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ORIGINAL RESEARCH

Microaneurysm Counting as a Biomarker for the Hyperperfusion Stage of Nonproliferative Diabetic Retinopathy

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ABSTRACT

Introduction: This study aimed to investigate the utility of microaneurysm (MA) counting as a tool for characterizing the hyperperfusion stage of nonproliferative diabetic retinopathy (NPDR) and to examine the hypothesis that MAs can serve as a surrogate biomarker for the presence of intraretinal microvascular abnormalities (IRMAs).

Methods: Forty-nine ($n=49$) eyes with type 2 diabetes mellitus with NPDR were included in this analysis: 12 with Early Treatment Diabetic Retinopathy Study (ETDRS) levels 43 and 37 with levels 47–53. Automated MA detection was performed using the RetmarkerDR software (Retmarker SA, Meteda Group, Italy), alongside manual detection, both done in the central retina (field 2). Based on MA counts, microaneurysm turnover (MAT) was computed. IRMAs were manually counted based on swept-source optical coherence tomography (SS-OCT) angiography on PLEX® Elite 9000 (ZEISS, Dublin, CA, USA). The statistically significant differences between ETDRS groups were studied by comparing Mann–Whitney *U* test *p* values (significance value < 0.05). The correlation between the presence of MAs and IRMAs and MAT and IRMAs was examined using Spearman correlation analysis.

Results: There was an observed increase in the number of IRMAs, MAs, and MAT values as NPDR progressed, independently of the counting method used. Specifically, this increase was noted when transitioning from ETDRS groups characterized by the predominance of the hypoperfusion stage (ETDRS 43) to those associated with the hyperperfusion stage (ETDRS 47–53). When MAs were counted manually, a moderate correlation was identified between the number of MAs and the presence of IRMAs ($\rho=0.40$; *p* value = 0.005). Additionally, a similar

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correlation was found between MAT and the presence of IRMAs (ρ = 0.43; *p* value = 0.002).
Conclusions: This study underscores the potential relevance of MAs as a pivotal indicator of the hyperperfusion stage of NPDR and supports their role as surrogate biomarkers for IRMAs. These results suggest a role for MA counting in the assessment and management of diabetic eye disease.

Keywords: Diabetes; Retinopathy; Microaneurysms; Intraretinal microvascular abnormalities; Fundus photography; Wide-field optical coherence tomography angiography

Key Summary Points

Why carry out this study?

This study was conducted to evaluate the role of microaneurysm (MA) counting as a potential biomarker for the hyperperfusion stage of nonproliferative diabetic retinopathy (NPDR).

Given the progressive nature of diabetic retinopathy (DR) and its stages of hypoperfusion (ETDRS 43) and hyperperfusion (ETDRS 47–53), identifying reliable biomarkers is crucial for better disease characterization and management.

The study aimed to explore whether microaneurysms could serve as a surrogate marker for intraretinal microvascular abnormalities (IRMAs), which are indicative of the hyperperfusion stage. Additionally, the utility of an automated microaneurysm counting tool was assessed, considering its potential benefits in clinical practice for reproducibility and efficiency in DR screening programs.

What was learned from the study?

The study demonstrated that microaneurysm turnover increases with the progression of NPDR, indicating its potential as a biomarker for the hyperperfusion stage (ETDRS 47–53).

A positive correlation was found between the presence of microaneurysms and IRMAs, supporting the hypothesis that microaneurysms can serve as a surrogate marker for IRMAs. Furthermore, while manual MA counting showed stronger correlations with IRMAs, automated methods provided reliable results with improved reproducibility, reinforcing their potential application in diabetic retinopathy monitoring and screening.

INTRODUCTION

Diabetic retinopathy (DR) is a frequent complication of diabetes and, through its vision-threatening complications, i.e., clinically significant macular edema (CSME) and proliferative diabetic retinopathy (PDR), may lead to blindness. It is estimated that by 2045, there will be 783 million people worldwide affected by diabetes [1]. DR is a progressive disease that can be divided into two main stages: nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). The gold standard method to perform the staging of DR, the Early Treatment Diabetic Retinopathy Study (ETDRS) Grading, relies on identifying specific lesions in color fundus photographs (CFP), mostly associated with microvascular disease [2].

