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Retina

Confocal MultiColor Signal Depends on Perfusion Characteristics of Retinal Microaneurysms in Diabetic Retinopathy as Detected by OCTA

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Purpose: To propose a noninvasive way of classifying multimodal imaging of retinal microaneurysms (MA) secondary to diabetic retinopathy (DR).

Methods: The research was designed as a cross-sectional, observational study of patients affected by DR. Multimodal imaging included confocal MultiColor imaging, optical coherence tomography (OCT) and OCT angiography (OCTA). MA green- and infrared-reflectance components were assessed by confocal MultiColor imaging, reflectivity properties by OCT, and MA perfusion features by OCTA. In addition, we included high-resolution (HR) and high-speed (HS) OCTA scans to assess HR–HS agreement in detecting retinal MA and to highlight different perfusion features detected by both OCTA acquisitions.

Results: We analyzed 216 retinal MAs, divided into green (46; 21%), red (58; 27%) and mixed types (112; 52%). Green MAs were mainly hyper-reflective on OCT, with no or poor filling on OCTA. Red MAs were characterized by an isoreflective signal on OCT and full filling on OCTA. Mixed MAs showed a hyper-reflective border and a hyporeflective core on OCT and partial filling on OCTA. No differences in red MA HR/HS size discrepancy and reflectivity were found, whereas these progressively increased as the MA MultiColor signal changed from infrared to green. MA types significantly correlated with visual acuity, DR duration, and DR severity.

Conclusions: Retinal MA can be classified reliably by means of a fully noninvasive multimodal imaging-based assessment. MA types are matched with visual acuity, DR duration and DR severity. Both HR and HS OCTA are highly effective in detecting MA, although HR OCTA is to be preferred in the presence of fibrotic evolution.

Translational Relevance: This study outlines a proposed novel MA classification based on noninvasive multimodal imaging. The findings presented in this paper endorse the clinical relevance of this approach, highlighting how this classification is associated with both DR duration and severity.

Introduction

Retinal microaneurysms (MA) are an early symptom of diabetic retinopathy (DR), and it is clinically useful to investigate them, because the morphological characteristics and turnover of retinal MAs are associated with the risk of DR complications and poor visual outcomes.[1] The primary method of analyzing retinal MAs has traditionally been based on fluorescein angiography examination, an invasive diagnostic technique that is highly sensitive in detecting MAs and is able to distinguish morphologically different types of MAs, including focal bulge, saccular, fusiform, mixed, pedunculated, and irregular subtypes.[2]

The recently introduced optical coherence tomography angiography (OCTA) provides noninvasive visualization of retinal and choroidal vascular networks,[3] yielding important insights into the pathogenesis of retinal diseases, including DR.[4] However, other noninvasive modalities have also proved useful in assessing MA characteristics. In a recent paper, Arrigo et al proposed a confocal MultiColor-based classification of retinal MAs, in which the signal coming from three simultaneous reflectance images is assessed using monochromatic blue (wavelength, 486 nm), green (518 nm), and infrared (IR) (815 nm) light.[5] The feature of the multichromatic MultiColor image enables the retinal MA to be broken down into red, green and mixed subtypes, correlating precisely with structural OCT properties and matching the previous histological classification.[6]

In the present study, we aim to expand our previously proposed confocal MultiColor-based classification of retinal MA, by incorporating the perfusion information obtained from OCTA examination and evaluating its relationship with the clinical stage of the DR.

Methods

This research was designed as a cross-sectional, observational study. Patients affected by type 2 diabetes mellitus were recruited at the Ophthalmology Unit of San Raffaele Hospital, Milan, Italy. All patients provided written informed consent. The entire study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Vita-Salute San Raffaele University in Milan. Patients with a confirmed diagnosis of DR, based on the presence of any stage of DR and clear funduscopic detection of MAs in the posterior pole, were included. Patients with significant media opacity, any other type of retinal or optic nerve disease, ocular surgery within the previous 3 months, or any systemic condition that could affect these analyses were excluded from the study.

All patients underwent a complete ophthalmic examination, including ETDRS logarithm of the minimum angle of resolution best-corrected visual acuity (BCVA), applanation tonometry, and anterior and posterior segment slit lamp evaluation.

