Diabetic retinopathy · Diabetes · Optical coherence tomography · Macular edema

Abstract
Purpose: To characterize the 1-year progression of retinal thickness (RT) increase occurring in eyes with subclinical macular edema in type 2 diabetes. ***Methods:*** Forty-eight type 2 diabetic eyes/patients with mild nonproliferative diabetic retinopathy (NPDR; levels 20 and 35 in the Early Treatment Diabetic Retinopathy Study) classified as presenting subclinical macular edema at baseline completed the 1-year follow-up period, from a sample of 194 followed in a 12-month observational and prospective study (ClinicalTrials.gov identifier: NCT01145599). Automated segmentation of the retinal layers in these eyes was performed, followed by verification and correction by a human grader. ***Results:*** The

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highest increase in RT over the 1-year follow-up period for the 48 eyes/patients with subclinical macular edema was found in the inner nuclear layer (INL). Progression to clinical macular edema was also associated with increased thickening of other retinal layers aside from the INL. The microvascular disease activity shown by microaneurysm (MA) turnover ≥6 was associated with progression from subclinical to clinical macular edema. ***Conclusions:*** Increases in RT occurring over a period of 1 year in diabetic eyes with mild NPDR and subclinical macular edema occur mainly in the INL. The development of clinical macular edema appears to be associated with increased thickening of other retinal layers and microvascular disease activity.

Introduction

Diabetic retinopathy has been identified as the leading cause of blindness in working-age adults [1]. The increase in the prevalence of diabetes around the world has resulted in an increase in the number of cases of diabetic retinopathy.

Diabetic retinal disease may lead to 2 sight-threatening complications: diabetic macular edema (DME) and proliferative diabetic retinopathy. It is, therefore, of major importance to identify the earliest stages of diabetic retinal disease and signs of progression, as well as the risk of development of these sight-threatening complications.

It has been shown that the presence of subclinical macular edema is a candidate biomarker for the development of DME [2]. Here we report the results of a 1-year follow-up study in a cohort of eyes with subclinical macular edema from a multicenter observational study which included only eyes with mild nonproliferative diabetic retinopathy (NPDR), using an automated segmentation algorithm for spectral-domain optical coherence tomography (SD-OCT).

Methods

Patients

This analysis was performed in the context of a prospective, multicenter, observational study designed to follow eyes/patients with mild NPDR [classified as 20 and 35 in the Early Treatment Diabetic Retinopathy Study (ETDRS)]. The results of that study and the procedures are described in the paper by Ribeiro et al. [3].

A total of 374 patients were recruited between September 2010 and June 2012 in 19 European clinical sites. Men and women with diagnosed adult-onset type 2 diabetes, aged 35–82 years, with mild NPDR (level 20 and 35 in the ETDRS classification), a best corrected visual acuity ≥75 letters (≥20/32), and refraction with a spherical equivalent of less than ±5 diopters were included in this study. 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).

Optical Coherence Tomography

Only the 194 patients who were examined with Cirrus SD-OCT are reported here because of the availability of a normative database and validated application of the automated segmentation algorithm [4].

To identify eyes/patients with increased RT in the central subfield (subclinical macular edema and clinical macular edema), the following reference values established by DRCR.net were used: for subclinical macular edema [2, 5, 6], RT >260 and <290 μm in women and >275 and <305 μm in men (for Cirrus SD-OCT), and for clinical macular edema (ClinicalTrials.gov identifier: NCT01909791) [7], RT ≥290 μm in women and ≥305 μm in men (for Cirrus SD-OCT).

Automated segmentation of the retinal layers in eyes with subclinical macular edema was performed [4], followed by verification and correction by a human grader.

Retinal thickness (RT) for the central subfield was calculated for each layer, allowing identification of changes occurring in the different retinal layers from baseline to the 12-month visit.

Color Fundus Photography

Color fundus photographs were taken according to the ETDRS protocol at the first and last visits. The 7-field photographs were obtained at 30/35° for diabetic retinopathy classification according to the ETDRS grading scale [8]. Additionally, 45/50° 2-field color fundus images (macula) were taken at all visits for automated MA analysis using an automated computer-aided diagnostic system (RetmarkerDR; RetmarkerDR SA, Coimbra, Portugal) at the reading center.