Nonproliferative diabetic retinopathy progresses through stages of hypoperfusion and hyperperfusion, as proposed by Curtis et al. [3]. These stages reflect the compensatory hemodynamic mechanisms seen in the microvasculature as the retinopathy progresses. First, a

hypoperfusion stage is identified in the early stage of NPDR, such as ETDRS levels 20, 35, and 43, characterized primarily by capillary closure. This stage is marked by a reduction in skeletonized vessel density (SVD) and perfusion density (PD), affecting both retinal capillary plexuses. This is followed by a hyperperfusion stage, which appears to be a response to the progression of hypoperfusion and seems to occur in ETDRS level 47. This stage is marked by dilated shunt vessels, intraretinal hemorrhages, and is apparently due to a compensatory response to hypoxia via vasodilation and increased blood flow. Hyperperfusion is likely a compensatory response. It manifests as dilation of some capillaries and the formation of abnormal shunts (e.g., intraretinal microvascular abnormalities, IRMAs), which serve as bypass pathways for the closing capillary bed [4].

One important question that this work aims to answer is whether microaneurysms (MAs) can be useful as a surrogate for the presence of IRMAs. Currently, the availability of automatic tools and artificial intelligence (AI) that allow the automatic detection of MAs remains limited. An example of an automatic MA detector is the RetmarkerDR software (Retmarker SA, Meteda Group, Italy). The main advantages of automated tools are the reduction of the time spent by an expert in the analysis of the raw data, as well as guaranteeing the reproducibility of the results. Automatic tools are especially needed in the scope of screening programs, where a very large amount of data is acquired in a very short time.

In this work, we also evaluate the performance of an automated method for counting MAs, the implications for turnover within a 6-month interval for characterizing the later stages of DR, and the implications for the correlation with the presence of IRMAs.

METHODS

Data preparation, cleaning, and analysis were performed using the Julia language (version 1.11) and Python (version 3.9) and its associated ecosystem that includes DataFrames.jl, CSV.jl, GLM.jl, MixedModels.jl, Statistics.jl, Distributions.jl, and summaryTables.jl.

Study Population

This study utilized data collected at the Association for Innovation and Biomedical Research on Light and Image (AIBILI), comprising 49 eyes with type 2 diabetes mellitus (T2DM), all classified as having ETDRS levels greater than 43. The selection criteria required patients who had baseline SS-OCTA PlexElite data with IRMA annotations from a prior study (NCT05112445), as well as CFP images at baseline (acquired on the same day as SS-OCTA) and at the 6-month follow-up. Data included details on age, diabetes duration, and sex. Demographic, clinical, and ophthalmological characteristics are described in Table 1. The dataset was categorized into two groups based on ETDRS grading: ETDRS level 43 (12 eyes) and ETDRS levels 47–53 (37 eyes). All patients were identified and recruited through AIBILI's electronic medical record system as part of the ongoing EYEMARKER project—*"Characterization of Potential Biomarkers of Eye Disease and Vision Loss"* (CEC/009/17). The study received approval from the local health ethics committee, adhering to the tenets of the Declaration of Helsinki. Each participant provided written informed consent after having all procedures thoroughly explained.

Criteria for Data Quality

The exclusion criteria for selection of patients includes if they had significant cataracts, glaucoma, recent eye surgery within the 6 months prior to the baseline visit, other retinal vascular diseases that in the opinion of the investigator may affect retinopathy status or alter visual acuity during the study, prior laser treatments or intravitreal injections, or if their pupil dilation was less than 5 mm. Additional exclusion criteria included glycated hemoglobin A1C (HbA1c) levels exceeding 10% (85.8 mmol/mol) and other systemic conditions that could impact ocular health, with particular attention to uncontrolled systemic hypertension and a history of heart or kidney disease.

