Progression of Diabetic Microaneurysms According to the Internal Reflectivity on Structural Optical Coherence Tomography and Visibility on Optical Coherence Tomography Angiography

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• PURPOSE: To investigate the progression of diabetic microaneurysms (MAs) according to the spectral-domain optical coherence tomography (SD-OCT) and OCT angiography (OCTA) characteristics and to evaluate their influence on the retinal extracellular fluid accumulation at 1 year follow-up in patients with nonproliferative diabetic retinopathy (NPDR).

• DESIGN: Prospective, observational case series.

• METHODS: Fourteen patients with NPDR underwent SD-OCT and OCTA at the baseline and at 1 year follow-up. For all the selected MAs the visibility, the changes of internal reflectivity, graded as hyporeflective, moderate, or hyperreflective, and the extracellular fluid accumulation surrounding each MA on SD-OCT at 1 year were evaluated. The changes in terms of visualization at the level either of superficial (SCP) or deep (DCP) capillary plexus and the presence of flow on the corresponding OCTA scan at 1 year were evaluated.

• RESULTS: Of 127 MAs selected at the baseline, 89 (70%) were still visible on SD-OCT at 1 year. The reflectivity pattern at baseline was strongly associated with extracellular fluid accumulation at 1 year, with 18% of hyporeflective vs 66% of hyperreflective MAs developing extracellular fluid ($P = .004$). Among OCTA findings, the presence of flow ($P = .001$), the visibility ($P < .001$), and the deep location (DCP or both DCP and SCP, $P = .007$) were strongly associated with the development of extracellular fluid at 12 months.

• CONCLUSIONS: This study suggests an association between the SD-OCT and OCTA characteristics of diabetic MAs and the retinal extracellular fluid accumulation at 1 year. A better interpretation of MA characteristics could improve the timing and the management of diabetic maculopathy. (Am J Ophthalmol 2019;198:8–16. © 2018 Elsevier Inc. All rights reserved.)

DIABETIC RETINOPATHY (DR) IS THE MAIN CAUSE of blindness among elderly individuals in the developed world, and its prevalence increases with increasing duration of the disease. Diabetic macular edema (DME) is one of the leading causes of vision loss in diabetic patients, and leaking microaneurysms (MAs) are one of the factors that lead to macular edema formation.[1,2]

MAs develop in areas of acellular capillaries and are the clinical marker for capillary nonperfusion in early DR.[3,4] The relationship between the capillary closure and the occurrence of chronic DME has previously been stressed in reports on MA turnover.[5,6] A high MA turnover has been associated with a higher risk of DME development in nonproliferative DR eyes, suggesting also a possible relationship between the capillary nonperfusion and the occurrence of chronic DME.[3,4] Therefore MAs can be considered a possible prognostic factor for the onset of macular edema. The identification of the early conditions that lead to the development of macular edema could be very important to focus on in those patients that are at risk of progression of diabetic maculopathy.

Several techniques exist to image the retinal vascular changes (such as MAs, foveal avascular zone (FAZ) changes, or nonperfusion areas) due to diabetes. Fluorescein angiography (FA) has long been the gold-standard examination to study the diabetic retinal changes, but it is invasive, costly, and time consuming. Recent improvements in imaging techniques brought a clear visualization of the different retinal layers, immediately highlighting the different diabetic lesions that could involve the retinal parenchyma and vasculature.

Spectral-domain optical coherence tomography (SD-OCT) is an examination that is now widely employed in our clinics, as it is noninvasive, cheap, and easy to perform.

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Recently, diabetic MAs were characterized using SD-OCT.[7,8]

Optical coherence tomography angiography (OCTA), a new noninvasive imaging technique, allows a fast visualization of macular vasculature and differentiation of different vascular layers, such as the superficial and deep vascular plexus.[9–12] Several studies have reported new findings of vascular changes in diabetic patients using OCTA, such as foveal avascular zone shape changes, presence of retinal nonperfusion, and MA evaluation.[13–16]

In a recent study[8] we demonstrated that MAs that appear hyporeflective on SD-OCT have a lower detection rate on OCTA images, suggesting that different patterns of blood flow dynamics could characterize different MAs. The purpose of this study was to investigate the progression of diabetic MAs according to the structural SD-OCT and OCTA characteristics and to evaluate their influence on the retinal extracellular fluid accumulation at 1 year follow-up in patients with nonproliferative DR.

METHODS

IN THIS PROSPECTIVE OBSERVATIONAL CASE SERIES, MAS were randomly selected and analyzed from type 2 diabetic patients with mild, moderate, or severe nonproliferative DR at the Department of Ophthalmology, G.B. Bietti Eye Foundation–IRCCS, Rome, between March 15, 2016, and July 15, 2017.