RetmarkerDR is a patented computer software certified as a CE mark class IIa medical device. The software was trained and tested prior to the CE medical device classification, and thus the algorithm (including the classifier) was objective and reproducible and was not modified or retrained to any individual data set.

The automated computer-aided diagnostic system consists of software earmarking microaneurysms (MA); 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 at each visit and the number of MA 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 good intergrader agreement for the total number of MA earmarked and MA turnover for 3 independent human graders. RetmarkerDR shows good intergrader agreement for the number of MA, the 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.

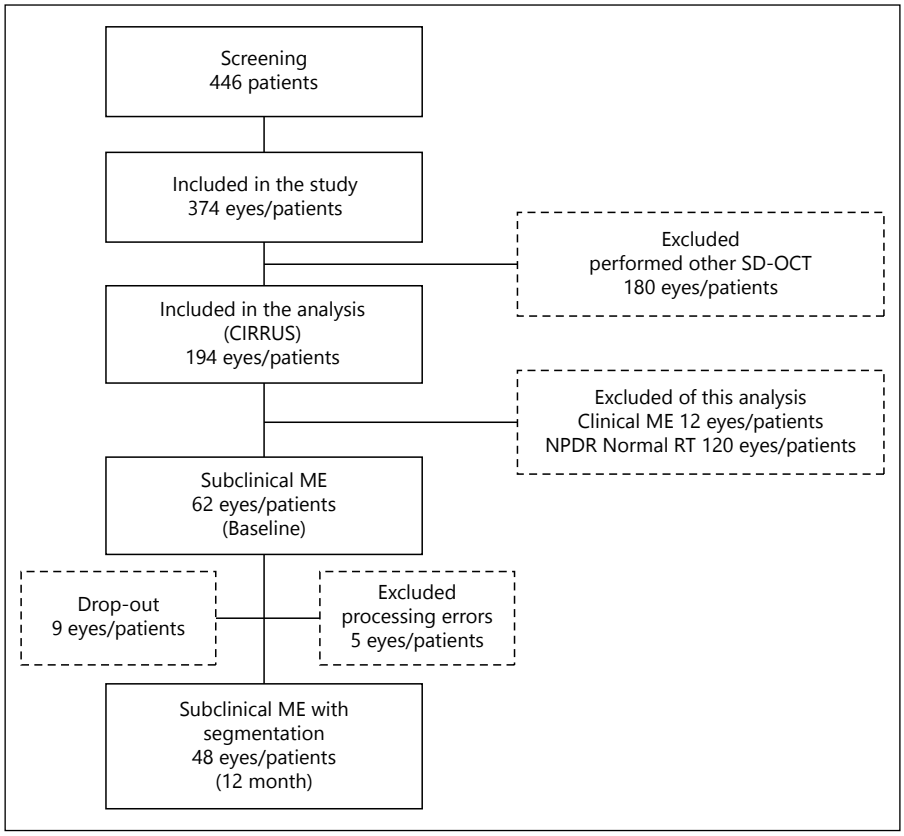

Fig. 1. Cohort flow chart.

In this study, the MA formation rate and MA turnover were computed for all of the eyes/patients at months 3, 6, and 12 (V3, V6, and V12, respectively). Patients were thereafter classified based on the presence of an MA formation rate ≥2 according to Nunes et al. [12] and Haritoglou et al. [11] and based on the presence of an MA turnover ≥6 according to Nunes et al. [13]. These cut-off values have been proposed as predictive of the progression of diabetic retinopathy [11–13].

Data Analysis

Categorical variables are reported as numbers (%), and numerical variables are reported as means ± SD. The paired Student t test or the nonparametric Wilcoxon test was used to compare the average evolution of retinal layers thickness between visits. The nonparametric Mann-Whitney test was used to assess differences in retinal layers thickness between groups. The χ^2 test was used for categorical variables. To analyze associations between ordinal and continuous variables, Spearman's correlation and the respective statistical significance were computed. A receiver operating characteristic analysis was conducted for the retinal layers to identify threshold values for progression. Two outliers were detected in the MA formation rate and MA turnover that were not considered in the analysis of these parameters.

All statistical analyses were performed using STATA version 12.1 (StataCorp LP, College Station, Tex., USA), and $p \leq 0.05$ was considered statistically significant.