Table 1 Demographic, clinical, and ophthalmological characteristics of included patients

	Total ($n = 49$)	ETDRS_baseline	
		43 ($n = 12$)	47–53 ($n = 37$)
Age (years)			
Mean (SD)	67.5 (8.86)	66.3 (9)	67.9 (8.9)
Median (min, max)	70 (50, 84)	68.5 (50, 79)	70 (51, 84)
Sex			
Female	10 (20.4%)	1 (8.33%)	9 (24.3%)
Male	39 (79.6%)	11 (91.7%)	28 (75.7%)
Diabetes duration (years)			
Mean (SD)	21.6 (7.93)	21.7 (7.05)	21.6 (8.28)
Median (min, max)	22 (4, 42)	21.5 (10, 32)	22 (4, 42)
BMI (kg/m^2)			
Mean (SD)	30 (4.7)	30.2 (5.32)	29.9 (4.56)
Median (min, max)	29 (24, 43)	28.5 (24, 43)	29 (24, 40)
HbA1c (%)			
Mean (SD)	7.61 (1.11)	7.67 (0.985)	7.59 (1.17)
Median (min, max)	7 (5, 10)	7.5 (6, 9)	7 (5, 10)
BCVA (letters)			
Mean (SD)	83 (5.39)	84.9 (5.26)	82.4 (5.35)
Median (min, max)	85 (68, 94)	85 (73, 90)	84 (68, 94)

Color Fundus Photography (CFP) and ETDRS Classification

Seven-field CFP images were obtained at 35° field of view, using a Topcon TRC-50DX mydriatic retinal camera (Topcon Medical Systems, Tokyo, Japan), with a resolution of 3596 × 2448 pixels. DR severity grading was performed according to ETDRS protocol based on the identification of lesions, such as microaneurysms, hemorrhages, IRMA, soft or hard exudates, and venous beading or presence of neovascularization. The DR severity scores were classified at Coimbra Ophthalmology Reading Centre (CORC), according to Diabetic Retinopathy Severity Scoring System. ETDRS classification was performed at baseline.

IRMAS Detection

IRMAs were identified in the swept-source optical coherence tomography (SS-OCTA) images by a single grader in the retina of the posterior pole (up to 50° field of view) using the PLEX® Elite 9000 (ZEISS, Dublin, CA, USA) SS-OCTA device and the Angio 15 mm × 15 mm acquisition protocol. This SS-OCTA system utilizes a tunable 1060-nm laser, achieving an axial resolution of 6.3 µm. The acquisition protocol covers a 50° retinal field, generating angiography slab images through two repetitions of 834 B-scans, each composed of 834 A-scans, at an acquisition rate of 100,000 A-scans/s.

According to the ETDRS severity scale, IRMAs are present in the more advanced stages of NPDR (ETDRS levels ≥ 43B). For the purposes of this study, IRMAs were defined as capillary tortuosities spanning a minimum equivalent circular area of 300 µm, corresponding to ETDRS standard photo 8A. IRMAs serve as a traditional biomarker indicative of the onset of the hyperperfusion stage.

Microaneurysms Detection and Turnover Assessment

The detection of MAs was performed using RetmarkerDR (Retmarker SA, METEDA company, Rome, Italy) and through manual grading of 50° field 2 images. Automated MA detection was conducted using RetmarkerDR, a computer-aided diagnostic software designed to earmark and identify macular red dot-like vascular lesions. Manual grading was performed on the same images analyzed by RetmarkerDR, using the annotation tools available in the software. In Figure 1 is an example of MAs detected manually for an eye classified as ETDRS 43 and an eye classified as ETDRS 47–53. To assess reproducibility, two graders manually evaluated a subset of nine eyes (18 images). In addition to automated MA detection, RetmarkerDR supports the registration of CFPs acquired at two different time instants (baseline and 6 months in this study), enabling the counting of new, old, and disappeared MAs. These counts are used to calculate the MA formation rate and the MA disappearance rate. The MAT is the sum of the MA formation and disappearance rates.

Statistical Analysis

Statistical analyses were performed using the Julia programming language (julialang.org; version 1.11) and Python (version 3.9) along with relevant packages (Statistics.jl, Distributions.jl, and summaryTables.jl). The statistical tables in this study report measures such as the mean, standard deviation, median, maximum, and minimum values.

