Single Retinal Layer Changes After Subthreshold Micropulse Yellow Laser in Diabetic Macular Edema

Stela Vujosevic, MD, PhD; Luisa Frizziero, MD; Ferdinando Martini, MD; Silvia Bini, MD; Enrica Convento, MSc; Fabiano Cavarzeran, ScD; Edoardo Midena, MD, PhD

ABSTRACT: A pilot prospective, interventional study has been conducted on 10 patients with diabetic macular edema (DME) treated with subthreshold micropulse laser (SMPL) to evaluate changes of individual retinal layers and to correlate with functional changes. All patients underwent complete ophthalmologic evaluation including spectral-domain optical coherence tomography (OCT) and microperimetry at baseline, 3 months, 6 months, 9 months, and 12 months. Compared with baseline, a significant decrease was found in inner nuclear layer (INL) and outer retinal layer (ORL) thickness in the central 1 mm ($P < .05$). Increase in best-corrected visual acuity was significantly and inversely correlated to central retinal thickness (CRT) ($P = .0027$), INL ($P = .0167$), and outer nuclear layer (ONL) thickness ($P = .0107$). Increase in retinal sensitivity was significantly and inversely correlated to CRT and ONL thickness ($P < .01$). Therefore, SMPL showed to improve firstly functional parameters and then morphologic parameters. Functional parameters were inversely correlated to CRT, INL, and ONL thickness. The exact mechanism of reduction of INL thickness induced by SMPL remains to be further evaluated.

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INTRODUCTION

Diabetic macular edema (DME) represents the first cause of legal blindness among diabetic patients.[1,2] The pathophysiology of DME is considered multifactorial. Breakdown of the inner and outer blood retinal barrier, alteration of the neurovascular unit in the retina, and chronic inflammation all play an important role in DME development.[3] Recent data from a large, multicenter clinical trial show that inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL) are sites of increased retinal thickness in patients with DME in type 2 diabetes mellitus.[4] Retinal pigment epithelium (RPE) is a constituent of the outer retinal barrier, and an early impairment in all RPE functions has been previously reported in patients with diabetes.[5-8]

Subthreshold micropulse laser (SMPL) has been recently proposed in DME. The mechanism of action of SMPL is to selectively stimulate RPE cells, avoiding any clinically visible damage to the inner or outer retina.[9-16] A recent meta-analysis of randomized controlled trials on the use of SMPL has confirmed that SMPL is as effective as conventional laser in the absorption of edema, but SMPL has superior visual acuity (VA) outcomes.[17] Moreover, SMPL preserves or increases retinal sensitivity as determined with microperimetry, whereas conventional laser reduces retinal sensitivity.[13]

From University Hospital Maggiore della Carità, Novara, Italy (SV); IRCCS-Fondazione Bietti, Rome (LF, EM); and the Department of Ophthalmology, University of Padova, Padova, Italy (FM, SB, EC, FC, EM).

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Address correspondence to Edoardo Midena MD, PhD, Department of Ophthalmology, University of Padova, Via Giustiniani 2, Padova 35128 Italy; email: edoardo.midena@unipd.it.