Results

Of the 194 eyes/patients (88 left eyes and 106 right eyes) examined with Cirrus SD-OCT at the baseline visit, 62 were classified as having subclinical macular edema. Semiautomated segmentation of the retinal layers on OCT was performed in this subclinical macular edema group. Due to processing errors of the segmentation tool, 5 eyes/patients classified as having subclinical macular edema were not included in the final analysis. Nine patients did not complete the 1-year follow-up. Therefore, a total of 48 eyes/patients (24.74%; 95% CI 18.67–30.81) with an OCT diagnosis of subclinical macular edema, according to DRCR.net standards [7], completed the 1-year follow-up and were included in this analysis (fig. 1). Of these 48 eyes/patients, 5 (10.42%) were classified as having clinical macular edema at the final visit. The baseline characteristics of these patients are presented in table 1.

Identification of Individual Retinal Layer Thickness Changes

The highest increase, in the 12-month period, in the 48 diabetic eyes with subclinical macular edema was found

Table 1. Baseline characteristics of the patients with subclinical macular edema that progressed and did not progress to clinical macular edema at the final visit

	Progression to clinical macular edema (n = 5)	No progression to clinical macular edema (n = 43)
Gender, n (%)		
Female	2 (40.00)	16 (37.21)
Male	3 (60.00)	27 (62.79)
ETDRS level, n (%)		
20	1 (20.00)	14 (32.56)
35	4 (80.00)	29 (67.44)
Age, years	61.60±10.11	60.44±8.43
HbA1c, %	7.52±1.67	7.92±1.65
Systolic blood pressure, mm Hg	137.60±23.37	135.30±17.67
Diastolic blood pressure, mm Hg	70.40±10.14	77.16±9.77
BMI	24.19±2.91	29.00±3.81
Letters on best corrected visual acuity, n	84.20±6.05	86.21±3.08
MA, n	4.80±3.90	3.30±5.66

Values are presented as means ± SD unless otherwise stated.

in the inner nuclear layer (INL). However, only 58.33% of the eyes/patients with subclinical macular edema at baseline showed an increase in the central subfield RT at the final visit (table 2).

For the 28 eyes/patients with subclinical macular edema at baseline that showed an increase in the central subfield RT at the final visit, the only statistically significant difference in central subfield RT between baseline and the 12-month visit was found in the INL (p = 0.048).

For the 5 eyes/patients with subclinical macular edema at baseline that progressed to clinical macular edema at the final visit, the mean RT at baseline was 287.20 μm (95% CI 275.51–298.89), which is slightly higher than that of the other 43 eyes/patients (i.e. 281.35 μm, 95% CI 278.24–284.45; p = 0.237). An association with progression to clinical macular edema in these 5 eyes/patients was only found with the increased RT registered in the outer nuclear layer (ONL) (p = 0.032; table 3).

A receiver operating characteristic analysis was conducted for the 7 layers to identify threshold values for progression. The only layer with a good discriminating value was the ONL (area under the curve = 0.83); all of the other layers showed an area under the curve <0.57. The best predictive value in the ONL was 126.70 μm, with 100% sensitivity and 71.4% specificity. This value is near the mean (+2 SD, i.e. 129.33 μm) of the ONL thickness in the healthy control group (mean ± SD = 112.63 ± 8.35).

The retinal layer thickness measured on Cirrus SD-OCT in eyes/patients with subclinical macular edema showed significant variations by gender (p = 0.003 for the INL and p = 0.001 for the inner segment + outer segment), and the increases were higher for females (37.50%).

MA Parameters

A higher MA formation rate and higher MA turnover values were registered in the eyes that showed increases in RT during the 1-year period. For the 28 eyes/patients with subclinical macular edema at baseline that showed an increase in central subfield RT at the final visit, the MA formation rate and MA turnover at the 3-, 6-, and 12-month visits were higher, though these differences were not statistically significant.

Using the cut-off value for MA turnover ≥6, a statistically significant difference was found for eyes/patients with subclinical macular edema at baseline that progressed to clinical macular edema at the final visit (p = 0.021). An MA turnover ≥6 was present in 80.0% of eyes/patients that showed progression and only in 28.2% of eyes/patients that did not progress to clinical macular edema at the final visit.

Discussion

This study is a subanalysis of a 1-year, observational, longitudinal study of 374 eyes/patients with type 2 diabetes, mild NPDR (ETDRS levels 20 and 35), and good visual acuity examined at baseline and at 3, 6, and 12 months in 19 clinical sites across Europe. This subanalysis focuses on characterization of the progression of diabetic subclinical macular edema during the period of 1 year.

