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ORIGINAL RESEARCH

Neovascular Age-Related Macular Degeneration Treated with Aflibercept: Five-Year Follow-Up and Correlation with Optical Coherence Tomography Biomarkers in a Real-World Setting

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ABSTRACT

Introduction: To evaluate long-term outcomes of neovascular age-related macular degeneration(nAMD) treatment with aflibercept in a treat-and-extend (T&E) regimen and explore correlations between optical coherence tomography (OCT) biomarkers and clinical evolution over 5 years in a real-world setting.

Methods: This retrospective monocentric study included patients diagnosed with type 1 or type 2 nAMD at the University Magna Graecia of Catanzaro between 2016 and 2024. Inclusion criteria were treatment-naïve status, diagnosis confirmed by OCT and optical coherence tomography angiography (OCT-A), exclusive treatment with intravitreal aflibercept following a T&E regimen, and a minimum 5-year follow-up. Best-correct visual acuity (BCVA) and OCT were assessed at baseline, after the third injection, and yearly up to year 5 (seven time points). Evaluated OCT biomarkers included subretinal and intraretinal fluid (SRF, IRF), hyperreflective spots (HS), drusenoid pigment epithelial detachment (dPED), subretinal hyperreflective material (SHRM), outer retinal tubulations (ORT), onion sign, retinal pigment epithelium (RPE) tears, and subretinal fibrosis.

Results: Among 59 cases, 57.6% were type 1 macular neovascularization (MNV) and 42.4% type 2. At the baseline, SRF was more common in type 1, SHRM in type 2. Central foveal thickness (CFT)

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decreased significantly in both groups after loading and remained stable. SRF decreased significantly in type 1 (p=0.001), but not in type 2. dPED decreased in both groups, significantly in type 1 (p=0.01). HS decreased in patients with type 1 MNV (p=0.009). RPE tears were more frequent in type 2 (12%) and linked to BCVA loss. For type 2 MNV, ORT (p=0.035) and subretinal fibrosis appeared from year 5 (p=0.006). BCVA improved after loading in both groups, declined after year 2, and was better preserved in those with better visual acuity after the loading dose.

Conclusions: Baseline SHRM in type 2 MNV may predict more IRF, ORT, and RPE tears over time. Type 1 MNV with baseline SRF often shows reduced HS and dPED but may develop subretinal fibrosis. BCVA gains after loading wane by year 5, and eyes needing ongoing treatment for persistent OCT biomarkers decline gradually in both subtypes (significant in type 1).

Keywords: Macular neovascularization; Age-related macular degeneration; Aflibercept; Treat-and-Extend; Optical coherence tomography biomarkers

Key Summary Points

Emphasize key findings—especially how optical coherence tomography (OCT) biomarkers (e.g., subretinal hyperreflective material [SHRM], intraretinal fluid [IRF], outer retinal tubulations [ORT], retinal pigment epithelium [RPE] tears) predict disease activity.

Our aim was to evaluate neovascular age-related maculopathy (nAMD) treatment outcomes and assess the potential correlations between OCT biomarkers and long-term clinical results over a 5-year follow-up period in a real-world setting.

The presence of SHRM and subretinal fluid (SRF) at baseline were associated, over the course of follow-up, with a greater incidence of retinal alterations and subretinal fibrosis, respectively.

Despite anti-vascular endothelial growth factor (anti-VEGF) treatment, the best-corrected visual acuity (BCVA) tended to worsen due to instauration of retinal alteration.

INTRODUCTION

Age-related macular degeneration (AMD) remains one of the leading causes of severe and irreversible vision loss worldwide [1, 2]. Advanced AMD is characterized by two different nosological entities: geographic atrophy (GA) and macular neovascularization (MNV). Although neovascular AMD (nAMD) accounts for approximately two-thirds of late-stage AMD cases, it contributes to over 80% of cases of legal blindness attributed to AMD [3]. The advent of intravitreal injection therapy (IVT) with anti-vascular endothelial growth factor (anti-VEGF) agents marked a paradigm shift in the management of nAMD [4, 5]. Landmark clinical trials demonstrated that intravitreal VEGF inhibition effectively interrupts the pathophysiological processes of nAMD, restores retinal morphology in the early stages of the pathology, and improves or stabilizes neurosensory function in most patients [6]. Currently, anti-VEGF agents are the cornerstone of nAMD management and are recommended as first-line therapy in international clinical guidelines [1]. However, in real-world practice, treatment outcomes with anti-VEGF agents are heavily influenced by the choice of dosing regimen [7, 8]. Studies have shown that fluctuations in central subfield thickness in patients treated with anti-VEGF therapy correlate with poor functional outcomes, while better disease control is linked to improved visual outcomes [6, 7]. Proactive treat-and-extend (T&E) regimen have been shown to reduce fluid fluctuations more effectively than reactive pro re nata dosing strategies [9–12].

Optical coherence tomography (OCT) plays a central role in the diagnosis and monitoring of nAMD. It is widely used to assess treatment response and guide clinical decision-making [13–16]. Several OCT-derived biomarkers—such as central foveal thickness (CFT), pigment epithelial detachment (PED), and the presence of intraretinal or subretinal fluid—have been associated with baseline visual acuity and the potential for visual improvement following anti-VEGF therapy [17–19]. Nevertheless, debates persist regarding the prognostic value of certain OCT biomarkers. Clarifying these uncertainties is

essential not only for understanding the progression of nAMD but also for optimizing anti-VEGF treatment strategies. Furthermore, knowledge gaps remain regarding the variability in treatment response among patients, as well as the criteria for switching between different anti-VEGF agents. Understanding why some patients respond poorly to therapy and determining the factors influencing treatment-switching decisions would better equip clinicians to refine individualized care [20]. In this study, we retrospectively analyzed treatment-naïve nAMD cases treated with aflibercept using a T&E regimen. Our aim was to evaluate nAMD treatment outcomes and assess the potential correlations between OCT biomarkers and long-term clinical results over a 5-year follow-up period in a real-world setting.

METHODS

This retrospective, monocentric study was conducted at the University Magna Graecia of Catanzaro. All patients diagnosed with nAMD between 2016 and 2024 were reviewed to identify those meeting the inclusion criteria, which were: treatment-naïve status, confirmed diagnosis of nAMD type 1 or type 2 using OCT and optical coherence tomography angiography (OCT-A), treatment exclusively with IVT of aflibercept following a T&E regimen, and a minimum follow-up period of 5 years. Type 3 MNV lesions were excluded from enrollment because they characteristically originate from the retina rather than the choroid. In cases of diagnostic uncertainty, indocyanine green angiography (ICGA) and/or fluorescein angiography (FA) were reviewed when available. Two unmasked ophthalmologists (M.B. and D.C.) independently evaluated the data. In case of disagreement, a senior ophthalmologist specialist in retinal diseases (A.C.) reviewed the assessments to resolve disagreement. Patients who met all of the aforementioned criteria were excluded if they presented with other ophthalmic conditions, such as advanced diabetic retinopathy secondary to diabetes mellitus type II, ocular inflammatory or neoplastic diseases, systemic neoplastic diseases, or neurodegenerative disorders affecting the quality of OCT imaging. The study was approved by the local ethics committee of the University Magna Graecia of Catanzaro and was conducted in accordance with the principles of the Declaration of Helsinki. Demographic data (e.g., age, sex), key ophthalmologic parameters (e.g., best-corrected visual acuity [BCVA]), and multimodal imaging (OCT scans and fundus photography) were analyzed. For OCT (Optovue, Fremont, CA, USA) and BCVA, the following time points were assessed: baseline, 1 month after completion of the first anti-VEGF injection cycle (three injections), and annually for up to 5 years post-treatment, for a total of seven time points. The cross-line and retina map scan protocols were used for OCT analysis. CFT was automatically measured by the device. CFT was defined as the distance between the vitreo-retinal interface and the interface between the retinal pigment epithelium (RPE) and Bruch's membrane. Additionally, the following biomarkers were evaluated via OCT: subretinal fluid (SRF), intraretinal fluid (IRF), presence of subretinal fibrosis, hyperreflective spots (HS), drusenoid PED (dPED), onion sign, outer retinal tubulation (ORT), subretinal hyperreflective material (SHRM), and RPE tears. BCVA was recorded using the Early Treatment Diabetic Retinopathy Study (ETDRS) letter score and subsequently converted to the logarithm of the minimum angle of resolution (logMAR) scale [21]. Moussa et al. described a formula for converting Snellen chart values into logMAR values, enabling standardized assessment of lower visual acuity levels such as counting fingers and hand motion [21]. In this study, we assigned values of 2.1 logMAR for counting fingers, 2.4 logMAR for hand motion, and 2.7 logMAR for light perception [21].