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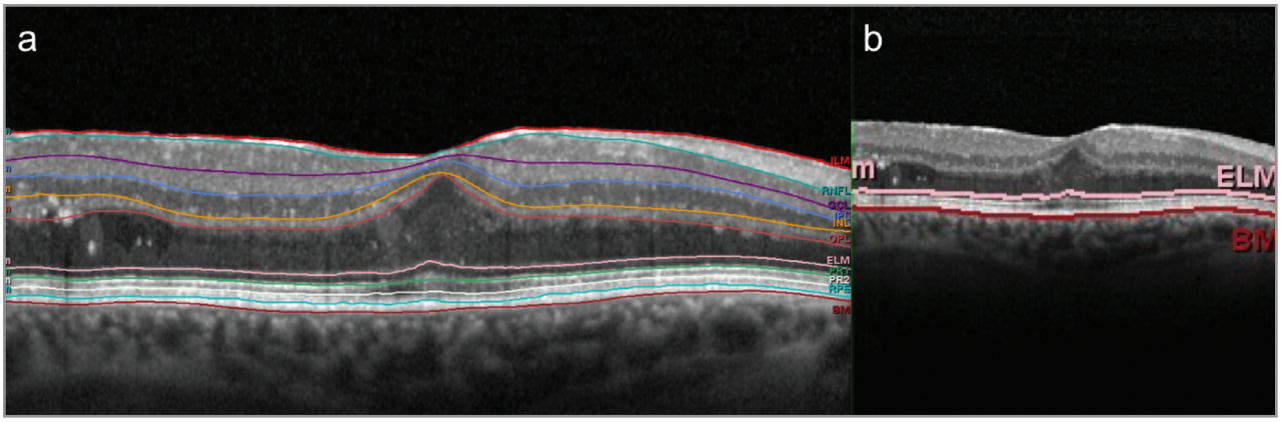

Figure 1. (A) OCT scan showing the automatic segmentation provided by the device's software: nerve fiber layer (ILM-RNFL), ganglion cell layer (RNFL-GCL), inner plexiform layer (GCL-IPL); inner nuclear layer (IPL-INL); outer plexiform layer (INL-OPL); Henle's fiber layer plus outer nuclear layer (OPL-ELM); outer retinal layers (ELM-BM, including external limiting membrane plus myoid zone of the photoreceptors plus ellipsoid zone of the photoreceptors plus outer segments of the photoreceptors plus cone interdigitation with RPE and RPE / Bruch's membrane complex). (B) The same OCT scan considering only the outer retinal layers as automatically provided by the device. OCT = optical coherence tomography; ILM = internal limiting membrane; RNFL = retinal nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ELM = external limiting membrane; PR1/PR2 = photoreceptors layers; RPE = retinal pigment epithelium; BM = Bruch's membrane.

The aim of this study was to evaluate modification of specific retinal layers in patients with DME treated with SMPL and to correlate these modifications with functional changes determined with microperimetry and VA during a 1-year follow-up.

PATIENTS AND METHODS

Patients

This was a pilot prospective, interventional study (EudraCT registration number: 2014-003660-20). All patients were enrolled and followed at the Diabetic Retinopathy Clinic from 2014 to 2016. The study was conducted in accordance with the tenets of the Declaration of Helsinki. A written consent form was obtained from all the patients, as well as the approval from our institutional ethics committee. The inclusion criteria were type 1 or type 2 diabetes mellitus (DM) and HbA1c of 10% or less, previously untreated center-involving macular edema with central retinal thickness of 400 µm or less (mild center-involving DME) confirmed with spectral-domain optical coherence tomography (SD-OCT), and best-corrected visual acuity (BCVA) of at least 35 letters on Early Treatment Diabetic Retinopathy Study (ETDRS) chart (logarithm of the minimum angle of resolution [logMAR] 1.0, Snellen 20/200). The exclusion criteria were proliferative diabetic retinopathy (DR), any type of previous macular treatment, refractive error of 6 diopter or greater, previous diagnosis of glaucoma or ocular hypertension, any other retinal disease besides DR, any intraocular surgery at least 6 months before treatment, ischemic or tractional maculopathy, and any significant media opacities precluding fundus examination or imaging. Only one eye was included and treated.

All eyes underwent a complete ophthalmologic evaluation including BCVA determination, slit-lamp biomicroscopy, SD-OCT, fundus autofluorescence (FAF), and microperimetry (MP) at baseline and 3 months, 6 months, 9 months and 12 months follow-up. Fundus fluorescein angiography (FA) was performed at baseline and at 12 months.