This study was approved by the Institutional Review Board of the G.B. Bietti Eye Foundation–IRCCS and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Inclusion criteria were as follows: patients with type 2 diabetes mellitus, age $>$ 18 years, and clinical evidence of DR on ophthalmologic examination, graded from mild to severe nonproliferative DR.

Exclusion criteria were as follows: MAs secondary to diseases other than DR (eg, retinal vascular occlusion); previous treatments for DR (intravitreal injection, laser treatment at the posterior pole); or diagnosis of other macular diseases, including central serous chorioretinopathy, vitreoretinal interface diseases, or age-related macular degeneration. Patients with diagnosis of diabetic macular edema, diagnosed clinically or with SD-OCT, which could change the contour of the segmentation on OCTA, or diffuse edema, which could mask the presence of MAs, were also excluded, as were patients with significant cataract, graded above NO3 or NC3.

All patients at the baseline underwent the same day 45-degree color fundus photograph (Topcon TRC 50DX; Topcon Corporation, Tokyo, Japan), simultaneous SD-OCT and FA (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany; version 6.4.7.0), and OCTA imaging (XR Avanti; Optovue Inc, Fremont, California, USA).

Color fundus photograph, FA, and near infrared were used to verify the identification of MAs detected on SD-OCT, as described previously.[8] Briefly, we superimposed the vascular landmarks to match the MAs on color fundus photographs to those of FA imaging where MAs appeared as hyperfluorescent dots in the early phase; additionally, the FA images were overlaid with the near infrared images of the Heidelberg Spectralis HRA+OCT.[8] The SD-OCT protocol performed included 49 horizontal cross-sectional B-scans per volume scan spanning 20 $\times$ 20 degrees of the macula volume with either the vertical or horizontal bisecting the center of each microaneurysm (each B-scan including an average of 16 frames).

SD-OCT imaging was repeated in all patients 7 days after the first acquisition in order to exclude from the selection MAs with fast turnover.[1,5] MAs present on both dates were included in the analysis. SD-OCT images with signal strength below 25 decibels were excluded.

The SD-OCT internal reflectivity within the lumen of each MA was graded as hyporeflective, moderate, or hyperreflective, as described previously.[8,17] The MA was classified as hyperreflective if the internal reflectivity within the lumen was similar to that of the MA wall, hyporeflective if it was similar to that of cystic intraretinal fluid, and moderate if the reflectivity was intermediate.

Finally, to explore the OCTA characteristics of MAs, we used the XR Avanti OCTA instrument (Optovue Inc, Fremont, California, USA) with split-spectrum amplitude-decorrelation angiography (SSADA) software.[12] A 6 $\times$ 6-mm scanning area, centered on the fovea, was obtained. Using the segmentation algorithm in the built-in software, the en face OCT angiograms were segmented to define the superficial capillary plexus (SCP) and deep capillary plexus (DCP).

Thinner slabs were also obtained with customized settings and were moved progressively from the outer retina to the inner plexiform layer to look for undetected MAs.

MAs were identified as focally dilated saccular or fusiform capillaries in the 6 $\times$ 6-mm area of the en face OCT angiograms. OCT angiograms with signal strength below 72 or with motion artifacts were excluded for low quality of images.

To compare the MAs seen on OCTA to those seen on Spectralis SD-OCT cross-sectional structural B-scan, we superimposed the OCTA SCP vascular landmarks onto the vascular landmarks of the near infrared, as described previously.[8] This allowed point-by-point correlations between both the SCP and DCP and corresponding Spectralis cross-sectional structural B-scan.

The same patients were imaged at 1 year follow-up ($\pm$ 15 days), when they underwent 45-degree color fundus photography, simultaneous FA and SD-OCT in the follow-up modality, setting the baseline macula volume

TABLE 1. Demographics and Characteristics of Patients

Characteristic	Result
Patients/eyes, n	14/26
Male/female, n	8/6
Age (y)	
Mean ± SD	66.3 ± 8.24
Range	52–77
Duration of diabetes (y)	
Mean ± SD	9.8 ± 5.4
Range	4–20
HbA1c (%)	
Mean ± SD	8.2 ± 1.7
Range	6.4–11.4
DR stage, n eyes (%)	
Mild nonproliferative	8 (30.8)
Moderate nonproliferative	10 (38.4)
Severe nonproliferative	8 (30.8)

DR = diabetic retinopathy; HbA1c = glycated hemoglobin.

images as reference and 6 × 6 OCTA. For all the selected MAs the visibility at 1 year and the extracellular fluid accumulation surrounding each MA on SD-OCT were evaluated. The extracellular fluid accumulation was defined as cystic intraretinal fluid surrounding each MA in the 4 superior and inferior macular volume scans (each 117 μm apart) not visible at baseline, corresponding to focal fluorescein leakage at FA. The follow-up changes in terms of MA internal reflectivity were also evaluated. Therefore the changes in terms of visualization at the level either of SCP or DCP on the corresponding OCTA images at 1 year follow-up and the presence of flow on the corresponding cross-sectional OCTA scan were evaluated.