Multimodal imaging included confocal MultiColor, IR, structural OCT and OCTA images, acquired by means of a Spectralis HRA+OCT2 device (Heidelberg Engineering, Heidelberg, Germany). OCT acquisitions included raster, radial, and dense scans with a high number of frames averaged (ART >25), and enhanced depth imaging. OCTA acquisitions included 30° scans using both high-resolution (HR) and high-speed (HS) modules. We decided to include both the HR OCTA scan (IR confocal scanning laser ophthalmoscopy scan angle of 15°, 5.73 µm/pixel, axial digital resolution 3.87 µm/pixel, ART 5 images) and the HS OCTA scan (scan angle 30°, 11.46 µm/pixel, axial digital resolution3.87 µm/pixel, ART 5 images) for the following reasons: first, to gauge the ability of HR and HS OCTA to detect retinal MA; and second, to assess the presence of different perfusion features revealed by both OCTA acquisitions.

We performed a qualitative MA analysis based on the acquired images to evaluate the relationship between confocal MultiColor imaging (green and red reflectance components), structural OCT (hyperreflective, hyporeflective, and mixed reflectivity) and OCTA findings. All detected MAs were classified according to the previous confocal MultiColor classification,[6] incorporating OCTA findings. The quantitative evaluation also included MA reflectivity as detected on OCTA, by loading segmented MAs in the ImageJ software package.[7] Each MA was carefully segmented by an expert grader and ImageJ scripts were used to measure MA signal intensity (namely MA reflectivity). Moreover, by including both HR and HS OCTA scans, we tested a new criterion, namely the measurement of MA size discrepancy between HR and HS modes of OCTA scanning (hereafter referred to as HR/HS size discrepancy). The aim was to ascertain whether HR and HS OCTA modalities might be able to provide different information regarding MA perfusion characteristics. First, MA reconstructions were segmented using the Otsu threshold approach.[8] Then, we calculated the HR/HS size discrepancy, superimposing HR-based and HS-based MA reconstructions and judging the percentage overlap of pixels belonging to the MA by means of custom scripts running on ImageJ. All the analyses were conducted by two expert graders (L.B., A.A.). The intraclass correlation coefficient (ICC) was calculated for all the measures performed. A two-tailed test was adopted to assess statistically significant differences in continuous variables. We used contingency tables to determine the agreement between the confocal MultiColor signal and OCTA perfusion characteristics, by considering red, green, and mixed components separately, as well as the OCTA finding (perfused or nonperfused). Moreover, we performed a Tau–Kendall correlation analysis to assess the relationship between the MA classification, involving one or more MA types, and the following parameters: hemoglobin A1c percent, DR duration, presence of diabetic macular edema, and logarithm of

the minimum angle of resolution BCVA. We considered a P value of <0.05 to be statistically significant. The SPSS software package (Version 18.0, SPSS Inc., Chicago, IL) was used to perform the entire statistical analysis.

Results

This study was conducted on 45 eyes of 45 DR patients (mean age 58 ± 9 years). All the eyes were exhibited nonproliferative DR, and 21 eyes (41%) were affected by diabetic macular edema. The main clinical characteristics are shown in Table 1.

We detected and analyzed 216 retinal MAs, divided into green (46; 21%), red (58; 27%) and mixed type (112; 52%). Green MAs were mainly hyper-reflective on structural OCT, with no or poor perfusion apparent on OCTA examination (ICC, 0.98; $P < 0.01$) (Fig. 1). Red MAs displayed an isoreflective signal on structural OCT; these were found to be fully filled on OCTA (ICC, 0.99; $P < 0.01$) (Fig. 2). Mixed MAs showed a hyper-reflective border and hyporeflective core on structural OCT and these were found to be partially perfused on OCTA (Fig. 3).

Comparing MultiColor findings with IR images, red MAs were mainly characterized by hyporeflectance on IR, whereas the more prominent the green in the MultiColor image, the greater the hyper-reflectance of retinal MAs on IR.