Diabetic subclinical macular edema has been shown to be a candidate biomarker for the risk of progression to clinical DME and the need for treatment [2]. In this study, an increase in RT over the 1-year period in eyes/patients with subclinical macular edema at baseline occurred in only 58.3% of eyes/patients, indicating that 1 year is a short interval to predict progression to clinical macular edema. In eyes/patients that showed an increase in overall RT during the 1-year period, this increase in RT occurred predominantly in the INL. However, for eyes/patients that developed clinical macular edema at the end of the 1-year follow-up period, the greatest differences occurred

Table 2. RT values in the central subfield obtained from the different segmented retinal layers of eyes/patients with subclinical macular edema (n = 28) showing an RT increase during the 12-month period

Layers	Baseline		V12		Difference between baseline and V12	
	RT, μm	increase over normative data[a], %	RT, μm	increase over normative data[a], %	RT, μm	%
RNFL	5.79±2.82	–17.43	6.00±3.49	–14.44	0.26±1.51	4.48
GCL + IPL	44.63±8.19	2.81	44.84±8.25	3.30	–0.38±3.44	–0.85
INL	23.61±3.42	35.78	24.92±2.89	43.33	1.07±2.38	4.53
OPL	28.70±4.55	30.79	28.92±5.53	31.82	–0.04±3.54	–0.15
ONL	120.01±10.89	6.55	121.05±10.84	7.48	1.52±4.78	1.27
IS + OS	44.47±4.15	–1.93	44.27±3.58	–2.37	–0.20±2.68	–0.45
RPE	24.80±2.22	–1.50	24.96±2.35	–0.88	0.16±3.14	0.63
Total RT	282.14±10.78	3.39	289.21±13.23	5.98	7.07±9.51	2.51

Values are presented as mean ± SD unless otherwise stated. RNFL = Retinal nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; OPL = outer plexiform layer; IS = inner segment; OS = outer segment; RPE = retinal pigment epithelium. [a] The healthy control group included 58 healthy eyes which were age matched with the eyes/patients from this study.

Table 3. RT values in the central subfield obtained from the different segmented retinal layers of eyes/patients that progressed and did not progress to clinical macular edema in the 12-month period

	No change to clinical macular edema (n = 43)		Change to clinical macular edema (n = 5)	
	RT, μm	increase over normative data, %	RT, μm	increase over normative data, %
Baseline				
RNFL	5.91±3.44	–15.74	6.36±1.57	–9.22
GCL + IPL	44.58±7.45	2.71	41.86±10.72	–3.58
INL	23.02±3.59	32.37	21.58±5.14	24.07
OPL	27.77±4.77	26.56	27.07±2.91	23.37
ONL	119.70±10.41	6.28	130.00±3.84	15.42
IS + OS	45.04±4.44	–0.67	45.98±1.82	1.42
RPE	25.12±2.12	–0.23	23.73±1.23	–5.76
Total RT	281.35±10.09	3.10	287.20±13.83	5.24
V12				
RNFL	5.61±3.31	–20.02	6.96±4.64	–0.70
GCL + IPL	44.32±8.04	2.11	46.52±6.48	7.16
INL	23.44±3.67	34.80	24.11±1.92	38.62
OPL	27.18±5.13	23.87	30.80±5.13	40.39
ONL	120.40±10.20	6.90	126.30±1.40	12.14
IS + OS	44.96±4.34	–0.84	45.93±3.15	1.29
RPE	24.75±2.30	–1.70	24.89±3.44	–1.14
Total RT	281.37±11.31	3.10	308.40±8.99	13.01

Values are presented as mean ± SD unless otherwise stated. RNFL = Retinal nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; OPL = outer plexiform layer; IS = inner segment; OS = outer segment; RPE = retinal pigment epithelium.

in the ONL, suggesting that the involvement of other retinal layers is a step associated with conversion from subclinical to clinical macular edema.

These observations show that in the early stages of development of DME the increase in RT occurs predominantly in the INL, probably due to colocalization of the deep retinal vascular net and extracellular accumulation of fluid due to alteration of the blood-retinal barrier in these retinal capillaries. A further increase in RT, resulting in clinical macular edema, on the other hand, appears to be also associated with an increase in RT in other retinal layers. The development of clinical macular edema in diabetes may, therefore, be associated with structural damage of the remaining retinal layers, allowing increased accumulation of fluid in the retinal layers located next to the INL, i.e. the inner plexiform layer, the outer plexiform layer, and the ONL.