OCT Biomarkers

Considering the predictive and prognostic significance of OCT biomarkers, it is essential for clinicians to understand the key characteristics underlying the various biomarkers described in the literature. Below are the classical definitions of the primary OCT biomarkers discussed in this

text, aimed at facilitating a better understanding (Fig. 1).

IRF is an accumulation of fluid within the retinal layers. On OCT, it appears as well-defined hyporeflective cystic spaces, primarily located in the inner and outer nuclear layers [22]. This phenomenon is commonly associated with various retinal pathologies, including exudative AMD, diabetic retinopathy, and retinal vein occlusion [22]. PED is defined as the anatomical separation of the RPE from the Bruch's membrane. The resultant sub-RPE space may contain serous exudate (e.g. sPED), blood, fibrovascular tissue (e.g. fPED), or drusenoid material (e.g. dPED) [23, 24]. dPED is characterized by coalescence of macular drusen, although its pathogenesis has not been clearly explicated [24–26]. HS is defined as small focal hyperreflective material scattered mainly in outer retinal layers but also spreading to all retinal layers. They are primarily around fluid accumulation in the intraretinal cystoid spaces [27]. SHRM in the context of MNV is primarily associated with exudation resulting from neovascularization above the RPE. It has been predominantly described in type 2 MNV as a marker of disease activity. However, SHRM can be observed in various retinal pathologies, with its composition varying depending on the underlying condition, including neovascular tissue, fibrosis, or hemorrhage [28, 29]. RPE tear is a retraction of the pigment epithelium, revealing bare Bruch's membrane. It is a mainly found in context of RPE detachment associated with nAMD. RPE tear has been reported to occur spontaneously and after treatment with anti-VEGF agents [30, 31]. ORT appears as branching, circular or ovoid tubular formations in the outer retina, displaying a hyporeflective core surrounded by a hyperreflective border on OCT imaging [22, 32]. This phenomenon is supposed to results from the reorganization of photoreceptors following damage and degeneration of the RPE. While it is most commonly associated with nAMD, ORT can also be observed in cases of other pathologies such as gyrate atrophy [22, 32]. The onion sign was first described as an OCT pattern characterized by multiple layered hyperreflective bands located in the sub-RPE space. This pattern has been identified in eyes with nAMD, specifically in cases with type 1 MNV. Histological studies suggest that the hyperreflective bands observed in the onion sign correspond to crystallized cholesterol deposits [32, 33] (Fig. 2).

Treat-and-Extend Regimen

For all patients treated exclusively with aflibercept, both clinical records and the institution's electronic database were reviewed to identify those who had maintained a T&E regimen with aflibercept for a continuous period of 5 years. Treatment management was based on well-defined criteria of disease activity and stability.

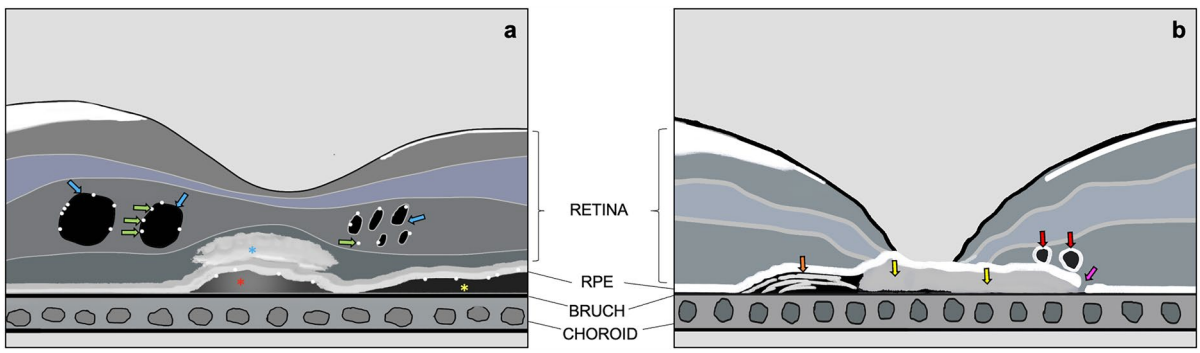

Fig. 1 Schematic illustration of the OCT biomarkers discussed in this article. **a** Blue arrows indicate intraretinal fluid; green arrows indicate hyperreflective spots; the blue asterisk marks subretinal hyperreflective material; the red asterisk marks a pigment epithelial detachment containing macular neovascularization; yellow asterisks indicate subretinal fluid. **b** The orange arrow indicates the "onion sign"; the yellow arrow indicates subretinal fibrosis; red arrows indicate outer retinal tubulations; the purple arrow indicates a retinal pigment epithelium tear

All patients received an initial loading phase consisting of three consecutive monthly injections. Patients who exhibited signs of disease activity were re-injected after 4 weeks from the fist injection [34]. At least one of the following criteria was required to define disease activity: the presence of new or persistent retinal fluid on OCT with stable or increased volume compared to the previous visit; a loss of five or more ETDRS letters compared to the prior visit in conjunction with recurrent fluid on OCT; an increase in CRT of 100 µm or more within the central 1 mm subfield relative to the previously recorded lowest value; the onset of new neovascularization, as determined at the discretion of the investigator and potentially supported by fundus examination or multimodal imaging; or the appearance of a new macular hemorrhage [34, 35]. In cases where improvement was observed—defined as a reduction, but not complete resolution, of SRF or IRF, stability of CRT, and a visual acuity loss of fewer than four ETDRS letters—the treatment interval was extended by 2 weeks. In the absence of the aforementioned signs of activity, the interval was extended by 4 weeks [34, 35].

Statistical Analysis

The analysis of the dataset under study involved the implementation of several statistical techniques. First, an exploratory analysis was performed using frequency (%) for dichotomous variables and mean and standard deviation for continuous variables. Subsequently, the normality of the distribution was assessed using both graphical methods, such as the QQ plot, and statistical methods, such as the Shapiro–Wilk test [36]. Non-normality was demonstrated for all variables, so the statistical tests were determined in accordance with this evidence. Specifically, the Kruskal–Wallis test and the Wilcox test were used for analysis. Other graphical tools such as scatter plots, histograms, and stack diagrams were used both to describe the link between CFT and logMAR and to assess the changes in visus and OCT biomarkers for various stages of follow-up.

We employed linear mixed-effects models with an autoregressive structure (AR1) to analyze the data, given the repeated-measures design of

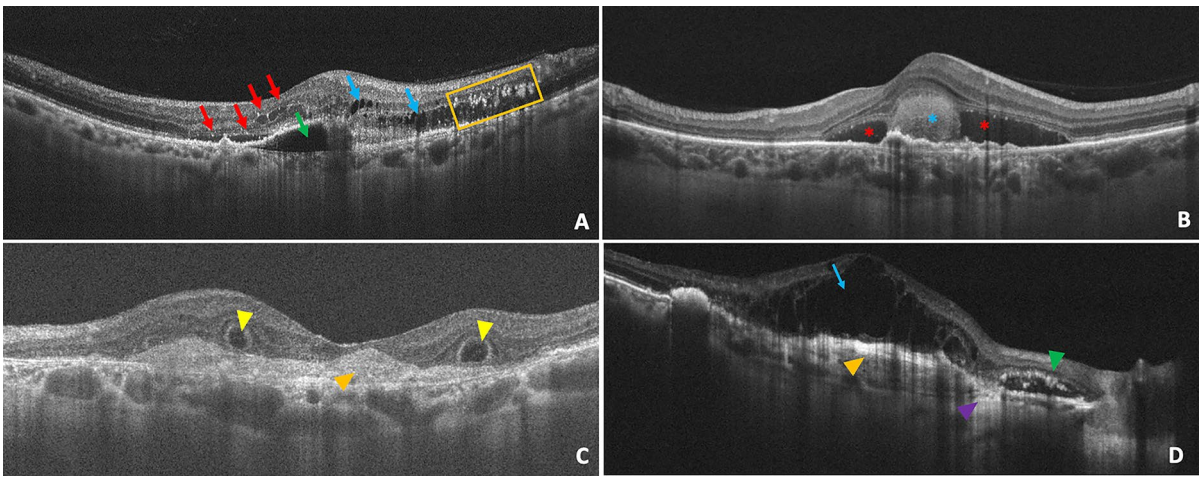

Fig. 2 Cross-sectional macular OCT images showing key retinal biomarkers: **A** Red arrows mark hyperreflective spots (HS) without posterior shadowing. The yellow box highlights retinal exudates with shadowing, shown here only to contrast with HS. Blue arrows point to intraretinal fluid (IRF), and the green arrow indicates fluid beneath the RPE. **B** Red asterisks denote subretinal fluid (SRF), while the blue asterisk identifies subretinal hyperreflective material (SHRM). **C** The yellow arrowhead indicates an outer retinal tubulation (ORT) just above the area of subretinal fibrosis, which is marked by the orange arrowhead. **D** Blue arrows again show IRF. The purple arrowhead marks a window defect from retinal atrophy, the orange arrowhead highlights subretinal fibrosis, and the green arrowhead points to the "onion sign."