Imaging

SD-OCT was performed using Spectralis (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). En face macula map scan pattern was used, with a 20° × 20° (5.90 × 5.90 mm) scan area centered onto the fovea. Ninety-seven horizontal scans 60 µm apart were obtained, allowing for high-resolution images. For each follow-up examination the follow-up modality was used, enabling to repeat the exam to a baseline reference examination.

SD-OCT Segmentation and Measurement: For each SD-OCT linear B-scan of the en face map, an automatic algorithm individualizes seven different retinal layers: nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), INL, OPL, Henle's fiber layer plus ONL (Henle's plus ONL), and outer retinal layers (ORL: external limiting membrane plus myoid zone of the photoreceptors plus ellipsoid zone of the photoreceptors plus outer segments of the photoreceptors plus cone interdigitation with RPE and RPE/Bruch's

TABLE 1

Morphological Parameters

		Changes From Baseline			
Parameter (Mean ± SD)	**Baseline**	**3 Months**	**6 Months**	**9 Months**	**12 Months**
1 mm Central Retinal Thickness, µm					
All Layers	360.10 ± 34.70	–14.90 ± 48.87	–7.70 ± 32.41	–10.10 ± 50.34	–23.00[a] ± 36.87
INL	43.61 ± 17.20	–1.80 ± 9.39	2.10 ± 15.10	–0.90 ± 10.67	**–9.56[b] ± 13.04**
ONL	141.53 ± 31.12	5.00 ± 23.03	–6.50 ± 39.06	–5.90 ± 48.30	–12.00 ± 44.21
ORL	89.30 ± 5.51	4.10 ± 18.20	–0.10 ± 9.02	**–2.20[c] ± 1.87**	–1.11 ± 2.47
Total Retinal Thickness*, µm					
All Layers	348.01 ± 17.32	–1.24 ± 15.33	2.59 ± 16.96	–1.92 ± 14.76	–6.28 ± 12.97
INL	41.03 ± 3.02	0.55 ± 1.96	–0.02 ± 2.00	–0.39 ± 1.81	–0.62 ± 1.37
ONL	76.10 ± 14.22	5.35[d] ± 5.71	4.50 ± 11.67	1.44 ± 7.64	1.08 ± 6.54
ORL	80.71 ± 3.04	–0.22 ± 1.45	–0.31 ± 1.31	–0.19 ± 1.28	–0.75 ± 1.42
Total Retinal Volume*, mm³					
All Layers	9.37 ± 0.52	0.070 ± 0.278	0.154 ± 0.395	0.054 ± 0.299	–0.051 ± 0.189
NFL	0.96 ± 0.09	0.003 ± 0.044	0.017 ± 0.034	0.019 ± 0.042	0.006 ± 0.039
GCL	1.19 ± 0.10	–0.002 ± 0.031	0.006 ± 0.017	–0.002 ± 0.019	–0.012[e] ± 0.014
IPL	0.99 ± 0.07	0.009 ± 0.039	0.014 ± 0.023	0.000 ± 0.023	–0.010 ± 0.020
INL	1.07 ± 0.06	0.010 ± 0.042	0.006 ± 0.041	0.000 ± 0.043	–0.017 ± 0.034
OPL	0.84 ± 0.06	–0.008 ± 0.072	0.005 ± 0.063	0.007 ± 0.064	–0.016 ± 0.069
ONL	2.07 ± 0.40	0.109[f] ± 0.140	0.103 ± 0.311	0.059 ± 0.272	0.004 ± 0.155
ORL	2.26 ± 0.09	0.004 ± 0.037	–0.006 ± 0.032	–0.006 ± 0.029	–0.019 ± 0.042

** Average total values in all sectors of the ETDRS-OCT map (covering 6 mm).*

Sign-rank test: a) P = .048; b) P = .012; c) P = .016; d) P = .062; e) P = .055; f) P = .065.

Statistically and borderline significant values, considering Bonferroni correction, have been reported in bold characters.