As for mixed MAs, OCTA proved remarkably sensitive in distinguishing different MA subregions. Indeed, the detection and distribution of blood flow observed on OCTA in these MAs, where both perfused and fibrotic subregions coexist, was clearly spatially correlated with the preponderance of a lesional IR and green signal on confocal MultiColor imaging (ICC, 0.96; $P < 0.01$) (Fig. 4).

Turning to MA HR/HS size discrepancy (exemplified in Fig. 5), we found few differences in size and reflectivity when considering red MAs, whereas variations progressively increased as the MA multicolor signal changed from IR to green. Actually,

Table 1. Clinical Data

Clinical Data	Mean ± Standard Deviation[a]
No. of eyes	45
Mean age, years	58 ± 9
Gender, male/female	24/21
Hemoglobin A1c %	7.4 ± 1.2
Mean DR duration, years	2.5 (1–6)
Diabetic macular edema, %	21 (41%)
Overall LogMAR BCVA	0.11 ± 0.22
LogMAR BCVA diabetic macular edema only	0.34 ± 0.38
No. of injections	18 ± 13

[a]Unless otherwise specified.
LogMAR, logarithm of the minimum angle of resolution.

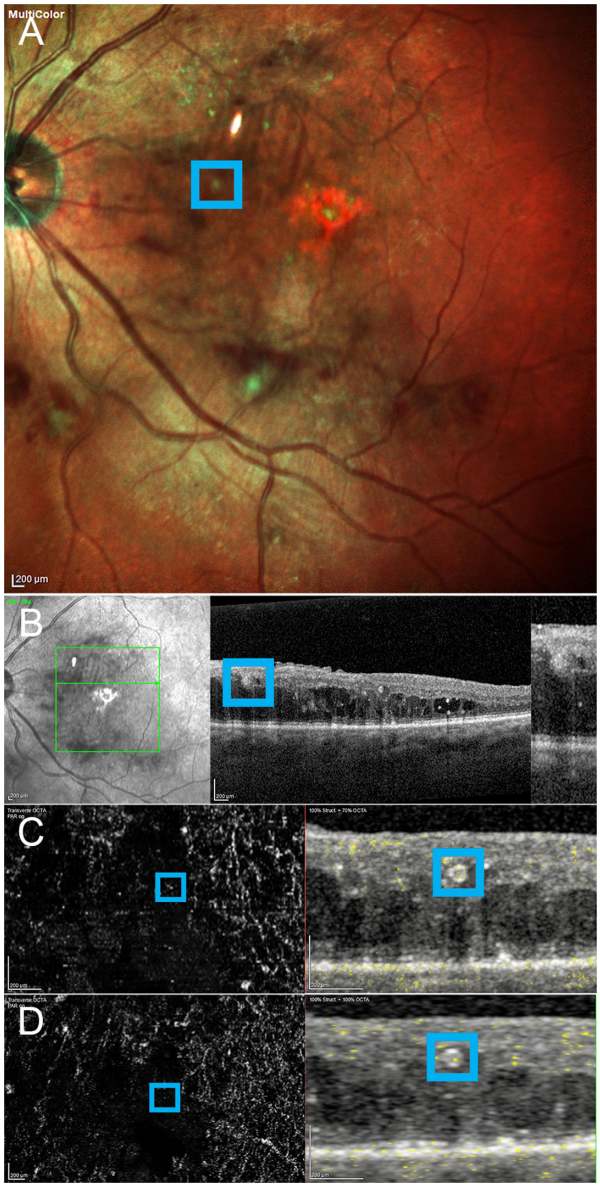

Figure 1. Multimodal imaging findings concerning green MAs. The fibrotic complication is well-detected by the green reflectance component on the confocal MultiColor image (A). On structural OCT, green MAs are mainly hyper-reflective and are almost undetectable on both HR (C) and HS (D) OCTA acquisitions, with just a spurious signal detected in HR OCTA.