the study. This approach was selected because multiple observations were collected from the same patients over a 5-year follow-up period, and standard multiple linear regression would not have been appropriate for handling the inherent within-subject correlations in such data [37]. The analysis aimed to evaluate visual acuity (measured using the logMAR scale) over time in a cohort of patients. Key covariates included year, sex, and various clinical and imaging variables, which were treated as fixed effects. Random effects were included to account for intra-patient variability, while the AR1 structure was chosen to model correlations between repeated measurements at successive time points.

All analyses were performed using SPSS software [38] and RStudio [39], with statistical significance set at $p<0.05$.

RESULTS

The study includes 59 eyes of 59 white patients on a 5-year follow-up. Of these 59 eyes, 34 had MNV type 1, with a mean age of 73.3 and standard deviation of 5.6 years, and a slight predominance of females was observed (53.9%). The remaining 25 patients had MNV type 2 with a similar age and gender distribution (73.1 ± 5.2 years and a slight predominance of females making up 52% of the sample) ($p>0.900$). Characteristics at baseline are shown in Table 1. Over a mean follow-up of 60 ± 3 months (range 54–66), patients underwent 26 ± 7 intravitreal aflibercept injections (range 18–34), corresponding to 5.2 ± 1.4 injections per year (range 3.6–6.8). This equates to a mean inter-injection interval of 9.3 ± 2.5 weeks, consistent with a treat-and-extend regimen.

At baseline, the group with MNV type 1 had predominantly the presence of SRF (76.5%), drusenoid PED (79.4%) and HS (64.7%). On the other hand, in the MNV type 2 sample, there was always a predominance of the presence of dPED (56.0%) and HS (80.0%), but also SHRM (68.0%) and IRF (56.0%). The only statistically significant differences between the two samples at baseline were the presence of SRF ($p<0.001$) and SHRM ($p<0.001$) characterizing type 1 and type 2, respectively. No significant differences were appreciated at baseline in terms of visus and CFT. Figure 3 shows the trend of logMAR and CFT throughout the study period.

As can be seen from Fig. 3, the BCVA tends to rise throughout the follow-up after an initial decline for both groups (group 1: 0.97 logMAR vs 0.77 logMAR; group 2: 0.93 logMAR vs 0.86 logMAR). In terms of logMAR, for the first group, a statistically significant difference was observed between the value at 5 years (1.05 logMAR) and the initial values (0.87 logMAR), i.e. at baseline ($p=0.047$), after the first cycle (0.77 logMAR, $p=0.007$) and 1 year (0.82 logMAR, $p=0.007$), but also between the value at 4 years (0.97 logMAR) and 1 year ($p=0.026$), and 4 ($p=0.002$) years and 3 (0.93 logMAR, $p=0.016$) years, while for the type 2 group, no statistically significant differences were observed. For CFT, on the other hand, a statistically significant difference was observed for type 1 in the baseline compared to

Table 1 Characteristics of the dataset at baseline

Variable	Type 1 $N=34$	Type 2 $N=25$
Age (years)	73.3 ± 5.6	73.1 ± 5.2
Gender		
M	16 (47.1%)	12 (48.0%)
F	18 (52.9%)	13 (52.0%)
SRF (1)	26 (76.5%)	6 (24.0%)
dPED (1)	27 (79.4%)	14 (56.0%)
Subretinal fibrosis (1)	0 (0.0%)	0 (0.0%)
HS (1)	22 (64.7%)	20 (80.0%)
IRC (1)	16 (47.1%)	14 (56.0%)
ORT (1)	0 (0.0%)	0 (0.0%)
RPE tear (1)	0 (0.0%)	0 (0.0%)
Onion sign (1)	2 (5.9%)	0 (0.0%)
SHRM (1)	2 (5.9%)	17 (68.0%)
CFT	349.8 ± 81.6	381.4 ± 72.6
BCVA (logMAR)	0.9 ± 0.3	0.9 ± 0.3

the whole sample ($p<0.01$), between the value at 1 year and that at 3 ($p=0.019$) and 4 ($p=0.011$) years, and the value at 2 years and that at 3 ($p=0.018$) years and 4 ($p=0.011$) years, while for type 2 there was a statistically significant difference in the comparison between the baseline and all other values recorded during follow-up ($p<0.001$).

With regard to the OCT biomarkers, the frequency at the various follow-up times and the result of the overall statistical comparison test are shown in Tables 2 and 3.

As can be seen from the results in the table, for type 1 there was a statistically significant decrease in the presence of the biomarkers SRF ($p=0.001$), dPED ($p=0.010$), and HS ($p=0.009$), while conversely, the incidence of subretinal fibrosis increased during follow-up ($p=0.033$). For type 2, the same trend was observed on the biomarker subretinal fibrosis ($p=0.006$), but an increase in the incidence of ORT ($p=0.035$) and RPE tear ($p=0.032$) was seen as well, while conversely, the incidence of IRF decreased ($p=0.013$).

The most effective study of biomarkers in both groups was carried out through linear mixed-effects model analysis. The type III test of fixed effects revealed five variables for the group with MNV type 1 and four variables for the group with MNV type 2 that were significantly associated with changes in visual acuity, as measured by logMAR. The results of the linear mixed-effects model analysis for patients with MNV type 1 (Table 4) and MNV type 2 (Table 5) highlight key factors associated with changes in visual acuity, measured by logMAR. In the MNV type 1 group, the absence of fibrosis was significantly associated with an increase in BCVA ($p<0.001$). Similarly, the presence of HS showed a significant correlation with improved vision ($p=0.044$). Conversely, the absence of RPE tears was linked to an increase in logMAR, suggesting a decline in visual acuity, as were greater CFT and longer follow-up duration. In the MNV type 2 group, the results showed a similar trend for certain variables. The presence of RPE tears and greater CFT were both significantly associated with an increase in logMAR, as observed in the MNV type 1 group. However, a key distinction emerged: the absence of an onion-sign pattern was strongly associated with a reduction in logMAR ($p<0.001$), reflecting an improvement in visual acuity. Additionally, age was a significant factor in this group, with increasing age correlating with higher logMAR values, indicating worsening vision.