To investigate a possible influence of different treatment (anti-VEGF or steroid injections) on internal reflectivity changes on SD-OCT and visualization changes on OCTA, we calculated how many and which MAs were treated after the baseline, excluding patients that received injections less than 2 months before the follow-up evaluation. Two different masked examiners (D.D.G., F.S.) evaluated independently the baseline and follow-up images, and in case of disagreement there was open adjudication until a consensus was established.

• **STATISTICAL ANALYSIS:** Continuous data were described as means (standard deviations) and categorical data as frequencies. The association of structural and OCTA variables with the development of extracellular fluid accumulation at 12 months was calculated using mixed logistic models, with subjects as a random effect to adjust for within-subject correlation.

All statistical analysis was performed using Stata 15.1 software (College Station, Texas, USA). A $P < .05$ was considered statistically significant.

RESULTS

ONE HUNDRED AND TWENTY-SEVEN RANDOMLY SELECTED MAs identified on color fundus photography, FA, near infrared, and cross-sectional structural SD-OCT B-scan were analyzed at baseline and at 1 year follow-up from 26 eyes of 14 type 2 diabetic patients with mild, moderate, and severe nonproliferative DR.

All 127 MAs selected and analyzed were detectable in the aforementioned techniques at the baseline. The quality of images was considered as sufficient for qualitative analysis in 100% (26/26) of eyes on SD-OCT. Patient demographics are shown in Table 1.

Of 127 MAs selected at the baseline, 89 (70%) were still visible and 38 (30%) were not visible on SD-OCT at 1 year follow-up. Extracellular fluid accumulation was detected in 44 out of 89 MAs (49.4%) at 1 year.

• **ASSOCIATION OF STRUCTURAL OPTICAL COHERENCE TOMOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY FINDINGS WITH DEVELOPMENT OF PERILESIONAL EDEMA:** Table 2 presents the analyses of the association of structural OCT and OCTA findings with the development of extracellular fluid accumulation at 12 months. This analysis is limited to the 89 MAs that were detectable with structural OCT at 12 months.

The reflectivity pattern at baseline was strongly associated with extracellular fluid accumulation, with 18% of hyporeflective MAs vs 66% of hyperreflective MAs developing extracellular fluid ($P = .004$). A similar cross-sectional association persisted at 12 months (Figures 1 and 2).

Among the OCTA findings, both the presence of flow ($P = .001$) and visibility ($P < .001$) were strongly associated with the development of extracellular fluid, an association that also persisted cross-sectionally at 12 months.

Regarding MA location, only deeply located MAs (DCP or both DCP and SCP, $P = .007$), but not those with superficial location (SCP or both SCP and DCP, $P = .592$), were associated with the development of extracellular fluid that was confirmed at 12 months.

• **CHANGE IN STRUCTURAL OPTICAL COHERENCE TOMOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY FROM BASELINE TO 12 MONTHS:** Figure 3 presents the cross-tabulation of structural OCT reflectivity at baseline vs 12 months and Figure 4 presents that of OCTA visibility at different plexa at baseline vs 12 months.

Overall, using structural OCT 38 out of 127 (30%) MAs were no longer visible at 12 months. In general, hyporeflective MAs remained hyporeflective (13/20, 65%). Of 65 MAs with intermediate reflectivity, 28 (44%) became hyperreflective and 24 (37%) became not visible.

At baseline 18 of 127 (14%) MAs were not visible using OCTA. Among those visible, few were located in the SCP only (5/109, 5%), whereas 70 (58%) and 28 (23%) were

TABLE 2. Association Between Spectral-Domain Optical Coherence Tomography and Optical Coherence Tomography Angiography Characteristics at Baseline and Spectral-Domain Optical Coherence Tomography Extracellular Fluid Accumulation at 1 Year Follow-up