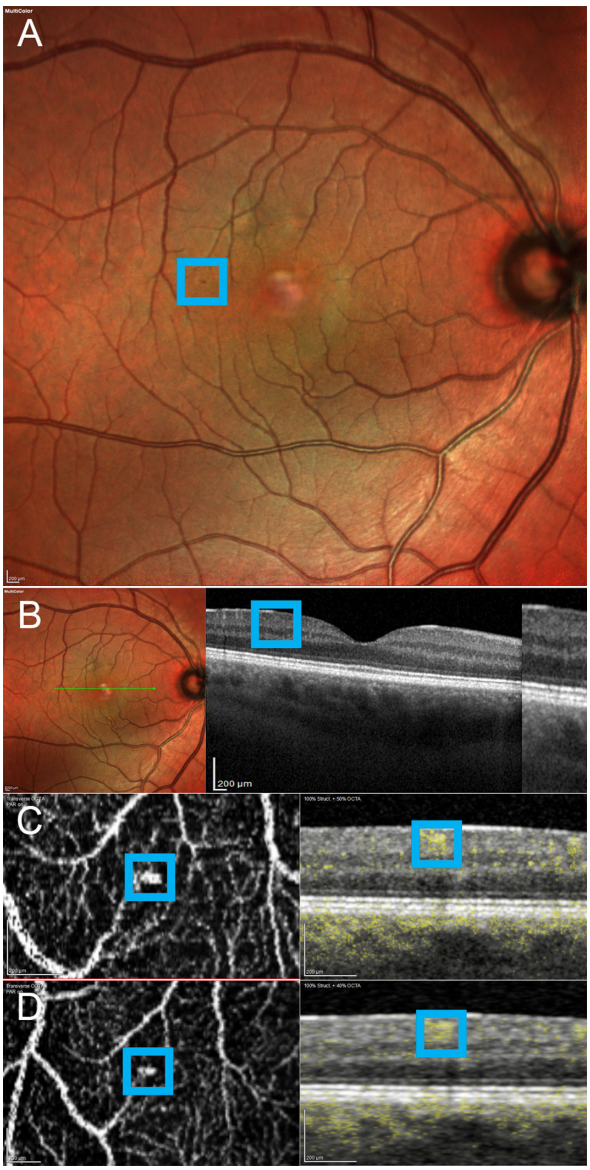

Figure 2. Red MA multimodal imaging findings. The IR reflectance component is predominant in the confocal MultiColor image (A). The red MA is mainly isoreflective on structural OCT, and clearly detected on both HR (C) and HS (D) OCTA acquisitions.

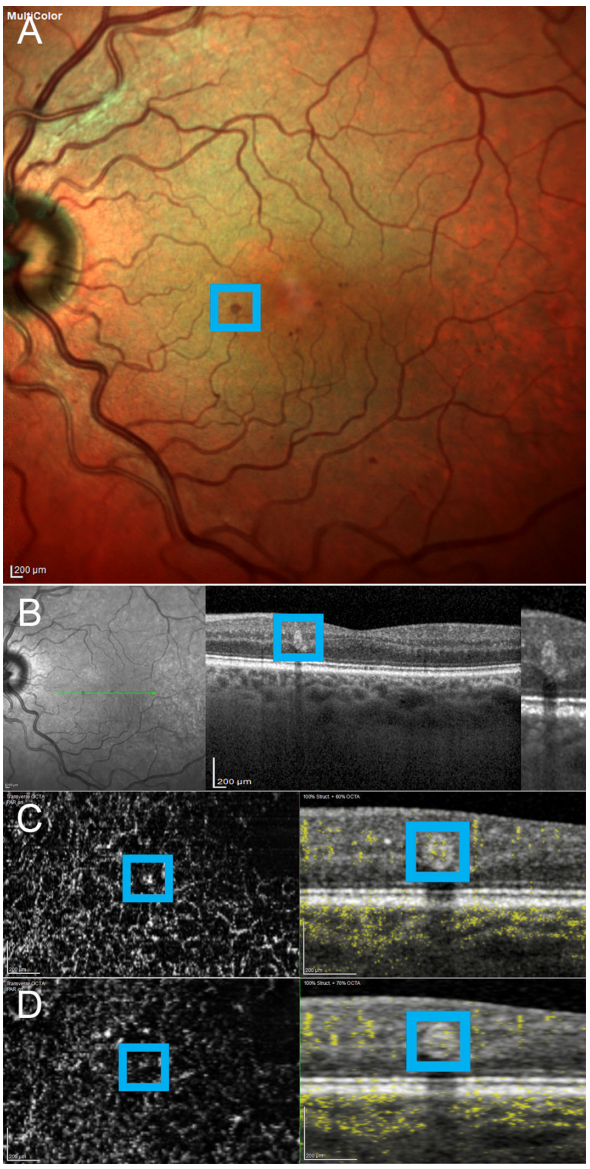

Figure 3. Mixed MA multimodal imaging findings. The confocal MultiColor image can detect both perfused (red) and fibrotic (green) MA subregions (A). Mixed MAs display hyper-reflective border and hyporeflective core on structural OCT, and a size mismatch visible on OCTA between the larger HR MA reconstruction (C) and the smaller HS one (D).