SD = standard deviation; INL = inner nuclear layer thickness; ONL = Henle's fiber layer plus outer nuclear layer thickness; ORL = outer retinal layer thickness (external limiting membrane + myoid zone of the photoreceptors + ellipsoid zone of the photoreceptors + outer segments of the photoreceptors + cone interdigitation with RPE + RPE/Bruch's membrane complex); NFL = nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; OPL = outer plexiform layer; OCT = optical coherence tomography

membrane complex)[18] (Figure). After automated segmentation, each scan was checked for the presence of segmentation errors, and in that case a manual correction was performed. Retinal thickness was automatically calculated in nine ETDRS areas (consisting in a central circular zone with a 1 mm diameter and inner and outer rings of 3 mm and 6 mm diameter, respectively). Mean total retinal thickness and mean thickness of INL, Henle's plus ONL, and ORL layers were recorded. Retinal volume data in the macula (total retinal volume and single retinal layer volume) was also recorded. On each linear B-scan, changes in the integrity and reflectivity of the external limiting membrane (ELM) and of the other ORL were also evaluated.

FAF: FAF and FA were recorded by a certified photographer with a confocal scanning laser ophthalmoscope (Heidelberg Retinal Angiograph, HRA2; Heidelberg Engineering, Heidelberg, Germany). To measure the extension of the areas of increased FAF, a circular area was manually outlined using the image analysis software (Heidelberg Eye Explorer HEYEX; Heidelberg Engineering, Heidelberg, Germany).[19] Using this tool, pixel area is automatically converted into square millimeters.

FA: FA images were evaluated for capillary loss, neovascularization, and presence of laser scars after treatment.

TABLE 2
Functional Parameters

		Changes From Baseline			
Parameter (Mean ± SD)	**Baseline**	**3 Months**	**6 Months**	**9 Months**	**12 Months**
BCVA					
ETDRS Letters Score	77.40 ± 10.06	2.90[g] ± 3.98	4.60 ± 8.25	5.30[h] ± 8.49	5.56 ± 9.11
logMAR	0.152 ± 0.201	-0.058[i] ± 0.080	-0.092 ± 0.165	-0.106[j] ± 0.170	-0.111 ± 0.182
RS (decibel)	25.90 ± 1.99	0.180 ± 1.386	0.780[k] ± 0.824	0.590 ± 1.349	0.644 ± 1.721
Fixation Area					
BCEA 63%	1.00 ± 0.88	0.400 ± 1.166	0.510[l] ± 0.709	0.450 ± 1.492	0.367 ± 0.762
BCEA 95%	3.92 ± 2.57	0.320 ± 3.265	0.550 ± 2.379	0.420 ± 4.176	0.600 ± 1.954

Sign-rank test: g) P = .047; h) P = .047; i) P = .047; j) P = .047; k) P = .008; l) P = .055

Statistically significant values considering Bonferroni correction have been reported in bold characters.

SD = standard deviation; BCVA = best-corrected visual acuity; RS = retinal sensitivity; BCEA = bivariate contour ellipse area

Functional Evaluation

Visual Acuity

Distance BCVA for each eye was measured by a certified tester using standard ETDRS protocol at 4 m distance with a modified ETDRS distance chart illuminator (Precise Vision, Bloomington, IL). BCVA was scored as the total number of letters read correctly (ETDRS score) and expressed also in logMAR.