		Baseline			1 Year Follow-up		
Covariate	Categories	Number With Extracellular Fluid/Total (%)	OR	*P* Value	Number With Extracellular Fluid/Total (%)	OR	*P* Value
Reflectivity	Hypo	3/17 (18%)	Reference		2/14 (14%)	Reference	
	Intermediate	20/40 (50%)	4.9	.030	16/20 (80%)	27.3	.001
	Hyper	21/32 (66%)	10.2	.004	26/55 (47%)	6.0	.036
Flow	Absent	15/46 (33%)	Reference		16/44 (36%)	Reference	
	Present	26/38 (68%)	4.9	.005	23/34 (68%)	7.4	.008
OCTA visibility	Visible	41/77 (53%)	Reference		36/51 (71%)	Reference	
	Not visible	0/9 (0)	0.07[a]	.004	3/27 (11%)	0.05	<.001
SCP	Other	30/60 (50%)	Reference		30/66 (45%)	Reference	
	SCP or SCP+DCP	11/26(42%)	0.77	.592	9/12 (75%)	4.2	.067
DCP	Other	1/14 (7%)	Reference		6/31 (19%)	Reference	
	DCP or both DCP+SCP	40/72 (56%)	21.8	.007	33/47 (70%)	9.8	<.001

DCP = deep capillary plexus; NV = not visible; OCTA = optical coherence tomography angiography; SCP = superficial capillary plexus.
[a]Exact logistic regression not adjusted for clustering on individuals.

located, respectively, in the DCP only or in both the SCP and DCP. Apart from the fact that the number of nonvisible MAs increased to 58 (45.7% of the original MA cohort), no other specific pattern was observed at 12 months.

Figure 5 shows the OCTA features of MAs at 12 months according to structural OCT reflectivity at baseline. Hyporeflective MAs became nonvisible at 12 months in 18 of 20 MAs (90%), as compared to 20 of 71 (28%) with intermediate and high baseline reflectivity, of which most remained located in the DCP (66%).

• **INFLUENCE OF INTRAVITREAL INJECTIONS ON MICROANEURYSM REFLECTIVITY CHANGES:** Of the total 89 MAs visible on SD-OCT at 1 year follow-up evaluation, 49 (55.1%) showed the same internal reflectivity as the baseline and 40 (44.9%) showed a different reflectivity in comparison with the baseline.

A similar percentage of MAs had received treatment (anti-VEGF or steroid injections) at 1 year follow-up in both groups. Of 49 MAs that maintained the same internal reflectivity, 21 (42.9%) had been treated and 28 (57.1%) did not receive any intravitreal injections. Of 40 MAs that showed a different internal reflectivity at 1 year follow-up, 22 (55%) had been treated and 18 (45%) had not been treated in the last year.

The treatment does not seem to influence the internal reflectivity of MAs, given that of a total of 43 MAs that had been treated in the year of follow-up, 21 (48.8%) showed a different internal reflectivity in comparison with the baseline and 22 (51.2%) presented the same internal reflectivity as the baseline.

DISCUSSION

IN THIS STUDY WE EVALUATED THE CHARACTERISTICS OF A series of diabetic MAs imaged with SD-OCT and OCTA in a group of diabetic patients at the baseline and at 1 year follow-up. The purpose was to study the main factors that could be associated with the intraretinal extracellular fluid accumulation after 1 year of follow-up.

Interestingly, we found that the reflectivity patterns at the SD-OCT of MAs, as well as the presence of flow on cross-sectional scan and the localization at the level of DCP on OCTA at baseline, were strongly associated with extracellular fluid accumulation at 1 year on SD-OCT.

We reported in a recent study[8] that MAs that appear hyporeflective on SD-OCT have a lower detection rate on OCTA images. We hypothesized that the reason could be that MAs that appear hyporeflective on SD-OCT have a low blood flow rate below the threshold necessary to register the flow in the OCTA system or, as previous authors reported, could be MAs with turbulent flow[16] or MAs that are not perfused and have extensive luminal fibrosis and lipid infiltration.[18] Conversely, we reported that the hyperreflective MAs, characterized by a high blood flow rate, are more likely to be visible in the OCTA images. In the current study we are able to support and confirm the hypothesis proposed in the previous study, where we speculated that the hyperreflective MAs, characterized by a high blood flow rate, is likely associated with the extracellular fluid accumulation resulting from alteration of the blood-retinal barrier in the DCP.

The strong association between the higher internal reflectivity of MAs on SD-OCT and the presence of flow