Beyond these associations, the covariance structure analysis provides additional insights into the temporal evolution of visual acuity in both groups. In the MNV type 1 group, the autoregressive parameter AR (1) was $\rho=0.811$, indicating a strong correlation between a patient's visual acuity in one year and the preceding year, suggesting relatively stable disease progression over time. Additionally, the variance in the intercept was 0.048, reflecting inter-subject variability in baseline visual acuity. In contrast, the MNV type 2 group exhibited a slightly lower autoregressive coefficient ($\rho=0.743$), indicating a weaker

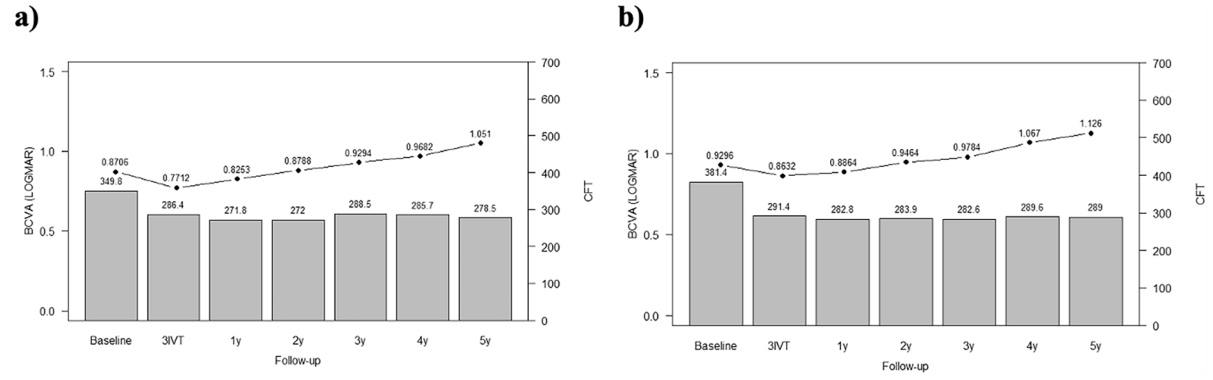

Fig. 3 Mean value of logMAR and CFT during the follow-up period for **a** type 1 and **b** type 2 MNV

Table 2 Study of the presence of biomarkers during follow-up: MNV type 1

Variable	Baseline	3 IVT	1 year	2 years	3 years	4 years	5 years	*p*-value
SRF (1)	26 (76.5%)	7 (20.6%)	9 (26.5%)	10 (29.4%)	9 (26.5%)	10 (29.4%)	13 (38.2%)	**0.001**
dPED (1)	27 (79.4%)	21 (61.8%)	17 (50.0%)	14 (41.2%)	14 (41.2%)	14 (41.2%)	16 (47.1%)	**0.010**
Subretinal fibrosis (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.9%)	3 (8.8%)	**0.033**
HS (1)	22 (64.7%)	9 (26.5%)	12 (35.3%)	9 (26.5%)	9 (26.5%)	11 (32.4%)	10 (29.4%)	**0.009**
IRF (1)	16 (47.1%)	5 (14.7%)	7 (20.6%)	8 (23.5%)	7 (20.6%)	10 (29.4%)	10 (29.4%)	0.075
ORT (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.9%)	2 (5.9%)	0.186
RPE tear (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.9%)	1 (2.9%)	0.538
Onion sign (1)	2 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.060
SHRM (1)	2 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.060

Bold values indicate statistically significant results ($p < 0.05$)

IVT intravitreal injection, *SRF* subretinal fluid, *dPED* drusenoid PED, *HS* Hyperreflective spots, *IRF* intraretinal fluid, *ORT* outer retinal tubulation, *RPE* retinal pigment epithelium, *SHRM* subretinal hyperreflective material

Table 3 Study of the presence of biomarkers during follow-up: MNV type 2

Variable	Baseline	3 IVT	1 year	2 years	3 years	4 years	5 years	*p*-value
SRF (1)	6 (24.0%)	6 (24.0%)	8 (32.0%)	5 (20.0%)	8 (32.0%)	7 (28.0%)	9 (36.0%)	0.882
dPED (1)	14 (56.0%)	6 (24.0%)	8 (32.0%)	9 (36.0%)	9 (36.0%)	7 (28.0%)	8 (32.0%)	0.326
Subretinal fibrosis (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (8.0%)	4 (16.0%)	**0.006**
HS (1)	20 (80.0%)	13 (52.0%)	16 (64.0%)	13 (52.0%)	16 (64.0%)	16 (64.0%)	15 (60.0%)	0.453
IRF (1)	14 (56.0%)	3 (12.0%)	5 (20.0%)	6 (24.0%)	7 (28.0%)	8 (32.0%)	4 (16.0%)	**0.013**
ORT (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (8.0%)	3 (12.0%)	**0.035**
RPE Tear (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	3 (12.0%)	**0.032**
Onion sign (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (8.0%)	0.059
SHRM (1)	17 (68.0%)	9 (36.0%)	10 (40.0%)	12 (48.0%)	8 (32.0%)	8 (32.0%)	7 (28.0%)	0.068

Bold values indicate statistically significant results ($p < 0.05$)

IVT intravitreal injection, *SRF* subretinal fluid, *dPED* drusenoid PED, *HS* hyperreflective spots, *IRF* intraretinal fluid, *ORT* outer retinal tubulation, *RPE* retinal pigment epithelium, *SHRM* subretinal hyperreflective material

but still significant correlation between consecutive yearly measurements. This suggests a potentially more variable disease course compared to MNV type 1. Moreover, the variance in the intercept was higher (0.090), suggesting greater variability in baseline visual acuity among patients in this group. The stronger autoregressive correlation in MNV type 1 suggests a more predictable disease course compared to the greater variability observed in MNV type 2. Finally, Fig. 4 shows the logMAR trend according to the three categories of improvement defined from the changes shown after completion of the first cycle 3 months after the start of therapy.

Table 4 Estimates of fixed effects: MNV type 1

Independent variable	Estimate	Std. error	*p*-value	95% Confidence interval	
				Lower bound	Upper bound
Intercept	−0.427	0.653	0.518	−1.759	0.905
Year	0.029	0.011	**0.011**	0.007	0.050
Gender (1)					
0	0.048	0.095	0.620	−0.147	0.242
Age	0.017	0.008	0.057	−0.001	0.034
Subretinal fibrosis (1)					
0	−0.024	0.020	0.248	−0.660	−0.282
dPED (1)					
0	−0.471	0.096	**< 0.001**	−0.026	0.042
HS (1)					
0	−0.033	0.016	**0.044**	−0.066	−0.001
SRF (1)					
0	−0.024	0.020	0.248	−0.064	0.017
IRF (1)					
0	−0.037	0.020	0.068	−0.078	0.003
ORT (1)					
0	−0.020	0.120	0.867	−0.257	0.217
Onion sign (1)					
0	0.014	0.068	0.834	−0.120	0.148
RPE tears (1)					
0	0.399	0.062	**< 0.001**	0.277	0.520
SHRM (1)					
0	−0.008	0.070	0.905	−0.146	0.129
CFT	0.001	0.000	**0.025**	0.000	0.001

Bold values indicate statistically significant results ($p < 0.05$)

SRF subretinal fluid, *dPED* drusenoid PED, *HS* Hyperreflective spots, *IRF* intraretinal fluid, *ORT* outer retinal tubulation, *RPE* retinal pigment epithelium, *SHRM* subretinal hyperreflective material

At 3 months, for the type 1 group, only two patients had no improvement (5.88%), while 27 (79.42%) showed limited improvement and five (14.70%) had strong improvement. The patients with no improvement started from a worse mean visus condition (0.82 + 0.056) than the limited improvement category (0.835 + 0.242) and the strong improvement category (1.08 + 0.301; $p = 0.217$). When looking at the condition at the end of follow-up, the

Table 5 Estimates of fixed effects: MNV type 2

Independent variable	Estimate	Std. error	*p*-value	95% Confidence interval	
				Lower bound	Upper bound
Intercept	−1.709	1.127	0.143	−4.039	0.620
Year	0.003	0.018	0.851	−0.034	0.041
Gender (1)					
0	−0.057	0.136	0.683	−0.341	0.228
Age	0.039	0.014	**0.013**	0.009	0.069
Subretinal fibrosis (1)					
0	−0.308	0.173	0.078	−0.651	0.035
dPED (1)					
0	0.034	0.030	0.262	−0.026	0.093
HS (1)					
0	0.007	0.031	0.834	−0.055	0.068
SRF (1)					
0	0.018	0.044	0.682	−0.069	0.105
IRF (1)					
0	0.031	0.026	0.235	−0.021	.083
ORT (1)					
0	−0.040	0.173	0.817	−0.383	0.303
Onion sign (1)					
0	−0.469	0.100	**< 0.01**	−0.667	−0.271
RPE tears (1)					
0	0.278	0.106	**< 0.01**	0.068	0.487
SHRM (1)					
0	−0.008	0.030	0.795	−0.066	0.051
CFT	0.001	0.000	**0.014**	0.000	0.001

Bold values indicate statistically significant results ($p < 0.05$)

SRF subretinal fluid, *dPED* drusenoid PED, *HS* Hyperreflective spots, *IRF* intraretinal fluid, *ORT* outer retinal tubulation, *RPE* retinal pigment epithelium, *SHRM* subretinal hyperreflective material

worst mean visus was associated with the limited improvement category (1.00+0.364) compared to the strong (1.20+0.681) and no improvement (1.29+0.127) categories, although not statistically significant ($p=0.180$). For the type 2 group, instead, four patients had no improvement (16.00%), 18 patients (72.00%) had limited improvement, and three (12.00%) had strong improvement. At baseline, the worst visus condition was associated with the no-improvement