Microperimetry

MP was performed on all subjects using the MAIA-2 microperimeter (CenterVue, Padova, Italy). Mean retinal sensitivity (RS) in the central area of 10° (37 tested points) was evaluated as well as fixation stability (the bivariate contour ellipse area [BCEA]) and site. BCEA analysis reflects the standard deviation (SD) of the horizontal and vertical eye movements during fixation. Smaller BCEA means more stable fixation than larger BCEA.[20]

Treatment Protocol

Macular laser treatment was performed after pupillary dilation and topical anesthesia. The lens used for the treatment was the Mainster Focal / Grid (Ocular Instruments, Bellevue, WA), with magnification of 1.05 times. SMPL treatment protocol was performed with a 577-nm yellow light (Iridex IQ 577 Laser System; Iridex Corp., Mountain View, CA), 5% duty cycle of 0.2 seconds, 250 mW power, and number of spots varying according to the extension of DME. Spots were delivered in a multiple and fully confluent fashion (high-density treatment) over all the areas of increased retinal thickness.[14] If needed, retreatment was performed according to the same protocol. Three months after any laser session, retreatment was considered if there was central subfield OCT macular thickness of 300 µm or greater, reduction of a subfield OCT macular thickening to less than 50% from baseline, or BCVA decrease of 5 letters or more on the ETDRS chart.

Statistics

To summarize the study parameters (age, duration of diabetes, systemic pressure, HbA1c, spherical equivalent of refraction) the usual methods of descriptive statistics (mean and standard deviation) were used.

Statistically significant variation of the evaluated parameters between baseline and follow-ups were tested using Wilcoxon signed-rank test.

Correlation between BCVA, RS, BCEA and morphologic parameters was performed by multiple linear regression model, adjusted for repeated measures over time.

For all analyses, a *P* value of .05 was considered statistically significant.

Moreover, we have applied the Bonferroni correction to the longitudinal evaluations of both functional and morphological parameters, considering four changes in time (baseline to 3 months; baseline to 6 months; baseline to 9 months; baseline to 12 months). All analyses were performed using SAS software SAS v. 9.3 (SAS, Cary, NC).

TABLE 3

Correlation Between Morphologic and Functional Parameters

Linear Regression Coefficients* (*P* Value)