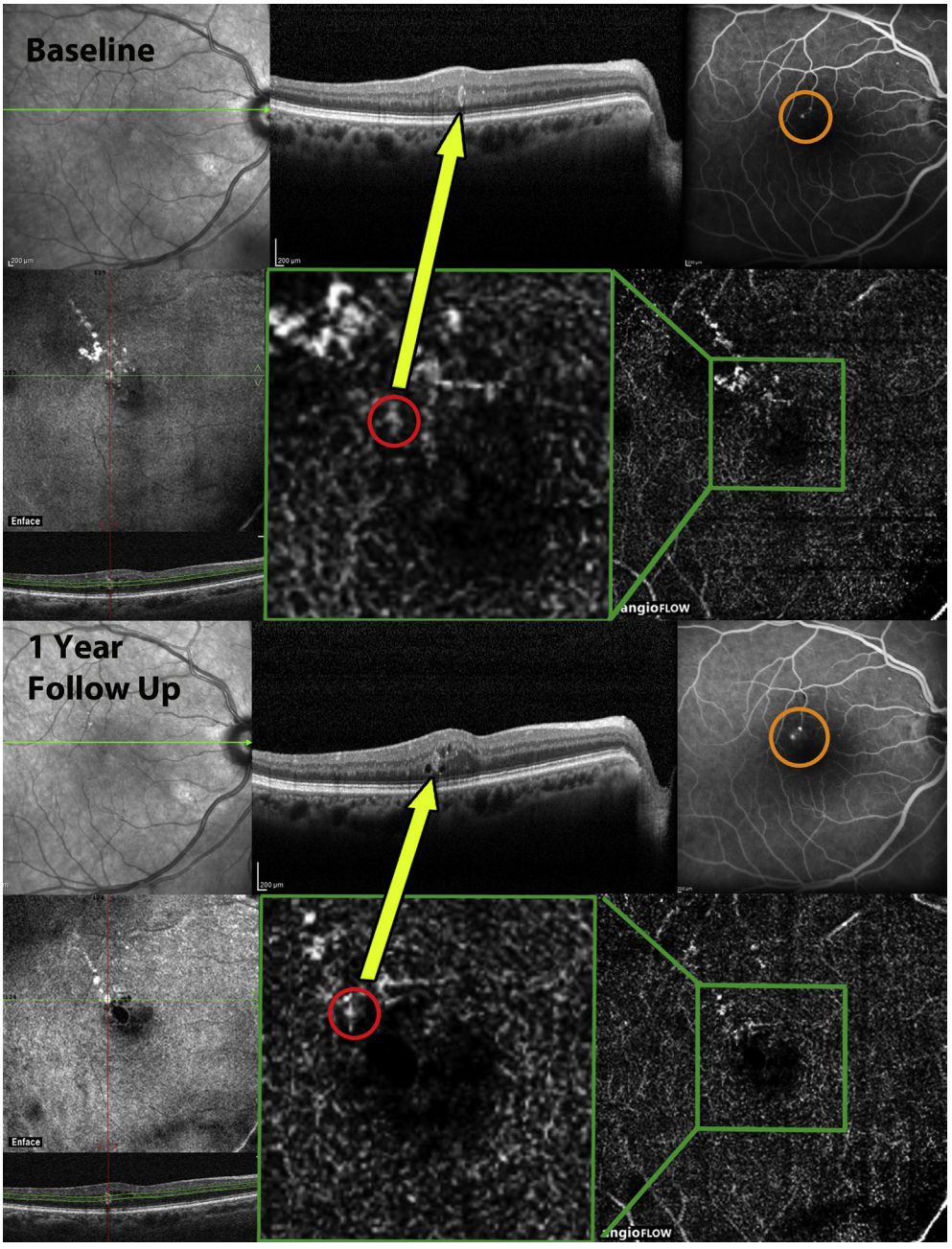

FIGURE 1. At baseline: (Top center) The Spectralis B-scan showing a hyperreflective microaneurysm (yellow arrow) and (Top left) the infrared image with the green line with arrow passing through the microaneurysm. (Top right) The late frame of fluorescein angiography shows the pooling of the dye in correspondence to the microaneurysm, with minimal leakage (orange circle). This exactly corresponds to the focally dilated microaneurysm highlighted by the red circle at the level of the deep capillary plexus (DCP) in the optical coherence tomography (OCTA) imaging (6 × 6 scanning area) (Bottom right); the inset shows the characteristics of the hypereflective microaneurysm in detail (Bottom center). (Bottom left) En face imaging with red and green lines indicating the location of B-scans (XR Avanti; Optovue Inc, Fremont, California, USA); the DCP segmentation boundaries (green lines) passing through the microaneurysm are visible. At 1 year follow-up: (Top center) The Spectralis B-scan showing the same hyperreflective microaneurysm (yellow arrow) surrounded by new extracellular fluid and (Top left) the infrared image with the green line with arrow passing through the microaneurysm. (Top right) The late frame of fluorescein angiography shows the pooling of the dye in correspondence to the microaneurysm with late leakage (orange circle). This exactly corresponds to the focally dilated microaneurysm highlighted by the red circle at the level of the DCP in the OCTA imaging (6 × 6 scanning area) (Bottom right); the inset shows the characteristics of the hypereflective microaneurysm in detail (Bottom center). En face imaging with red and green lines indicating the location of B-scans (Optovue). The DCP segmentation boundaries (green lines) passing through the microaneurysm are visible (Bottom left).