Colocalization of the INL with the deep vascular unit suggests a vascular origin for edema fluid accumulation. This interpretation is well supported by the increased microvascular disease activity identified in eyes that showed an increase in RT over the 1-year period of follow-up. The MA parameters studied, i.e. the MA formation rate and MA turnover, showed positive correlations with increases in RT. Higher MA formation rates and MA turnover values were registered in eyes that showed higher increases in RT during the 1-year period.

In conclusion, eyes with subclinical macular edema in type 2 diabetic patients with mild NPDR showed little progression during a 1-year follow-up period, and higher RT values in the INL alone are not predictive of progression or development of clinical macular edema. This occurrence appears to be mainly associated with extension of the edema to others retinal layers, particularly to the ONL. Involvement of the outer retinal layers such as the ONL appears to indicate a spread of extracellular fluid to the outer retina and a more rapid conversion from subclinical to clinical macular edema.

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Disclosure Statement

None of the authors has any conflict of interests.

References

1 Fong DS, Aiello LP, Ferris FL, Klein R: Diabetic retinopathy. Diabetes Care 2004;27:2540–2553.

2 Pires I, Santos AR, Nunes S, Lobo C, Cunha-Vaz J: Subclinical macular edema as a predictor of progression to clinically significant macular edema in type 2 diabetes. Ophthalmologica 2013;230:201–206.

3 Ribeiro L, Bandello F, Tejerina AN, Vujosevic S, Varano M, Egan C, et al: Characterization of retinal disease progression in a one-year longitudinal study of eyes with mild non-proliferative retinopathy in diabetes type 2. Invest Ophthalmol Vis Sci, in press.

4 Bandello F, Tejerina AN, Vujosevik S, Varano M, Egan C, Sivaprasad S, Menon G, Massin P, Verbraak FD, Lund-Andersen H, Martinez JP, Jürgens I, Smets E, Coriat C, Wiedemann P, Ágoas V, Querques G, Holz FG, Nunes S, Alves D, Neves C, Santos T, Ribeiro L, Cunha-Vaz J: Retinal layer location of increased retinal thickness in eyes with subclinical and clinical macular edema in diabetes type 2. Ophthalmic Res 2015;54:112–117.

5 Bressler NM, Miller KM, Beck RW, Bressler SB, Glassman AR, Kitchens JW, et al: Observational study of subclinical diabetic macular edema. Eye (Lond) 2012;26:833–40.

6 Browning DJ, Fraser CM: The predictive value of patient and eye characteristics on the course of subclinical diabetic macular edema. Am J Ophthalmol 2008;145:149–154.

7 Chalam K V, Bressler SB, Edwards AR, Berger BB, Bressler NM, Glassman AR, et al: Retinal thickness in people with diabetes and minimal or no diabetic retinopathy: Heidelberg Spectralis optical coherence tomography. Invest Ophthalmol Vis Sci 2012;53:8154–8161.

8 Grading diabetic retinopathy from stereoscopic color fundus photographs: an extension of the modified Airlie House classification – ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991;98:786–806.

9 Bernardes R, Nunes S, Pereira I, Torrent T, Rosa A, Coelho D, et al: Computer-assisted microaneurysm turnover in the early stages of diabetic retinopathy. Ophthalmologica 2009;223:284–291.

10 Ribeiro ML, Nunes S, Cunha-Vaz J: Microaneurysm turnover at the macula predicts risk of development of clinically significant macular edema in persons with mild nonproliferative diabetic retinopathy. Diabetes Care 2013;36:1254–1259.

11 Haritoglou C, Kernt M, Neubauer A, Gerss J, Oliveira CM, Kampik A, et al: Microaneurysm formation rate as a predictive marker for progression to clinically significant macular edema in nonproliferative diabetic retinopathy. Retina 2014;34:157–164.

12 Nunes S, Pires I, Rosa A, Duarte L, Bernardes R, Cunha-Vaz J: Microaneurysm turnover is a biomarker for diabetic retinopathy progression to clinically significant macular edema: findings for type 2 diabetics with nonproliferative retinopathy. Ophthalmologica 2009;223:292–297.

13 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;54:4595–4604.