the HR/HS size discrepancy analysis of green MAs was considered unreliable because they were found to be almost undetectable with both HR and HS OCTA acquisitions. Conversely and significantly, a MA HR/HS size discrepancy was detected for mixed MAs, both in terms of size and reflectivity. Table 2 summarizes the HR/HS size discrepancy results. In more detail, the HR/HS size discrepancy was 10.2 ± 2.1% for red MAs, albeit without significant differences in terms of MA reflectivity between HR and HS OCTA acquisitions ($P > 0.05$). In contrast, the mixed MA HR/HS size discrepancy was much greater (39.6 ± 8.7%), with significantly lower MA reflectivity detected for HS OCTA acquisition ($P < 0.05$).

We have used these results, incorporating OCTA findings, to expand the previously published confocal MultiColor-based MA classification, where multimodal imaging findings were compared with MA histological classification (Table 3). Our MA multimodal imaging categorization showed significant correlation between the presence of one or more MA types and both visual acuity (Tau–Kendall coefficient, −0.623; $P < 0.01$) and DR severity

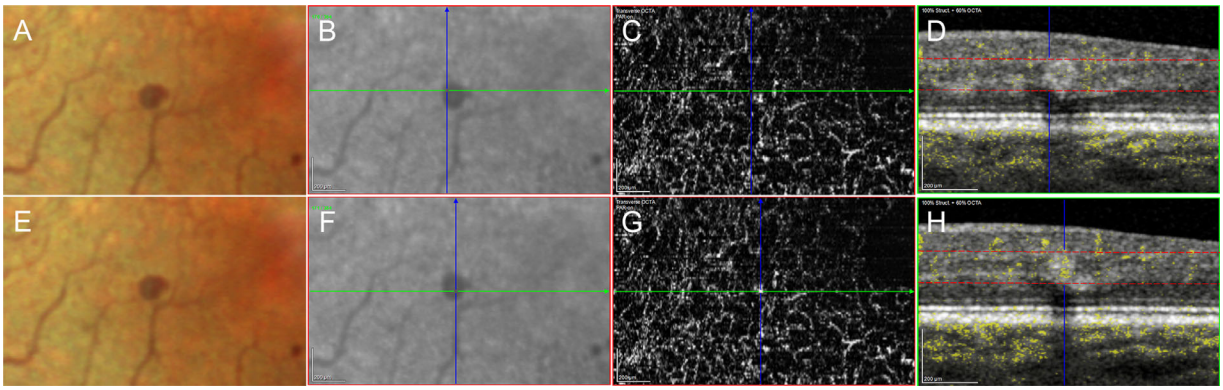

Figure 4. Different MA subregion detection. Confocal MultiColor image, showing mixed MA type (A, E). Corresponding enface OCT reconstruction shown in (B) and (F). The absence of perfusion signal, as recorded by both OCTA reconstruction (C) and structural OCT with superimposed motion signal (D), is detectable by selecting the OCTA slab on the green portion of the MA. In contrast, the perfused part of the MA is clearly detectable by placing the OCTA slab on the red portion, as recorded by the OCTA reconstruction (G) and structural OCT, with positive motion signal within the MA (H).