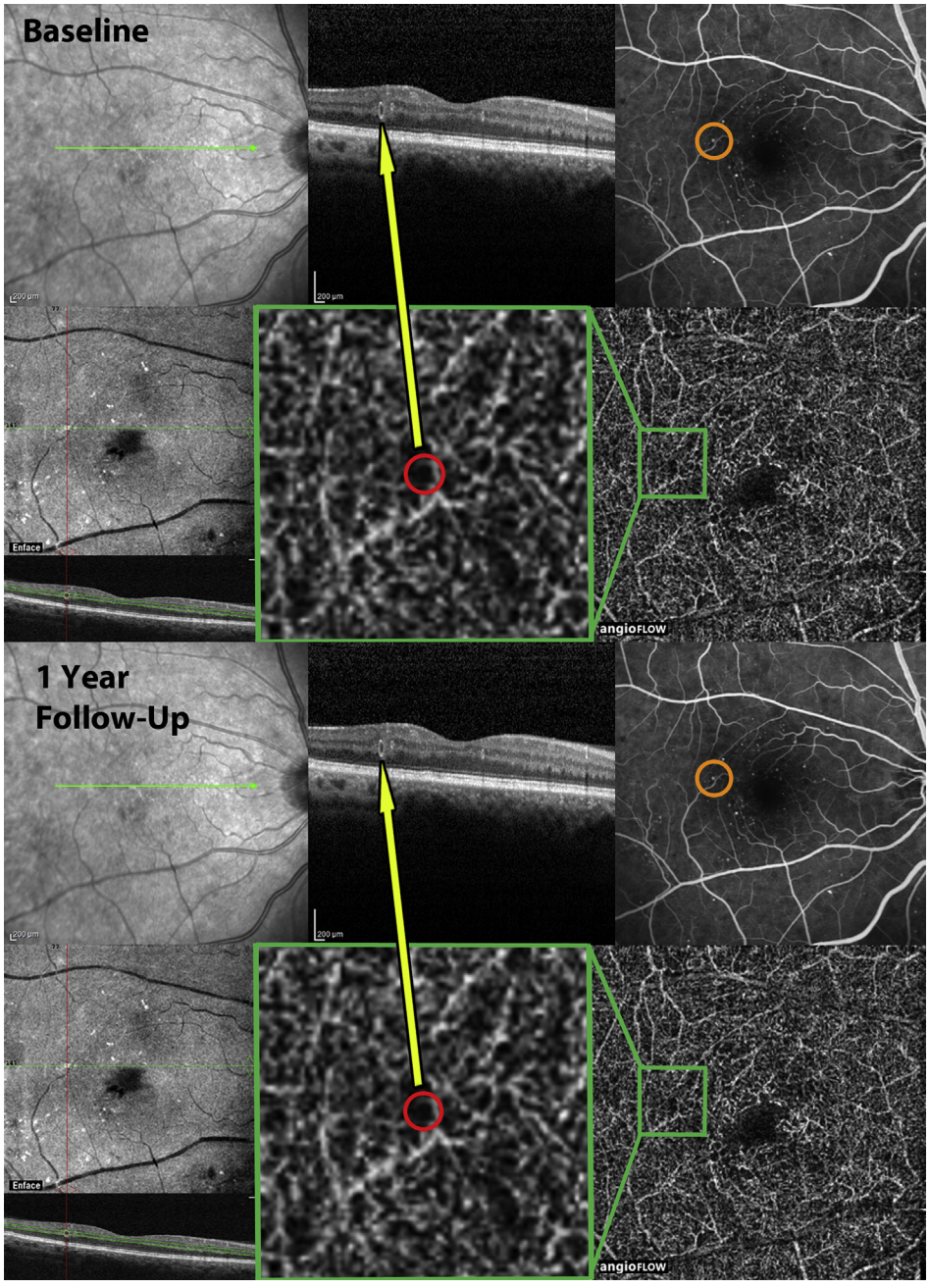

FIGURE 2. At baseline: (Top right) Spectralis B-scan showing a hyporeflective microaneurysm (yellow arrow) and (Top left) the infrared image with the green line with arrow passing through the microaneurysm. (Top right) The late frame of fluorescein angiography shows the pooling of the dye in correspondence to the microaneurysm, with no evident leakage (orange circle). The microaneurysm (red circle) cannot be detected at the level of the deep capillary plexus (DCP) by means of optical coherence tomography angiography (OCTA) imaging (6 × 6 scanning area) (Bottom right); the inset (green box) shows the area of interest with more detail (Bottom center). (Bottom left) En face imaging with red and green lines indicating the location of B-scans (XR Avanti; Optovue Inc, Fremont, California, USA). The DCP segmentation boundaries (green lines) passing through the microaneurysm are visible. At 1 year follow-up: (Top right) The Spectralis B-scan showing the same hyporeflective microaneurysm (yellow arrow) without any new extracellular fluid and (Top left) the infrared image with the green line with arrow passing through the microaneurysm. (Top right) The late frame of fluorescein angiography shows the pooling of the dye in correspondence to the microaneurysm, with still no leakage (orange circle). After 1 year it still appears not visible (red circle) at the level of the DCP by means of OCTA imaging (6 × 6 scanning area) (Bottom right); the inset (green box) shows the area of interest with more detail (Bottom center). En face imaging with red and green lines indicating the location of B-scans (Optovue). The DCP segmentation boundaries (green lines) passing through the microaneurysm are visible (Bottom left).