Descriptive statistics were stratified by ETDRS severity levels and include the number of microaneurysms (MAs) at baseline (reflecting available ETDRS levels) and the microaneurysm turnover (MAT) calculated over 6 months. The agreement between manual and automated detection methods was evaluated using the intraclass correlation coefficient (denoted as ICC; single fixed raters). Additionally, the ICC was employed to assess interobserver agreement between two independent graders. Correlation strength for ICC was categorized as follows: very weak ($0.00 \le ICC < 0.20$), weak ($0.20 \le ICC < 0.40$), moderate ($0.40 \le ICC < 0.60$), strong ($0.60 \le ICC < 0.80$), and very strong ($0.80 \le ICC \le 1.00$).

The ability of the two detection methods to have statistical differences between ETDRS groups was examined using the Mann–Whitney U test, and the corresponding p values were compared. Spearman's rank correlation coefficient (denoted as ρ) was utilized to explore the relationship between MA and MAT counts and the presence of IRMAs. The strength of

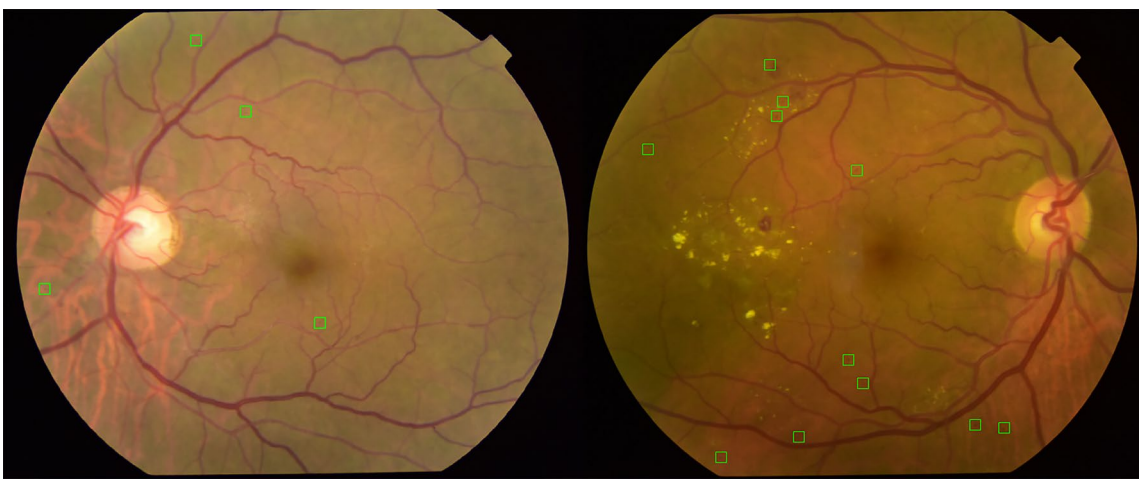

Fig. 1 MAs detected manually for an eye classified as ETDRS 43 (*left*) and an eye classified as ETDRS 47–53 (*right*)

Spearman's correlation (ρ) was classified as very weak ($0.00 \le \rho < 0.20$), weak ($0.20 \le \rho < 0.40$), moderate ($0.40 \le \rho < 0.60$), strong ($0.60 \le \rho < 0.80$), or very strong ($0.80 \le \rho \le 1.00$).

In this study, p values below 0.05 were considered statistically significant. The interobserver agreement between the two graders was also analyzed using the intraclass correlation coefficient (single fixed raters).

RESULTS

IRMAs and MAs Counts

A statistically significant difference was observed in the manually counted IRMAs between ETDRS groups 43 and 47–53 (Table 2). The median IRMA count increased from 0 IRMAs in ETDRS group 43 to 2 IRMAs in ETDRS group 47–53 ($p = 0.014$).

In terms of MAs, the overall median number counted by the manual grader was 3.6 times higher than the count obtained using the automated method (11 vs. 3, respectively). The increase in the median MA count for manual grading between ETDRS groups 47–53 and 43 was 1.5 times higher (12.5 vs. 8), while for the automated method, it was twice as high (4 vs. 2). However, no statistically significant difference was detected between ETDRS groups 43 and 47–53 using either method (manual: p value = 0.068 and automated: p value = 0.103).

The ICC was employed to assess the agreement between MA counts obtained by the manual grader and the automated method, indicating good consistency of measurements (ICC = 0.80; 95% confidence interval, 0.66; 0.88). Interobserver agreement between two manual graders was very strong, as reflected by an ICC value of 0.88 (Table 3).