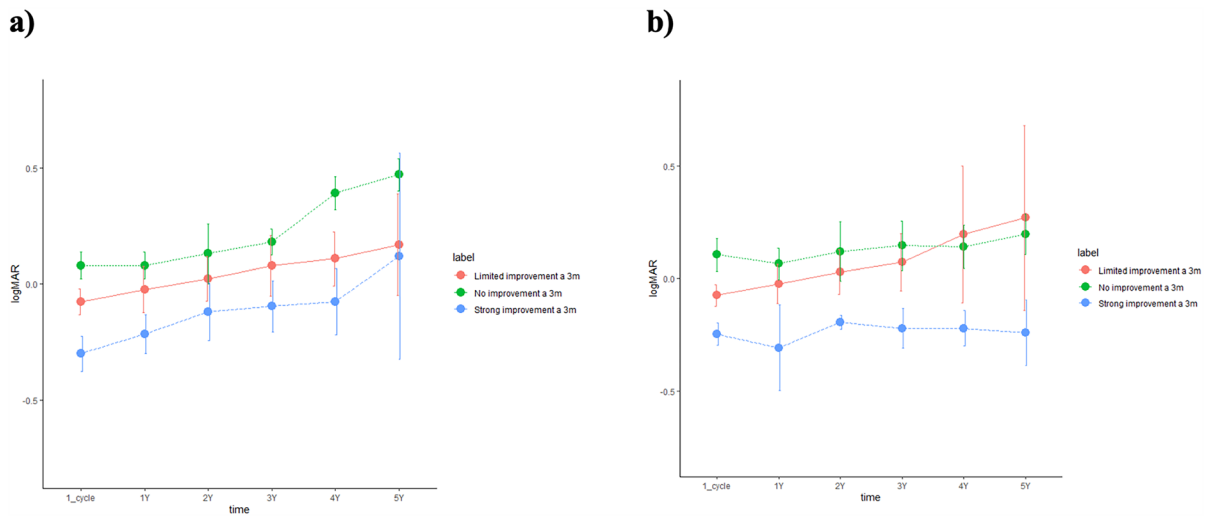

Fig. 4 Development of BCVA compared to baseline at different time points, divided according to treatment response at month 3

class (0.65 + 0.129), followed by the limited (0.967 + 0.332) and strong improvement classes (1.08 + 0.05, $p = 0.083$), while at the end of follow-up, similar conditions in terms of mean visus were found between the no (0.84 + 0.097) and strong (0.84 + 0.104) improvement classes, with the highest result for the limited improvement class (1.24 + 0.684) although the difference was not significant ($p = 0.972$). In contrast, there were no statistically significant differences in the visus observed for the three classes in the two study groups.

DISCUSSION

Despite the introduction of various anti-VEGF injections for nAMD, patient response to treatment remains unpredictable. This suggests the potential presence of specific predictive factors that may vary among patients and could influence treatment outcomes. In this retrospective study, patients with diagnosis of treatment-naïve nAMD treated with the T&E regimen with aflibercept with 5-year follow-up were evaluated. The study objective was to evaluate treatment outcomes and assess the potential correlations between OCT biomarkers and long-term clinical results over a 5-year follow-up period in a real-world setting.

Of the 59 MNV cases, 57.6% were classified as type 1 and 42.4% as type 2. According to baseline OCT biomarkers, SRF was more frequently observed in the MNV type 1 group than the type 2 group, whereas SHRM was more commonly found in patients with type 2 MNV. This finding is consistent with the anatomical characteristics of type 2 MNV, which typically involve neovascularization located above the RPE, making the presence of SHRM more likely in this subtype [28, 29].

Central Foveal Thickness

Throughout the entire follow-up period, CFT showed a significant reduction from baseline following the loading dose in both MNV type 1 and type 2 groups. Thereafter, CFT remained relatively stable, with only minor fluctuations observed over the subsequent 4 years of follow-up. Several studies have demonstrated that CFT significantly decreases after the initial anti-VEGF treatment, with the reduction remaining stable for up to 90 days [40]. In the ARIES study, patients with nAMD treated with aflibercept under both early and late T&E protocols exhibited a significant reduction in CFT at the

2-year follow-up [41]. In the current study, CFT was shown to correlate with BCVA, with higher CFT values being associated with lower visual outcomes [42]. In line with this, a positive correlation has also been demonstrated between reductions in CFT and improvements in BCVA over time in patients with nAMD undergoing aflibercept treatment [43]. Studies investigating anti-VEGF therapies have reported that greater CFT fluctuations are linked to poorer BCVA at the end of follow-up, whereas more stable CFT measurements are associated with better visual outcomes. This suggests that CFT variability may serve as a more reliable prognostic indicator than the mere presence of retinal fluid [42].

Subretinal and Intraretinal Fluid

Regarding MNV type 1, SRF showed a significant reduction following the loading dose. Throughout follow-up, a slight tendency toward re-accumulation was observed, particularly during the fourth and fifth years. Nevertheless, SRF levels remained consistently lower than baseline across the 5-year follow-up period. In contrast, in patients with MNV type 2, SRF did not exhibit a statistically significant change. Baseline SRF levels in this group were already lower than those observed in type 1, and showed a gradual, nonsignificant increasing trend over time. It is known that SRF typically decreases following the loading phase and tends to remain stable during long-term follow-up [34]. A similar trend was observed for IRF, which decreased significantly after the loading dose in both study groups. Over the subsequent 5 years of follow-up, IRF levels remained low, with MNV type 2 patients demonstrating a more pronounced and sustained reduction than those with type 1.

After the initial 2 years of CFT reduction, stabilization of retinal thickness was accompanied by persistent SRF suppression. IRF showed a sustained and statistically significant reduction in the MNV type 2 group, and a mild, nonsignificant decline in the MNV type 1 group. These findings support the hypothesis that the gradual decline in visual acuity observed from year 1 to year 5 is likely attributable to the progression of the dry AMD component and subretinal fibrosis, rather than to recurrent MNV activity [34]. The presence of subretinal fibrosis and minimal residual IRF likely contributes to the overall stabilization of retinal thickness at 5 years, as these components progressively replace the previously observed SRF. A similar pattern was reported in a study by Jajji et al., which analyzed 82 eyes of patients with nAMD [34].

Subretinal Hyperreflective Material

SHRM is confirmed to be a key OCT biomarker and prognostic factor in the management of neovascular nAMD [44]. Its presence typically decreases following intravitreal anti-VEGF therapy [45]. Previous studies have reported a baseline SHRM prevalence of approximately 70% in patients with nAMD [44–46]. In the current study, SHRM was present in 68% of patients within the MNV type 2 group, whereas its baseline prevalence in the MNV type 1 group was 5.9%. Among patients with MNV type 2, SHRM showed a decreasing trend over the 5-year follow-up, although the reduction was not statistically significant. Interestingly, patients who exhibited SHRM at baseline and experienced an improvement in BCVA following the loading dose tended to maintain stable visual function over the long term. Conversely, patients with stable or worsening vision after the loading dose were more likely to show progressive visual decline. However, previous studies have reported that SHRM was not consistently associated with BCVA improvement after 1 year of intravitreal aflibercept treatment [46]. Similarly, in the present study, SHRM did not demonstrate a significant correlation with visual acuity outcomes over the 5-year period.

Drusenoid PED

dPED is typically considered a risk factor for the progression to advanced forms of AMD, including both neovascular and atrophic subtypes. It has been observed that patients with dPED tend to develop type 2 MNV more frequently than type 1 MNV [47]. On OCT imaging, it is common to identify an MNV located beneath the RPE in the context of a PED. During anti-VEGF

treatment for MNV, a reduction in the height of dPED is often observed, which can eventually lead to its collapse and the development of retinal atrophy [26, 48]. In the present study, dPED showed a progressive tendency to decrease in both groups throughout the treatment period; however, this reduction reached statistical significance only in the MNV type 1 group. Regarding the relationship between dPED and visual acuity, patients with MNV type 2 who experienced an improvement in BCVA following the loading dose tended to maintain better visual acuity over time. This trend was less evident in patients with MNV type 1. Nevertheless, no significant correlation was found between the presence or evolution of dPED and visual acuity improvement at the end of the 5-year follow-up.