		Functional Parameters						
Morphological Parameter	**BCVA**		**RS**		**BCEA (63%)**		**BCEA (95%)**	
	Baseline	**12 Months**	**Baseline**	**12 Months**	**Baseline**	**12 Months**	**Baseline**	**12 Months**
FAF	10.962 (0.7336)	−7.042 (0.5426)	−0.561 (0.9300)	−11.406 (0.1553)	−0.414 (0.8834)	−1.662 (0.7451)	2.624 (0.7497)	−4.782 (0.7575)
1 mm Central Retinal Thickness								
Al Layers	−0.224 (0.0027)	−0.024 (0.3717)	−0.044 (0.0036)	−0.007 (0.7266)	0.005 (0.5693)	0.021 (0.0373)	0.007 (0.7647)	0.065 (0.0357)
INL	−0.426 (0.0167)	−0.042 (0.5590)	−0.052 (0.1953)	0.073 (0.1430)	0.026 (0.1411)	0.058 (0.0344)	0.029 (0.5841)	0.174 (0.0347)
ONL	−0.246 (0.0107)	−0.002 (0.9480)	−0.050 (0.0083)	−0.041 (0.0527)	−0.008 (0.4442)	0.008 (0.5484)	−0.013 (0.6656)	0.027 (0.5284)
ORL	0.456 (0.4844)	0.070 (0.7491)	0.075 (0.5603)	−0.165 (0.2901)	−0.035 (0.5347)	−0.161 (0.0562)	−0.182 (0.2605)	−0.484 (0.0578)
Total Retinal Thickness**								
All Layers	0.338 (0.0784)	0.022 (0.7259)	0.025 (0.5392)	0.004 (0.9354)	−0.003 (0.8630)	0.031 (0.2213)	0.054 (0.3055)	0.095 (0.2236)
INL	0.527 (0.6645)	0.060 (0.8200)	0.021 (0.9317)	0.153 (0.4257)	0.113 (0.2706)	0.029 (0.8031)	0.247 (0.4180)	0.094 (0.7886)
ONL	0.234 (0.3520)	0.110 (0.1948)	−0.014 (0.7794)	−0.058 (0.3688)	−0.001 (0.9757)	0.037 (0.3308)	0.092 (0.1347)	0.111 (0.3346)
ORL	−0.223 (0.8562)	−0.313 (0.2307)	0.135 (0.5745)	0.029 (0.8870)	−0.054 (0.6166)	−0.136 (0.2320)	−0.426 (0.1470)	−0.403 (0.2444)
Total Retinal Volume**								
All Layers	10.832 (0.0940)	0.920 (0.6501)	0.170 (0.9031)	0.666 (0.6568)	0.092 (0.8818)	0.779 (0.3695)	2.380 (0.1604)	2.333 (0.3750)
NFL	−4.176 (0.9179)	−8.514 (0.4511)	−6.521 (0.4044)	10.266 (0.2001)	−0.881 (0.8032)	2.107 (0.6745)	3.682 (0.7207)	6.117 (0.6875)
GCL	41.669 (0.2171)	−0.779 (0.9298)	2.487 (0.7217)	6.888 (0.2692)	2.331 (0.4438)	3.145 (0.4040)	6.610 (0.4574)	9.404 (0.4103)
IPL	95.957 (0.0372)	3.769 (0.7811)	10.326 (0.3062)	9.866 (0.3065)	1.617 (0.7255)	3.501 (0.5509)	6.865 (0.6079)	10.394 (0.5590)
INL	44.864 (0.4225)	2.247 (0.8379)	1.591 (0.8879)	6.896 (0.3820)	5.500 (0.2501)	0.700 (0.8843)	13.163 (0.3530)	2.320 (0.8734)
OPL	100.067 (0.0839)	−8.9E−12 (1.0000)	7.426 (0.5509)	−9.321 (0.6256)	1.647 (0.7672)	16.728 (0.1064)	14.933 (0.3447)	49.753 (0.1143)
ONL	9.309 (0.2951)	3.866 (0.2014)	−0.422 (0.8165)	−1.310 (0.5769)	−0.117 (0.8843)	1.367 (0.3131)	3.128 (0.1555)	4.084 (0.3200)
ORL	1.270 (0.9760)	−11.113 (0.2465)	5.968 (0.4672)	1.316 (0.8597)	−1.418 (0.7000)	−4.912 (0.2411)	−13.181 (0.1971)	−14.514 (0.2540)

** Coefficient of the multiple linear regression model, adjusted for repeated measures over time.*

*** Average total values in all sectors of the ETDRS-OCT map (covering 6 mm).*

Statistically significant coefficients have been reported in bold characters.

BCVA = best-corrected visual acuity (number of letters); RS = retinal sensitivity (decibel); BCEA = fixation area (63% and 95% of fixation points); FAF = area of fundus autofluorescence; INL= inner nuclear layer; ONL = Henle's fiber layer plus outer nuclear layer; ORL= outer retinal layer (external limiting membrane + myeloid zone of the photoreceptors + ellipsoid zone of the photoreceptors + outer segments of the photoreceptors + cone interdigitation with RPE + RPE/Bruch's membrane complex); NFL= nerve fiber layer; GCL= ganglion cell layer; IPL= inner plexiform layer; OPL= outer plexiform layer; OCT = optical coherence tomography

RESULTS

Demographic Data and Characteristics of the Patients

Ten patients with diabetes mellitus type 2 (eight men and two women) with nonproliferative DR were enrolled. Mean age of patients was 61 years ± 7.81 years, and mean duration of diabetes was 14.72 years ± 10.70 years. At baseline, mean HbA1c was 7% ± 2.73%, mean arterial systolic pressure was 132 mm Hg ± 9.21 mm Hg, and mean diastolic pressure was 78 mm Hg ± 5.92 mm Hg. HbA1c and systemic pressure remained stable for the entire follow-up period. All eyes were affected by diffuse DME. The mean number of laser spots for each treatment was 359.62 ± 102.21 at the first treatment (10 eyes), 374.84 months ± 168.94 at 3 months (10 eyes), 462.82 ± 238.70 at 6 months (nine eyes), and 469.91 ± 138 at 9 months (10 eyes).