MAT (Between Baseline and 6 Months)

The overall absolute median value for MAT obtained through manual grading was 31.1, whereas for the automated method it was 9.15 (3.4 times higher). MAT values demonstrated an increase in accordance with the severity of diabetic retinopathy (DR), regardless of the detection method.

For manual grading, the median MAT increased by 2.6 times between ETDRS group 43 and ETDRS group 47–53 (18 vs. 35, with $p = 0.008$). Using automated grading, the median MAT increased by 2.32 times, from 4.74 in ETDRS group 43 to 11 in ETDRS group 47–53 ($p = 0.050$). Across all measurement methods, MAT showed a statistically significant difference between ETDRS groups analyzed. The agreement between manual and automated methods was very strong with an ICC value of 0.88.

Correlation Between the Presence of MAs in CFP and the Presence of IRMAs in OCTA

Spearman correlation analysis was performed to investigate the relationship between the presence of MAs in CFP and the presence of IRMAs in OCTA (Table 4). Statistically significant correlations were observed only with manual grading. The correlation coefficient (ρ) for the relationship between MAs and IRMAs was 0.40 ($p = 0.005$), reflecting a moderate correlation. Similarly, for the correlation between MAT and IRMAs, a coefficient (ρ) of 0.43 ($p = 0.002$) was recorded, indicating moderate correlation. In Figure 2 is presented MAs and IRMAs manually detected on an eye classified as ETDRS 47.

DISCUSSION

In this study, we investigated the hypothesis that MAs can serve as surrogate biomarkers for the presence of IRMAs and as a tool for characterizing the transition between the hypoperfusion and hyperperfusion stages of DR.

An elevated MA count has been associated with increased retinal ischemia and vascular instability [5–7]. Previous studies have shown that MA counts correlate with the progression of DR [8–10], making them valuable for both diagnosis and disease monitoring. Additionally, MAs have been categorized by their morphology and appearance as focal bulge, saccular or pedunculated, fusiform, and mixed saccular/fusiform

Table 2 Descriptive statistics of the MAs, MAT, and IRMAs counted by human grader or automatic method

	Total (***n*** **= 49**)	**ETDRS_baseline**		***p*** **value**
		43 (***n*** **= 12**)	**47–53** (***n*** **= 37**)	
IRMAS				**0.014**
Mean (SD)	2.1 (3.21)	0.91 (1.73)	2.49 (3.49)	
Median (min, max)	1.0 (0, 19)	0 (0, 6)	2.0 (0, 19)	
MA_Manual				0.068
Mean (SD)	12.5 (9.23)	8.58 (5.04)	13.9 (9.96)	
Median (min, max)	11.0 (0, 56)	8.00 (0, 17)	12.5 (2, 56)	
MA_RETMARKER				0.103
Mean (SD)	5.43 (6.99)	2.92 (2.54)	6.24 (7.77)	
Median (min, max)	3.0 (0, 42)	2.00 (0, 9)	4 (0, 42)	
MAT_Manual				**0.008**
Mean (SD)	35.9 (29.5)	20.7 (14.7)	41 (31.6)	
Median (min, max)	31.1 (0, 188)	18.00 (0, 49.1)	35 (10.8, 188)	
MAT_RETMARKER				**0.050**
Mean (SD)	13.7 (20.7)	6.2 (5.9)	16.2 (23.2)	
Median (min, max)	9.1 (0, 133)	4.7 (0, 18.7)	11 (0, 133)	

The statistical differences between ETDRS groups were examined using the Mann–Whitney *U* test, and the corresponding *p* values were compared

Table 3 Human grader variability. Descriptive statistics for the manual detection of MAs by two different human graders

	Total ($n = 18$)	Grader		ICC	95% CI
		1 ($n = 9$)	2 ($n = 9$)		
MA				0.88	(0.59, 0.97)
Mean (SD)	19.9 (19.6)	16.1 (15.8)	23.8 (23.1)		
Median (min, max)	15 (4, 82)	11 (4, 56)	17 (7, 82)		
MAT				0.96	(0.85, 0.99)
Mean (SD)	52.9 (57.1)	49.6 (53.3)	56.3 (63.8)		
Median (min, max)	33.4 (9.26, 224)	36.5 (9.26, 188)	31.3 (25.9, 224)		