Hyperreflective Spots

In OCT analysis, the hyperreflective nature of the so-called HS has been hypothesized to be associated with microglia activation, lipoprotein deposition in the neuroretinal layers, or RPE migration; however, its exact origin remains uncertain [49, 50]. Other authors have hypothesized that the increased presence of HS in the subretinal layer is associated with blood–retinal barrier impairment and is considered a marker of disease activity [50]. The presence of HS was equally distributed among all types of MNV [27]. Previous studies have reported HS regression after treatment [27, 50]. In the current study, reduction was observed after the loading dose followed by a significant decrease of approximately up to 5 years. Moreover, data on the correlation between HS and visual acuity remain conflicting. Some authors reported no association between HS and visual acuity changes at 12 months [49]. Coscas et al. reported that HS resolution was associated with better final visual acuity in nAMD and tended to recur based on the evolution of the MNV activity [23]. On the contrary, Lee et al. found that a higher presence of HS at baseline was negatively correlated with visual acuity in patients with nAMD [49, 50]. In our study, a reduction in HS in MNV type 1 was associated with better visual outcomes at 5 years. Lee et al. demonstrated that a higher number of HS at baseline correlated with a smaller reduction in SRF during follow-up. These findings are consistent with the hypothesis that HS reflect an inflammatory component that is more pronounced in the early stages of disease, prior to treatment initiation. HS tend to decrease significantly with the progression of intravitreal anti-VEGF therapy, although they rarely disappear completely. This may suggest that, at the time of diagnosis, HS represent a marker of active disease, closely associated with increased SRF and fluid extravasation. However, in more advanced stages, HS may acquire a degenerative nature, reflecting RPE debris or lipofuscin deposits that remain stable within the retinal layers over time [49].

Retinal Degeneration OCT Biomarkers

Regarding OCT biomarkers of retinal degeneration, RPE tears were observed in only 2.9% of eyes in the type 1 MNV group at the fifth year of follow-up. In contrast, in the type 2 MNV group, RPE tears were present in 12% of patients at year 5, representing a significant increase compared to baseline. In both groups, the presence of RPE tears was associated with a reduction in BCVA at 5 years. Conversely, no statistically significant differences were found for the presence of the onion sign in either group. However, in the type 2 MNV group, 8% of patients ($n=2$) exhibited this sign at year 5. Although not statistically significant in the overall analysis, this finding in the type 2 group was notably associated with a decrease in BCVA.

The development of ORT is believed to be driven by progressive photoreceptor degeneration, which leads to the activation of Müller cells. This activation induces the expression of glial fibrillary acidic protein, contributing to the formation of ORT structures [32, 51]. ORT showed an increased prevalence during the fourth and fifth years of follow-up in the MNV type 2 group, whereas in the MNV type 1 group, the prevalence remained low and did not show a significant change over the 5-year period. Several authors have reported that the prevalence of ORT tends to increase over time and is associated with a decline in visual acuity

[52, 53]. Although still observed in a relatively small number of cases, the higher prevalence of ORT in the MNV type 2 group appears to be anatomically driven. In type 2 MNV, the pathological process also affects the neuroretinal layers located above the RPE, which may result in greater damage to the inner retinal structures [53, 54]. Over time, this could lead to a higher likelihood of ORT formation compared to MNV type 1, which showed a prevalence of only 2.9% at 5 years.

Subretinal fibrosis was not observed in either group during the first 4 years of follow-up. However, starting from the fifth year, a few cases emerged, reaching statistical significance. This late onset is likely related to the chronic nature of the disease, which over time may lead to the development of subretinal fibrosis and retinal atrophy, ultimately contributing to a decline in visual acuity. In the MNV type 1 group, the absence of subretinal fibrosis was associated with better BCVA.

Functional Outcome

In terms of visual acuity, both groups presented with similar BCVA at baseline. Following the loading dose of aflibercept, both MNV type 1 and type 2 groups demonstrated an improvement in BCVA, with the visual gain maintained up to the second year. From the second to the fifth year, however, a gradual and statistically significant decline in BCVA was observed in MNV type 1 group when compared to both baseline and post-loading values, while type 2 did not show any statistical significance. Visual acuity progressively decreased in a linear fashion throughout the follow-up, suggesting a slow disease progression despite adequate intravitreal treatment under a T&E regimen. One potential factor contributing to this trend may be the long-term effects of anti-VEGF therapy on photoreceptor integrity, as proposed in previous studies [55, 56]. In the MNV type 1 group, BCVA showed a more linear pattern of decline over time, whereas the trajectory in the type 2 group appeared more variable and therefore less predictable. In the type 1 group, approximately 94% of patients experienced a BCVA improvement at 3 months following the loading dose, with 14.7% showing a marked visual gain. In the type 2 group, about 84% of patients improved at the same time point, of whom 12% achieved a nonsignificant improvement. Across both groups, patients who began treatment with better baseline visual acuity were more likely to maintain higher BCVA values at 5 years, compared to those who started with poorer visual function. Additionally, age was identified as a significant factor influencing long-term visual outcomes, with increasing age correlating with higher logMAR scores, indicating progressive deterioration of visual acuity over time.

The main limitations of this study include its retrospective design, relatively small sample size, the subjective assessment of OCT biomarkers rather than the use of artificial intelligence (AI)-based models, and the inability to establish a precise correlation between OCT biomarkers and the histopathological analysis of the human retina. Strengths of the study include the uniform treatment regimen, as all patients underwent a T&E protocol with aflibercept, and the extended 5-year follow-up period.

CONCLUSIONS

The main differences observed in this study highlight that, in type 2 MNV, the presence of SHRM at baseline was associated, over the course of follow-up, with a greater incidence of retinal alterations. This is evidenced by the higher occurrence of biomarkers such as IRF, the presence of retinal tubulations, and RPE tears. This pattern may plausibly be linked to the inflammatory response occurring in the neuroretina above the neovascular membrane—precisely where SHRM is located—which, over time, may lead to retinal degeneration and the emergence of the aforementioned biomarkers. Conversely, this association does not appear to be present in type 1 MNV. In these cases, which more frequently exhibit SRF at baseline, a reduction in HS and dPED is observed, along with a slight increase in subretinal fibrosis. These findings suggest that the presence of SRF at baseline

may more frequently evolve into subretinal fibrosis over time, in patients with a partial anti-VEGF response. Moreover, patients who show an improvement in BCVA after the loading dose tend to maintain lower visual acuity levels at 5-year follow-up. Despite adherence to an appropriate treatment regimen and good patient compliance, when criteria for treatment suspension are not met due to the persistent presence of OCT biomarkers, BCVA tends to progressively decline in both groups, with significance in group 1. Further investigation is needed into the direct effects of anti-VEGF agents on photoreceptor integrity.

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Declarations

Conflict of Interest. Massimiliano Borselli, Mutasem Elfalah, Teresa Angela Trunfio, Arianna Scala, Domenico Chisari, Alessandra Mancini, Andrea Lucisano, Giovanna Carnovale Scalzo, Vincenzo Mollace, Giovanni Improta, Sandrine Zweifel, Vincenzo Scorcia, Mario Damiano Toro, and Adriano Carnevali declare that they have no competing interests.

Ethical Approval. This study was conducted in accordance with the Declaration of Helsinki (1964) and its subsequent amendments. Approval was obtained from the University of Catanzaro Ethics Committee. Written informed consent was obtained from all participants. Consent for publication was also obtained where identifying information is included.

REFERENCES

1. Schmidt-Erfurth U, Chong V, Loewenstein A, et al. Guidelines for the management of neovascular age-related macular degeneration by the European Society of Retina Specialists (EURETINA). Br J Ophthalmol. 2014;98(9):1144–67. https://doi.org/10.1136/bjophthalmol-2014-305702. (**PMID: 25136079**).

2. Urbańska K, Stępień PW, Nowakowska KN, et al. The role of dysregulated miRNAs in the pathogenesis, diagnosis and treatment of age-related macular degeneration. Int J Mol Sci. 2022;23(14): 7761. https://doi.org/10.3390/ijms23147761. (**PMID: 35887109**).

3. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology. 2008;115(1):116–26. https://doi.org/10.1016/j.ophtha.2007.03.008. (**PMID: 17675159**).