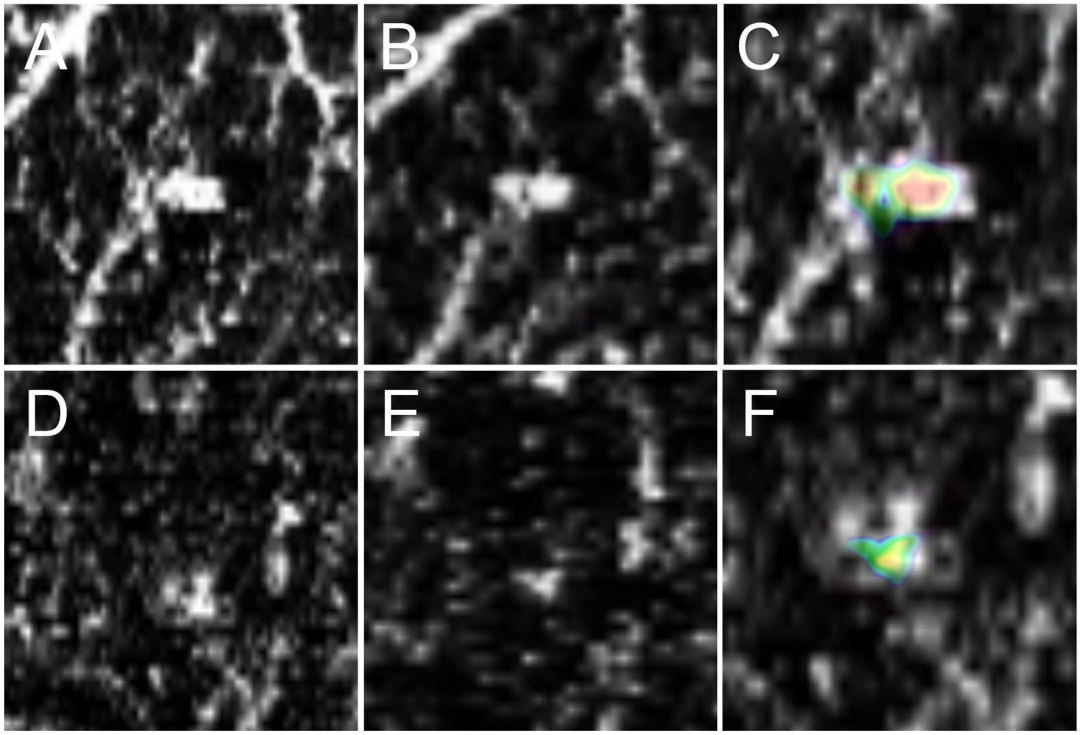

Figure 5. HR/HS OCTA gap. The first example is a confocal MultiColor red MA, detected by HR (A) and HS (B) OCTA acquisitions. The magnified overlapping image (C) shows the small HR/HS gap, with the HR-based MA considerably larger than the HS-based one. The second example shows a confocal MultiColor mixed red–green MA, which is detected better by HR (D) than HS (E) OCTA acquisition. The overlapping magnified image (F) shows the greater HR/HS gap, with HR-based MA proving to be much bigger than the HS-based one.

(presence/absence of diabetic macular edema) (Tau–Kendall coefficient, 0.588; $P < 0.01$). Moreover, the presence of mixed and green MAs was significantly associated both with DR duration (Tau–Kendall coefficient: 0.602; $P < 0.01$) and hemoglobin A1c percent (Tau–Kendall coefficient: 0.502; $P < 0.01$). The overall ICC was 0.96 (range, 0.94–0.98; $P < 0.01$).

Table 2. Quantitative Analysis of HR/HS Gap on OCTA

Quantitative Analysis of HR/HS Gap			
Multicolor MA Type	Parameter	Mean ± SD	*P* Value
Green MA	MA delta size %	Almost undetectable	
	MA reflectivity HR		
	MA reflectivity HS		
Red MA	MA delta size %	10.2 ± 2.1	
	MA reflectivity HR	118.2 ± 9.4	0.05
	MA reflectivity HS	113.5 ± 10.6	
Mixed MA	MA delta size %	39.6 ± 8.7	
	MA reflectivity HR	110.4 ± 7.3	0.05
	MA reflectivity HS	99.6 ± 11.8	

Table 3. Expanded Retinal MA Classification

Retinal MA Classification				
Histological Classification		Confocal MultiColor	Structural OCT	OCTA
Type I	Extensive accumulation of polymorphonuclear cells into the lumen, with intact endothelium and absent pericytes.	Red	Hyper-reflective margin and unevenly reflective core.	Fully perfused.
Type II	Large numbers of red blood cells in the lumen, with completely absent endothelial cells and pericytes.			
Type III	Nonperfused MA, with aggregates of irregularly shaped red blood cells profiles and breakdown products.	Mixed	Hyper-reflective border and hyporeflective core.	Partially perfused, with most of blood flow signal localized in the red portion of the MA.
Type IV	Almost or completely sclerosed, with extensive fibrosis and lipid infiltration into the lumen and basement membrane wall.	Green	Mainly hyper-reflective.	Poorly or not perfused.