The ICC was employed to assess interobserver agreement between two independent graders. Correlation strength for ICC was categorized as follows: very weak ($0.00 \leq ICC < 0.20$), weak ($0.20 \leq ICC < 0.40$), moderate ($0.40 \leq ICC < 0.60$), strong ($0.60 \leq ICC < 0.80$), and very strong ($0.80 \leq ICC \leq 1.00$)

Table 4 Association between the presence of IRMA (manual counted in SS-OCTA) and number of MA or MAT counted by a human grader or using Retmarker (up to 50 degrees of the retina)

	ρ	p value
MA		
Manual	0.40	**0.005**
RETMARKER	0.25	0.079
MAT		
Manual	0.43	**0.002**
RETMARKER	0.24	0.100

Spearman correlation coefficient (ρ) categorization: very weak ($0.00 \leq \rho < 0.20$), weak ($0.20 \leq \rho < 0.40$), moderate ($0.40 \leq \rho < 0.60$), strong ($0.60 \leq \rho < 0.80$), or very strong ($0.80 \leq \rho < 1.00$)

Values represent statistically significant alterations with $p < 0.05$ using Spearman correlation

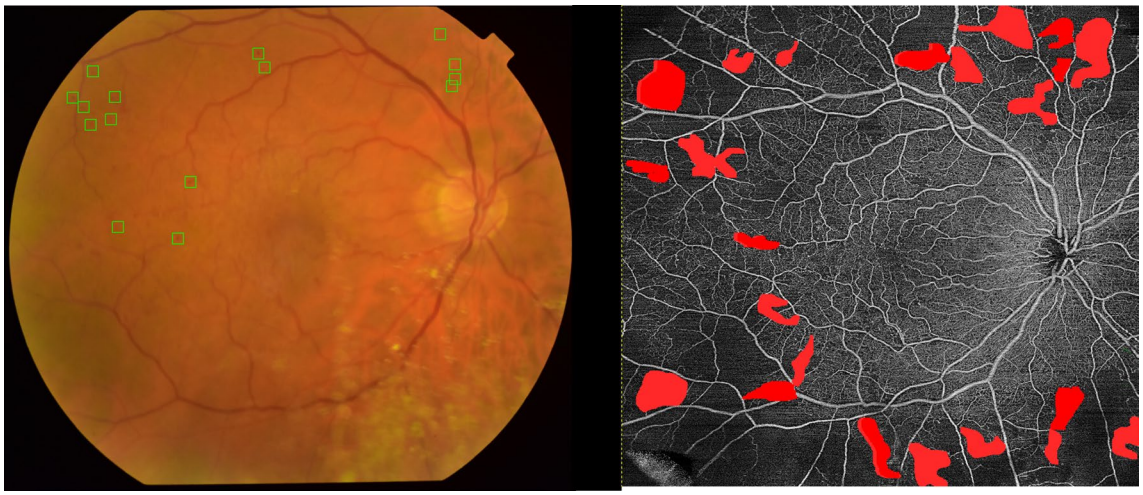

Fig. 2 MAs (left) and IRMAs (right) manually detected on an eye classified as ETDRS 47. The green box corresponds to the MAs detected. The red ROIs correspond to the IRMAs manually detected

[11]. These different morphologies explain why their identification may vary depending on the presence of different MA subtypes and the methods of examination used. IRMAs are observed only in advanced stages of nonproliferative NPDR and are associated with increased severity of the disease. In this study, we observed that the number of IRMAs, MAs, and MAT increased with the progression of DR, particularly when transitioning from the predominance of the hypoperfusion stage (ETDRS group 43) to the predominance of the hyperperfusion stage (ETDRS groups 47–53, Table 2). MAs, regardless of the counting method (manual or automated), demonstrated an increase as the progression of DR moved from predominant hypoperfusion to predominant hyperperfusion stages. Although this increase was not statistically significant, the findings suggest that MAs may play a crucial role as biomarkers of the hyperperfusion stage.