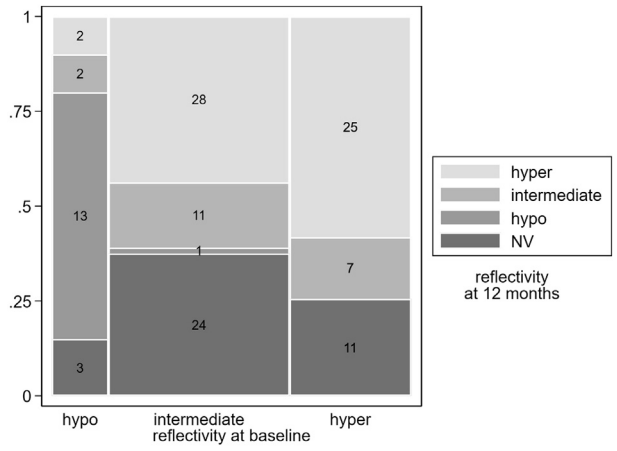

FIGURE 3. Graphical representation of the cross-tabulation of the reflectivity of the visible microaneurysms on structural optical coherence tomography at baseline and at 12 months.

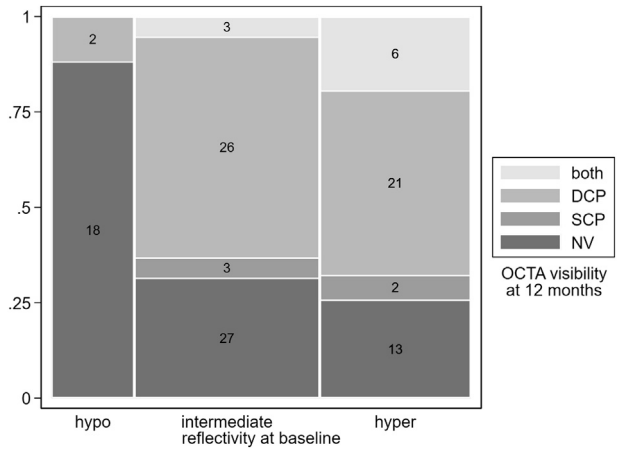

FIGURE 5. Graphical representation of the cross-tabulation of reflectivity of the visible microaneurysms at structural optical coherence tomography at baseline vs optical coherence tomography angiography visibility at 12 months.

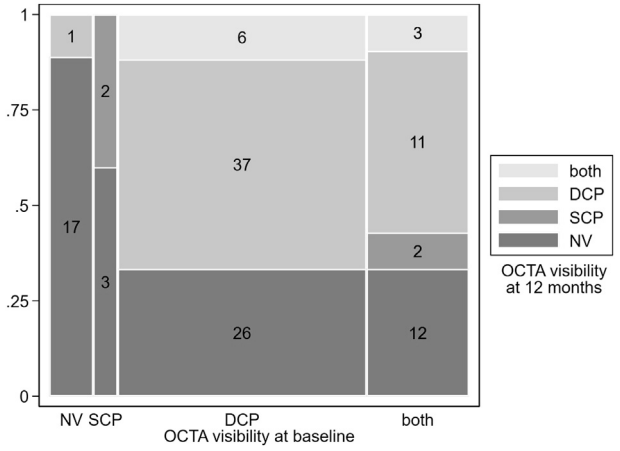

FIGURE 4. Graphical representation of the cross-tabulation of optical coherence tomography angiography visibility at baseline vs 12 months, including microaneurysms, which were reflective at baseline.

on OCTA images at the baseline and the extracellular fluid accumulation on SD-OCT after 1 year follow-up that we reported in this study confirm that the different reflectivity pattern of MAs on SD-OCT corresponds to the different pattern of blood flow dynamics in MAs. Therefore hyperreflective MAs, strongly associated with extracellular fluid accumulation in the long term resulting from alteration of the blood-retinal barrier in the DCP, can be considered a possible prognostic factor for onset of macular edema, and patients that present a higher number of hyperreflective MAs could be considered as patients with a higher risk of progression of diabetic maculopathy.