Morphologic Outcomes

Retinal Thickness Modifications: Table 1 shows changes in retinal thickness and volume (in various layers) over 1-year follow-up. At baseline, mean central retinal thickness (CRT, in the 1 mm) was 360.10 µm ± 34.70 µm. A significant decrease in the 1 central mm was found in CRT (-23 µm ± 36.87 µm, $P = .048$) and INL thickness (-9.56 µm ± 13.04 µm, $P = .012$) at 12 months and ORL thickness (-2.20 µm ± 1.87 µm, $P = .016$) at 9 months. After Bonferroni correction, significance was retained only for INL and ORL thickness (Table 1). No significant changes were found in any retinal layer thickness in any of the sectors in the 3 central mm or 6 central mm.

Retinal Volume Modifications: Total retinal volume was 9.37 ± 0.52 mm^3 at baseline. No significant changes were found after Bonferroni correction (Table 1).

FAF Changes: There was a decrease in the area of increased foveal autofluorescence at 12 months versus baseline value (0.081 ± 0.082 mm^2 vs. 0.165 ± 0.113 mm^2, decrease of 0.09 ± 0.153 mm^2, $P = .039$). No visible secondary effects of the laser spots on the retina were observed on fundus examination, FAF, or FA at follow-up. No changes in the integrity of the ELM and other ORL were found in any patient.

Functional Outcomes

Visual Acuity: Table 2 shows visual function modifications during the follow-up. At baseline, mean BCVA was 77.40 ± 10.06 ETDRS score. There was an increase in 2.90 letters ± 3.98 letters ($P = .047$) at 3 months, 5.30 letters ± 8.49 letters ($P = .047$) at 9 months, and 5.56 letters ± 9.11 letters ($P = .086$) at 12 months.

Microperimetry: At baseline, mean 10° central RS was 25.9 dB ± 1.99 dB. After treatment, there was a significant increase in RS at 6 months, +0.78 dB ± 0.82 dB, $P = .008$. The increase did not reach statistical significance at other follow-up time points. All eyes had central and stable fixation. No changes after SMPL treatment were found in fixation stability.

Association Between Functional and Morphologic Parameters: BCVA was significantly and inversely correlated to CRT ($P = .0027$), INL thickness ($P = .0167$), and Henle's plus ONL thickness in the central 1 mm ($P = .0107$) (Table 3). RS was significantly and inversely correlated to CRT ($P = .0036$) and Henle's plus ONL thickness in the central 1 mm ($P = .0083$). BCEA (both 63% and 95%) was significantly and directly associated with CRT ($P = .0373$ for BCEA 63%; $P = .0357$ for BCEA 95%) and INL thickness ($P = .0344$ for BCEA 63%; $P = .0347$ for BCEA 95%) in the central 1 mm (Table 3).

DISCUSSION

In this study, we have evaluated in detail, for the first time, changes in specific retinal layer thickness in eyes with DME treated with SMPL in the central 1 mm, 3 mm, and 6 mm of the macula ETDRS map. INL thickness in the 1 central mm significantly decreased at 12 months follow-up, whereas ORL thickness significantly decreased at 9 months follow-up in the 1 central mm. No changes in any retinal layer thickness were recorded at earlier follow-up visits. As previously reported in prospective, randomized studies evaluating SMPL for DME, a decrease in CRT considering all layers was also found in this study, even if not maintaining significance ($P = .048$) after Bonferroni correction.[13,14] No data are currently available on the effect of SMPL on specific retinal layer thickness.