Our results align with the hypothesis that MAs are predominantly located in dilated shunt vessels resulting from progressive capillary nonperfusion [12]. These dilated shunt vessels appear to act as precursors to IRMAs, which are themselves associated with the hyperperfusion stage and are preferential sites for the development of new vessels and proliferative retinopathy [13]. This hypothesis is further supported by MAT analysis. MAT values, which represent the formation and disappearance rates of MAs, significantly increased with DR severity, reinforcing its association with the hyperperfusion stage (ETDRS groups 47–53). Overall, the number of MAs and MAT values obtained through manual grading was approximately twice as high as those derived from the automated RetmarkerDR tool. A similar trend was observed in the separation of ETDRS groups. The intraclass correlation coefficient analysis demonstrated strong agreement between manual and automated methods for both MA and MAT quantification. However, it must be realized that one advantage of using RetmarkerDR for counting MAs is that it ensures the reproducibility of the results. Despite the strong correlation between two manual graders (MA ICC = 0.88; 95% confidence interval (0.59, 0.97); MAT ICC = 0.96; 95% confidence interval (0.85, 0.99)), manual MA identification remains prone to errors, as reflected by discrepancies in counts median values between graders (Table 3).

Quantification of IRMAs is particularly challenging due to their irregular shapes and variable dimensions [14]. Using manual MA counts, we found a moderate correlation (Table 4) between the number of MAs and the presence of IRMAs, as well as between MAT and IRMAs. These

findings highlight the potential role of MAs as surrogate biomarkers for IRMAs. However, the automated method for MA counting failed to achieve statistically significant correlations, suggesting that its ability to detect MA types associated with IRMAs may be lower compared to manual methods. One of the limitations of this study is the restricted field of view. Although we acquired SS-OCTA images using one of the widest fields currently available in OCTA technology, the field of view is still smaller compared to other imaging modalities, such as wide-field acquisitions like OPTOS (~ 200 degrees). This limitation is particularly relevant for IRMA detection, as IRMAs are expected to appear more frequently in the peripheral retina in advanced stages of NPDR. Additionally, the automated technology used for detecting MAs is limited to CFP with a 50-degree field of view.

CONCLUSIONS

The present study underscores the importance of MAs as pivotal indicators for characterizing the hyperperfusion stage of NPDR. This study supports the hypothesis that MAs can serve as a surrogate biomarker for the presence of IRMAs in NPDR, reinforcing their role in monitoring DR's progression and severity. Our results also show that while the automated method detects fewer MAs compared to manual grading, it still yields reliable results that closely align with human grader assessments, demonstrating the utility of such technology in routine clinical practice. The agreement between manual and automated methods further substantiates the validity of the automated approach. Moreover, the turnover of MAs has been identified as a critical indicator of disease severity, aiding clinicians in identifying the patients who are at higher risk for progression to vision-threatening complications. Overall, these findings advocate the incorporation of MAs counting in the assessment and management of diabetic eye disease, contributing to better patient care and outcomes.

Author Contributions. Luís Mendes and José Cunha-Vaz designed and conducted the study, collected, analyzed, and interpreted the data, wrote, revised, and edited the manuscript. Ana Rocha, Marta Lopes, Ana Almeida, Nicole Duarte, Débora Reste-Ferreira, António Martinho, Pedro Pereira, Inês Marques, and Conceição Lobo assisted in the analysis and interpretation of the data and revised the manuscript.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Luís Mendes, Ana Rocha, Marta Lopes, Ana Almeida, Nicole Duarte, Débora Reste-Ferreira, António Martinho, Pedro Pereira, Inês Marques, and Conceição Lobo declare there are no conflicts of interest. Pedro Pereira was a Retmarker employee. José Cunha-Vaz reports grants from Bayer, Boehringer Ingelheim and Carl Zeiss Meditec and is a consultant for Alimera Sciences, Bayer, Boehringer Ingelheim, Carl Zeiss Meditec and Roche. José Cunha-Vaz is an Editorial Board member of Ophthalmology and Therapy. José Cunha-Vaz was not involved in the selection of peer reviewers for the manuscript nor any of the subsequent editorial decisions.

Ethical Approval. The tenets of the Declaration of Helsinki were followed, and approval was obtained from the AIBILI Ethics Committee for Health with the number CEC/009/17- EYEMARKER. Written informed consent was obtained by each participant agreeing to participate in the study.

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