4. Spilsbury K, Garrett KL, Shen WY, Constable IJ, Rakoczy PE. Overexpression of vascular endothelial growth factor (VEGF) in the retinal pigment epithelium leads to the development of choroidal neovascularization. Am J Pathol.

2000;157(1):135–44. https://doi.org/10.1016/S0002-9440(10)64525-7. (**PMID: 10880384**).

5. Krzystolik MG, Afshari MA, Adamis AP, et al. Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. Arch Ophthalmol. 2002;120(3):338–46. https://doi.org/10.1001/archopht.120.3.338. (**PMID: 11879138**).

6. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006;355(14):1419–31. https://doi.org/10.1056/NEJMoa054481.

7. Maguire MG, Martin DF, Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, et al. Five-year outcomes with anti-vascular endothelial growth factor treatment of neovascular age-related macular degeneration: the Comparison of Age-Related Macular Degeneration Treatments Trials. Ophthalmology. 2016;123(8):1751–61. https://doi.org/10.1016/j.ophtha.2016.03.045. (**PMID: 27156698**).

8. Dolar-Szczasny J, Bucolo C, Zweifel S, et al. Evaluation of aqueous flare intensity in eyes undergoing intravitreal bevacizumab therapy to treat neovascular age-related macular degeneration. Front Pharmacol. 2021;12: 656774. https://doi.org/10.3389/fphar.2021.656774. (**PMID: 33995079**).

9. Giannakaki-Zimmermann H, Ebneter A, Munk MR, Wolf S, Zinkernagel MS. Outcomes when switching from a pro re nata regimen to a treat and extend regimen using aflibercept in neovascular age-related macular degeneration. Ophthalmologica. 2016;236(4):201–6. https://doi.org/10.1159/000452929. (**PMID: 27907924**).

10. Lanzetta P, Loewenstein A. Fundamental principles of an anti-VEGF treatment regimen: optimal application of intravitreal anti-vascular endothelial growth factor therapy of macular diseases. Graefes Arch Clin Exp Ophthalmol. 2017;255(7):1259–73. https://doi.org/10.1007/s00417-017-3647-4. (**PMID: 28527040**).

11. Freund KB, Korobelnik JF, Devenyi R, et al. Treat-and-extend regimens with anti-VEGF agents in retinal diseases: a literature review and consensus recommendations. Retina. 2015;35(8):1489–506. https://doi.org/10.1097/IAE.0000000000000627. (**PMID: 26076215**).

12. Brinkmann M, Viggiano P, Boscia G, et al. Analysis of choriocapillaris reperfusion topography following faricimab treatment for neovascular age-related macular degeneration in therapy-naïve patients. Ophthalmol Ther. 2024;13(7):1981–92. https://doi.org/10.1007/s40123-024-00967-2. (**PMID: 38801614**).

13. Schmidt-Erfurth U, Waldstein SM, Deak GG, Kundi M, Simader C. Pigment epithelial detachment followed by retinal cystoid degeneration leads to vision loss in treatment of neovascular age-related macular degeneration. Ophthalmology. 2015;122(4):822–32. https://doi.org/10.1016/j.ophtha.2014.11.017. (**PMID: 25578255**).

14. Bolz M, Simader C, Ritter M, et al. Morphological and functional analysis of the loading regimen with intravitreal ranibizumab in neovascular age-related macular degeneration. Br J Ophthalmol. 2010;94(2):185–9. https://doi.org/10.1136/bjo.2008.143974. (**PMID: 19692384**).

15. Bloch SB, la Cour M, Sander B, et al. Predictors of 1-year visual outcome in neovascular age-related macular degeneration following intravitreal ranibizumab treatment. Acta Ophthalmol. 2013;91(1):42–7. https://doi.org/10.1111/j.1755-3768.2011.02268.x. (**PMID: 22008284**).

16. Koman-Wierdak E, Róg J, Brzozowska A, et al. Analysis of the peripapillary and macular regions using OCT angiography in patients with schizophrenia and bipolar disorder. J Clin Med. 2021;10(18): 4131. https://doi.org/10.3390/jcm10184131. (**PMID: 34575242**).

17. Schmidt-Erfurth U, Waldstein SM. A paradigm shift in imaging biomarkers in neovascular age-related macular degeneration. Prog Retin Eye Res. 2016;50:1–24. https://doi.org/10.1016/j.preteyeres.2015.07.007. (**PMID: 26307399**).

18. Holz FG, Tadayoni R, Beatty S, et al. Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. Br J Ophthalmol. 2015;99(2):220–6. https://doi.org/10.1136/bjophthalmol-2014-305327. (**PMID: 25193672**).

19. Mehta H, Tufail A, Daien V, Lee AY, Nguyen V, Ozturk M, Barthelmes D, Gillies MC. Real-world outcomes in patients with neovascular age-related macular degeneration treated with intravitreal vascular endothelial growth factor inhibitors. Prog Retin Eye Res. 2018;65:127–46. https://doi.org/10.1016/j.preteyeres.2017.12.002. (**Epub 2018 Jan 2. PMID: 29305324**).

20. Hanson RLW, Airody A, Sivaprasad S, Gale RP. Optical coherence tomography imaging biomarkers associated with neovascular age-related macular degeneration: a systematic review. Eye (Lond). 2023;37(12):2438–53. https://doi.org/10.1038/s41433-022-02360-4. (**Epub 2022 Dec 16. PMID: 36526863; PMCID: PMC9871156**).

21. Moussa G, Bassilious K, Mathews N. A novel excel sheet conversion tool from Snellen fraction to LogMAR including "counting fingers", "hand

movement", "light perception" and "no light perception" and focused review of literature of low visual acuity reference values. Acta Ophthalmol. 2021;99(6):e963–5. https://doi.org/10.1111/aos.14659. (**PMID: 33326177**).

22. Zweifel SA, Engelbert M, Laud K, Margolis R, Spaide RF, Freund KB. Outer retinal tubulation: a novel optical coherence tomography finding. Arch Ophthalmol. 2009;127(12):1596–602. https://doi.org/10.1001/archophthalmol.2009.326. (**Erratum in: Arch Ophthalmol. 2012 Jul 1;130(7):856. PMID: 20008714**).

23. Cheong KX, Teo KYC, Cheung CMG. Influence of pigment epithelial detachment on visual acuity in neovascular age-related macular degeneration. Surv Ophthalmol. 2021;66(1):68–97. https://doi.org/10.1016/j.survophthal.2020.05.003. (**Epub 2020 May 16. PMID: 32428539**).

24. Carnevali A, Mastropasqua R, Gatti V, et al. Optical coherence tomography angiography in intermediate and late age-related macular degeneration: review of current technical aspects and applications. Appl Sci. 2020;10: 8865. https://doi.org/10.3390/app10248865.

25. Roquet W, Roudot-Thoraval F, Coscas G, Soubrane G. Clinical features of drusenoid pigment epithelial detachment in age related macular degeneration. Br J Ophthalmol. 2004;88(5):638–42. https://doi.org/10.1136/bjo.2003.017632. (**PMID: 15090415**).

26. Shijo T, Sakurada Y, Tanaka K, et al. Incidence and risk of advanced age-related macular degeneration in eyes with drusenoid pigment epithelial detachment. Sci Rep. 2022;12:4715. https://doi.org/10.1038/s41598-022-08626-x.

27. Coscas G, De Benedetto U, Coscas F, Li Calzi CI, Vismara S, Roudot-Thoraval F, Bandello F, Souied E. Hyperreflective dots: a new spectral-domain optical coherence tomography entity for follow-up and prognosis in exudative age-related macular degeneration. Ophthalmologica. 2013;229(1):32–7. https://doi.org/10.1159/000342159. (**Epub 2012 Sep 19 PMID: 23006969**).

28. Dansingani KK, Tan ACS, Gilani F, et al. Subretinal hyperreflective material imaged with optical coherence tomography angiography. Am J Ophthalmol. 2016;169:235–48. https://doi.org/10.1016/j.ajo.2016.06.031. (**PMID: 27349411**).

29. Shah VP, Shah SA, Mrejen S, Freund KB. Subretinal hyperreflective exudation associated with neovascular age-related macular degeneration. Retina. 2014;34(7):1281–8. https://doi.org/10.1097/IAE.0000000000000166. (**PMID: 24695062**).