Identification of risk factors for progression and visual loss with a calculations of risk for progression in each patient over a given time period are crucial steps in the decision process on the initiation of treatment. As previously demonstrated, the presence of a DCP impairment, characterized by reduction of vessel density, high MA density, and enlargement of foveal avascular zone, could be related to poor response to anti-VEGF therapy in patients with diabetic macular edema.[19] In addition, according to our results the presence of a particular SD-OCT reflectivity pattern could be considered as an additional possible biomarker to the diabetic macular edema treatment response.

We hypothesize that hyperreflective MAs, which present active internal blood flow dynamics and are associated with extracellular fluid accumulation, could have a higher inflammatory factor component, responsible for blood-retinal barrier changes. Therefore, in the presence of a higher number of hyperreflective MAs, either anti-VEGF or steroid treatments could allow for better morphologic and functional outcomes, thanks to the action on the vascular permeability as well as on the inflammation pathway.

On the contrary, it has been previously suggested[13] that MAs could appear hyporeflective because they contain only plasma without cellular components such as erythrocytes or leukocytes. Therefore, we could suppose that hyporeflective MAs, which were poorly associated with extracellular fluid accumulation in our cohort of patients, could be poorly perfused because of low blood flow dynamics and without the presence of inflammatory factors, and for this reason poorly responding to the conventional DME treatment.

Summarizing, we suggest that hyperreflective MAs might be considered a recent sign of blood-retinal barrier changes better responding to anti-VEGF or steroid treatments, and hyporeflective MAs might be considered an indirect sign of advanced nonperfusion changes in diabetic

maculopathy with a poor functional and morphologic response to the DME treatments. Indeed, it has been found in patients with DME treated with intravitreal injection either of triamcinolone or bevacizumab that the presence of macular ischemia has a negative impact in terms of visual acuity outcomes, in comparison to patients without macular ischemia.[20,21]

The association between the reflectivity pattern of MAs on SD-OCT and the blood-retinal barrier changes means that with SD-OCT, which is a noninvasive imaging technique routinely used in our clinics, we can very quickly obtain not only static but also dynamic information that improves our interpretation of MAs detected on SD-OCT.

Hasegawa and associates[22] for the first time reported an association between the distribution of MAs on OCTA and the macular volume in patients with DME, highlighting the key role of MAs located at the level of the DCP in the pathogenesis of DME. Our results are in agreement with these findings, considering that most MAs that at 1 year showed a hyperreflectivity or moderate internal reflectivity on SD-OCT remained visible on OCTA (55/71; 77.5%) and most of them (45/55; 81.8%) were located at the level of the DCP.

Ito and associates[23] reported that structural changes of MAs such as the absence of a capsular structure or intraluminal hyperreflective spots seen on SD-OCT are significantly associated with focal fluorescein leakage in the MAs, suggesting the pathogenesis of vascular permeability in MAs. The authors hypothesized that hyperreflective spots could represent leukocytes that contribute to vascular hyperpermeability.

The results of our study could confirm this hypothesis. As mentioned above, hyperreflective MAs could be MAs with a higher flow but also with a high component of inflammatory cells that could be more likely to be associated with blood-retinal barrier changes and, in the long term, with extracellular fluid accumulation.

Interestingly, in this group of MAs randomly selected and followed over time with multimodal imaging, we found that most of the hyperreflective and moderately reflective MAs were still visible on SD-OCT after 1 year follow-up (74.4% and 62.5%, respectively) and did not show important changes in terms of internal reflectivity. These data suggest a low turnover in our cohort of hyperreflective and moderately reflective MAs over the entire period of follow-up. With regard to this, considering MAs that maintained the same internal reflectivity after 1 year, only 42.9% of them showed extracellular fluid accumulation that required intravitreal injection over 1 year.

These results are somehow in agreement with previous data reported in literature,[1] in which the MA turnover has been proposed as a prognostic biomarker of development of clinically significant macular edema.

The limitations of this study include that patients with different disease stages and duration were included, and that a relatively small number of MAs was evaluated.

In conclusion, this is the first study that suggests an association between the SD-OCT and OCTA characteristics of MAs and the alterations of the blood-retinal barrier leading to extracellular fluid accumulation in diabetic patients. A better interpretation of SD-OCT and OCTA characteristics of MAs could in the near future improve the timing and management of diabetic maculopathy.

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