Starting from the first confocal MultiColor-based MA classification,[6] we have now integrated OCTA information. The histological classification is adapted from Stitt et al. (1995).[9]

Discussion

This study presents a novel multimodal imaging classification of retinal MA that incorporates data obtained from MultiColor imaging, structural OCT, and OCTA. We confirm the findings described in our previous paper,[6] further highlighting the potentially useful role of confocal MultiColor imaging in DR diagnostics. MultiColor imaging is a useful, noninvasive way of detecting retinal MAs, which IR imaging is known to detected effectively. However, considering the red–green components on MultiColor, which correspond well to hyporeflectance or hyper-reflectance signals on IR, respectively, MultiColor may somehow make it easier to visualize retinal MAs and their perfusion–fibrosis components, reflecting the histological characteristics associated with MAs stages.[9] Furthermore, we now provide fresh details regarding MA perfusion obtained through OCTA. In our

previous study, the use of fluorescein angiography-led green MAs to be described as featuring poor filling and poor leakage phenomena, whereas red and mixed MAs both showed full filling and variable leakage.[6] Using OCTA, this study furnishes a precise picture of the blood flow features characterizing retinal MAs. Red MAs consistently seemed to be well-perfused, whereas mixed MAs showed variable perfusion, being more common in the region characterized by a red confocal MultiColor signal. The perfusion signal was almost absent in green MAs, as detected on OCTA. A comparison of the two different OCTA acquisitions, namely, HR and HS modes, yielded interesting additional information. Indeed, whereas red MAs showed a small HR/HS size discrepancy (10%) with no statistically significant differences in reflectivity values, mixed MAs were characterized by a greater HR/HS size discrepancy (40%) and significantly higher reflectivity signal for HR OCTA, compared with HS OCTA. HR OCTA may be more sensitive to temporary reductions of erythrocyte displacement (intermittent flow), to the extent that OCTA cannot detect these displacements, given the four-fold greater sampling density and consequently approximately four-fold longer total retention time of the OCT probe beam in the same retinal areas. The latter affords more time for local erythrocyte displacement and/or changes in the structural configuration of groups of blood cells during OCTA image acquisition, that is, in the context of turbulent flow. Under this assumption, HR OCTA may identify temporarily undetectable erythrocyte displacement better than HS OCTA, and this might occur more frequently in MAs. This hypothesis is consistent with our noninvasive quantitative evidence concerning blood flow intensity and MA size discrepancy (the HR/HS size gap), which is greater in red MAs. Red MA filling is also not affected by obstacles, whereas sclerotic complications are present in green MAs, leading to filling delays that may be more readily detected by HR OCTA and remain unrecognized by HS OCTA. Furthermore, the turbulent nature of the flow within the MA might play a small part in detecting the HR/HS gap, for both red and green MAs,[2] owing to OCTA signal underdetection mainly affecting HS OCTA acquisition, under the hypothesis outlined elsewhere in this article. From this standpoint, HS OCTA can be considered a feasible and less time-consuming OCTA acquisition, to be preferred for patients displaying unstable fixation, although the HS OCTA deficiencies in detecting mixed MA subtypes should be taken into account. As for the differences in MA reflectivity, these might be associated with the higher number of particles detected by HR OCTA than HS OCTA, including both faster and slower ones.

It is worth noting that the combined use of confocal MultiColor and OCTA examinations was very useful in distinguishing the differently perfused regions typical of mixed MAs, thus adding to the information contributed by fluorescein angiography. The other meaningful result of the present investigation is the significant relationship detected between our proposed MA multimodal imaging classification and clinically relevant data, including BCVA, DR severity, DR duration, and glycemic control. This finding paves the way for new diagnostic pipelines for improving the categorization of DR patients and follow-up strategies.