In the present study, visual function changes were recorded as from the third month of follow-up: an increase in visual acuity (at 3 months and 9 months) and an increase in RS at 6 months after the SMPL treatment. An early increase in visual function as from the third month after SMPL treatment in DME had already been reported.[14,16] Lavinsky et al. reported a significant increase in BCVA as from the third month after high-density SMPL.[14] Vujosevic et al., reported a significant increase in central RS as from the third month of follow-up after SMPL treatment.[16] Therefore, data from the present study may indicate that SMPL treatment induces visual function changes precociously then morphologic changes. Therefore, OCT evaluation in these patients may have a limited value in the early period after the treatment, as retinal thickness decrease should be expected after approximately 1 year from the first SMPL treatment.

This finding would merit further studies in order to be fully confirmed.

In a recent study evaluating patients with subclinical and clinically relevant DME, an increase in thickness of all retinal layers (except the ORL, IS+OS, and RPE) was documented when compared with eyes without DME.[4] Moreover, a major increase in retinal thickness in the INL in the 1 central mm was reported.[4] These data may confirm hypotheses on multifactorial pathogenesis of DME: involvement of both glial and vascular components.[3,4] Müller cell (the most important macroglial cells in the retina) involvement with increased INL thickness, even in diabetic eyes without clinical signs of diabetic retinopathy, has been previously reported.[21] Moreover, experimental studies showed the increase of GFAP, AQP4, and specific cytokines in the aqueous humor of diabetic human eyes even without signs of DR or at early stages of DR as a sign of retinal glial cells activation.[22,23] On the other hand, an alteration of the blood-retinal barrier in the deep retinal vascular plexus with extracellular fluid accumulation was suggested as the possible cause of retinal edema in early stages of nonproliferative DR in patients with diabetes type 2.[4] Several studies have shown that RPE plays an important role in DME pathogenesis.[5-8] RPE is a constituent of the outer retinal barrier and has many functions including regulation of the transport of ions, nutrients, oxygen, and water between retina and choroid, and secretion of many factors important for the homeostasis, integrity, and survival of all retinal elements.[5-8] Thus, treatment theoretically targeting RPE may have a beneficial effect on resolution of DME.[9,10]

In the present study, SMPL reduces retinal thickness more in the INL than in the Henle's plus ONL. This may indicate that SMPL also has an effect on the function of INL resident cells. Whether this is a direct effect on Müller cells or indirect (through stimulation of RPE inducing specific physiologic changes in cytokine expression and growth factors secretion, which ultimately affect Müller cells, as reported in experimental studies in vitro or in animals) is unknown.[24]

Correlation between morphologic and functional parameters showed that CRT, INL, and ONL thickness in the 1 central mm are the parameters mostly correlated to BCVA and RS. In fact, an increase in CRT, INL, and ONL thickness values was inversely correlated to BCVA and RS values. These data confirm previously reported data by Deák et al.[25] These authors reported greater reduction in RS when giant retinal cysts are located in the ONL.[25] Therefore, increased retinal thickness/volume in the ONL (and / or presence of cysts in the ONL) may become an imaging prognostic biomarker of visual function in patients with DME. Moreover, fixation remained stable for the entire follow-up period, confirming that patients with DME have stable fixation and that SMPL treatment does not alter fixation stability.

The strength of the present study lies in its rigorous methodology in the evaluation of single retinal layer thickness. The main limit of this pilot study is the limited number of evaluated eyes/patients, even if a detailed approach to segmentation and evaluation of single retinal layers offers valuable data. Further study may validate and confirm these results.

In conclusion, the present study shows the effect of SMPL on single retinal layers in the macula, with improvement of both morphological and functional parameters over the 12-month follow-up period. Moreover, we report that INL and Henle's plus ONL thickness are majorly correlated to visual function data and that SMPL has the major effect in reducing retinal thickening in the INL. SMPL shows to be a safe treatment, not inducing any alteration on the outer retina, as demonstrated by SD-OCT and by FAF.[13] The exact mechanism of action remains to be further evaluated with experimental studies in human eyes.

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