30. Rachitskaya AV, Goldhardt R. Retinal pigment epithelium tear. Curr Ophthalmol Rep. 2015;3(1):26–33. https://doi.org/10.1007/s40135-014-0057-1. (**PMID: 32051789**).

31. Pauleikhoff D, Löffert D, Spital G, et al. Pigment epithelial detachment in the elderly. Clinical differentiation, natural course and pathogenetic implications. Graefes Arch Clin Exp Ophthalmol. 2002;240(7):533–8. https://doi.org/10.1007/s00417-002-0505-8. (**PMID: 12136282**).

32. Dansingani KK, Balaratnasingam C, Klufas MA, et al. Optical coherence tomography angiography of shallow irregular pigment epithelial detachments in pachychoroid spectrum disease. Am J Ophthalmol. 2015;160(6):1243-54.e2. https://doi.org/10.1016/j.ajo.2015.08.028. (**PMID: 26319161**).

33. Pang CE, Messinger JD, Zanzottera EC, et al. The onion sign in neovascular age-related macular degeneration represents cholesterol crystals. Ophthalmology. 2015;122(11):2316–26. https://doi.org/10.1016/j.ophtha.2015.07.008. (**PMID: 26298717**).

34. Jaggi D, Nagamany T, Ebneter A, et al. Aflibercept for age-related macular degeneration: 4-year outcomes of a "treat-and-extend" regimen with exit-strategy. Br J Ophthalmol. 2022;106(2):246–50. https://doi.org/10.1136/bjophthalmol-2020-316514. (**PMID: 33127830**).

35. Ohji M, Takahashi K, Okada AA, et al. Efficacy and safety of intravitreal aflibercept treat-and-extend regimens in exudative age-related macular degeneration: 52- and 96-week findings from ALTAIR: a randomized controlled trial. Adv Ther. 2020;37(3):1173–87. https://doi.org/10.1007/s12325-020-01236-x. (**PMID: 32016788**).

36. Das KR, Imon AHMR. A brief review of tests for normality. Am J Theor Appl Stat. 2016;5(1):5–12. https://doi.org/10.11648/j.ajtas.20160501.12.

37. Schober P, Vetter TR. Linear mixed-effects models in medical research. Anesth Analg. 2021;132(6):1592–3. https://doi.org/10.1213/ANE.0000000000005541. (**PMID: 34032662**).

38. IBM Corp. Released 2023. IBM SPSS Statistics for Windows. Version 29.0.2.0 Armonk. NY: IBM Corp

39. RStudio Team. RStudio: Integrated Development for R. 2023. RStudio. PBC. Boston. MA URL http://www.rstudio.com/.

40. Told R, Reiter GS, Schranz M, et al. Correlation of retinal thickness and swept-source optical coherence tomography angiography derived vascular changes in patients with neovascular age-related macular degeneration. Curr Eye Res. 2021;46(7):1002–9. https://doi.org/10.1080/02713683.2020.1849734. (**PMID: 33211556**).

41. Mitchell P, Holz FG, Hykin P, et al. Efficacy and safety of intravitreal aflibercept using a treat-and-extend regimen for neovascular age-related macular degeneration: the ARIES study: a randomized clinical trial. Retina. 2021;41(9):1911–20. https://doi.org/10.1097/IAE.0000000000003128. (**PMID: 33782365**).

42. Dugel PU, Jhaveri CD, Chakravarthy U, et al. Effect of retinal thickness variability on visual outcomes and fluid persistence in neovascular age-related macular degeneration: a post hoc analysis of the HAWK and HARRIER studies. Retina. 2022;42(3):511–8. https://doi.org/10.1097/IAE.0000000000003349. (**PMID: 34923515**).

43. Stepanov A, Nemcansky J, Veith M, et al. Two-year results of a combined regimen of aflibercept treatment in three types of choroidal neovascular membrane in the wet form of age-related macular degeneration: real-life evidence in the Czech Republic. Eur J Ophthalmol. 2021;31(5):2488–95. https://doi.org/10.1177/1120672120971190. (**PMID: 33198503**).

44. Daniel E, Toth CA, Grunwald JE, et al. Risk of scar in the comparison of age-related macular degeneration treatments trials. Ophthalmology. 2014;121(3):656–66. https://doi.org/10.1016/j.ophtha.2013.10.019. (**PMID: 24314839**).

45. Willoughby AS, Ying GS, Toth CA, et al. Subretinal hyperreflective material in the comparison of age-related macular degeneration treatments trials. Ophthalmology. 2015;122(9):1846-53.e5. https://doi.org/10.1016/j.ophtha.2015.05.042. (**PMID: 26143666**).

46. Leung KFC, Downes SM, Chong V. A retrospective analysis of the effect of subretinal hyper-reflective material and other morphological features of neovascular age-related macular degeneration on visual acuity outcomes in eyes treated with intravitreal aflibercept over one year. Vision. 2018;2(1):5. https://doi.org/10.3390/vision2010005. (**PMID: 31735869**).

47. Muth DR, Toro MD, Bajka A, et al. Correlation between macular neovascularization (MNV) type and druse type in neovascular age-related macular degeneration (AMD) based on the CONAN classification. Biomedicines. 2022;10(10): 2370. https://doi.org/10.3390/biomedicines10102370. (**PMID: 36289632**).

48. Saitta A, Dliseo LA, Dliseo D. Efficacy and safety of brolucizumab for serous drusenoid pigment epithelium detachment non-responder to bevacizumab and aflibercept. Eur J Ophthalmol. 2023;33(6):NP109–12. https://doi.org/10.1177/11206721221143154. (**PMID: 36448189**).

49. Riazi-Esfahani H, Faghihi H, Bazvand F, et al. Predictive value of different baseline optical coherence tomography biomarkers for visual acuity changes in neovascular age-related macular degeneration. Int J Retina Vitreous. 2025;11:12. https://doi.org/10.1186/s40942-025-00633-0.

50. Lee H, Ji B, Chung H, Kim HC. Correlation between optical coherence tomographic hyperreflective foci and visual outcomes after anti-VEGF treatment in neovascular age-related macular degeneration and polypoidal choroidal vasculopathy. Retina. 2016;36(3):465–75. https://doi.org/10.1097/IAE.0000000000000645. (**PMID: 26076214**).

51. Dolz-Marco R, Litts KM, Tan ACS, et al. The evolution of outer retinal tubulation, a neurodegeneration and gliosis prominent in macular diseases. Ophthalmology. 2017;124(9):1353–67. https://doi.org/10.1016/j.ophtha.2017.03.043. (**PMID: 28456420**).

52. Kovacs A, Kiss T, Rarosi F, et al. The effect of ranibizumab and aflibercept treatment on the prevalence of outer retinal tubulation and its influence on retreatment in neovascular age-related macular degeneration. BMC Ophthalmol. 2018;18(1):298. https://doi.org/10.1186/s12886-018-0960-6. (**PMID: 30428856**).

53. Lee JY, Folgar FA, Maguire MG, Ying GS, Toth CA, Martin DF, Jaffe GJ, CATT Research Group. Outer retinal tubulation in the comparison of age-related macular degeneration treatments trials (CATT). Ophthalmology. 2014;121(12):2423–31. https://doi.org/10.1016/j.ophtha.2014.06.013. (**Epub 2014 Jul 23. PMID: 25064723; PMCID: PMC4254295**).

54. Wrzesińska D, Nowomiejska K, Nowakowska D, et al. Secondary vitrectomy with internal limiting membrane plug due to persistent full-thickness macular hole OCT-angiography and microperimetry features: case series. J Ophthalmol. 2020;2020:2650873. https://doi.org/10.1155/2020/2650873. (**PMID: 33029387**).

55. Alis A, Guler AM. Long-term effect of intravitreal aflibercept treatment on choroidal vascularity index in neovascular age-related macular degeneration. Photodiagn Photodyn Ther. 2021;36:102582. https://doi.org/10.1016/j.pdpdt.2021.102582. (**PMID: 34648995**).

56. Vo TA, Abedi S, Schneider K, Chwa M, Kenney MC. Effects of bevacizumab, ranibizumab, and aflibercept on phagocytic properties in human RPE cybrids with AMD versus normal mitochondria. Exp Eye Res. 2018;177:112–6. https://doi.org/10.1016/j.exer.2018.07.025. (**Epub 2018 Jul 30. PMID: 30071215; PMCID: PMC7105352**).