Our study has had to come to terms with some potential weaknesses, the first of which concerns the possible presence of imaging artifacts.[3] Of course, we are aware that all imaging modalities are potentially affected by artifacts, and we restricted their impact by including only high-quality data. Another undoubted limitation is the fact that a considerable percentage of retinal MAs (at least 20%) are not detected by noninvasive multimodal retinal imaging, compared with fluorescein angiography, as already assessed in our previous investigation.[6] These MAs are very small and often poorly perfused, and this factor might be a possible cause of the poor resolution and sensitivity of noninvasive approaches for these specific lesions. As a result of this shortcoming, fluorescein angiography continues to be regarded as the gold standard for retinal MA detection. Although this characteristic has an effect on the current performance of noninvasive imaging and points to the need for further technological improvements, it should not affect our proposed classification, which is buttressed powerfully by previous histological evidence. Moreover, owing to the cross-sectional nature of the present investigation, we were unable to provide data regarding the relationship between our MA classification and DR evolution. For this reason, further prospective studies are warranted to assess definitively the clinical usefulness of this MA multimodal imaging classification. We decided not to consider the contribution of fluorescein angiography here because, first, our previous study showed high agreement with confocal MultiColor imaging, and second, our main goal was to perform a completely noninvasive assessment of retinal MA. We also acknowledge that postprocessing analyses, including those performed through Image-J tools, are not standardized and, therefore, are prone to operator-based errors. Additionally, we suggest future studies might be dedicated to assessing how far MA histology matches our proposed MA classification. Last, further studies are also warranted to definitively clarify the underlying physical basis of MA HR/HS size discrepancy.

In conclusion, retinal MA secondary to DR can be reliably categorized by means of a fully noninvasive multimodal imaging-based classification. This approach has a considerable bearing on clinical practice, because it has proved to be associated with BCVA, DR stage and DR duration. HR and HS OCTA acquisitions are reliable ways of obtaining MA blood flow perfusion information, although HR OCTA was found to be highly sensitive in detecting a wider range of perfusion signals, especially in the presence of fibrotic evolution. Our data have given a new lease of life to the role of MA assessment in DR diagnostics, and offer a glimpse of intriguing future prospects regarding the development of artificial intelligence-based approaches.

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References

1. Pappuru RKR, Ribeiro L, Lobo C, Alves D, Cunha-Vaz J. Microaneurysm turnover is a predictor of diabetic retinopathy progression. *Br J Ophthalmol*. 2019;103(2):222–226.
2. Dubow M, Pinhas A, Shah N, et al. Classification of human retinal microaneurysms using adaptive optics scanning light ophthalmoscope fluorescein angiography. *Invest Ophthalmol Vis Sci*. 2014;55(3):1299–1309.
3. Arrigo A, Aragona E, Battaglia Parodi M, Bandello F. Quantitative approaches in multimodal fundus imaging: state of the art and future perspectives. *Prog Retin Eye Res*. 2022;92:101111.
4. Sun Z, Yang D, Tang Z, Ng DS, Cheung CY. Optical coherence tomography angiography in diabetic retinopathy: an updated review. *Eye (Lond)*. 2021;35(1):149–161.
5. Tan AC, Fleckenstein M, Schmitz-Valckenberg S, Holz FG. Clinical application of MultiColor imaging technology. *Ophthalmologica*. 2016;236(1):8–18.
6. Arrigo A, Teussink M, Aragona E, Bandello F, Battaglia Parodi M. MultiColor imaging to detect different subtypes of retinal microaneurysms in diabetic retinopathy. *Eye (Lond)*. 2021;35(1):277–281.
7. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;9(7):676–682.
8. Arrigo A, Aragona E, Saladino A, et al. The impact of different thresholds on optical coherence tomography angiography images binarization and quantitative metrics. *Sci Rep*. 2021;11(1):14758.
9. Stitt AW, Gardiner TA, Archer DB. Histological and ultrastructural investigation of retinal microaneurysm development in diabetic patients. *Br J Ophthalmol*. 1995;79(4):362–367.