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ORIGINAL ARTICLE

Psychophysical, electrofunctional, and morphological evaluation in naïve neovascular AMD patients treated with intravitreal anti-VEGF

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Abstract

Objectives: The aim of this study was to investigate the retinal morpho-functional characteristics of patients with neovascular wet age-related macular degeneration (nAMD) treated with intravitreal injection (IV) of aflibercept (AFL).

Methods: The study was conducted on 35 patients previously diagnosed with type 1 nAMD who received a fixed-dosing regimen of aflibercept injections over 12 months. The goal was to assess trends in visual abilities over time by measuring visual acuity (VA), contrast sensitivity (CS), visual evoked potentials (VEPs), and spectral domain-optical coherence tomography (SD-OCT). The same psychophysical, electro-functional, and morphological tests administered at baseline (T0) were repeated 4 to 8 weeks after the last aflibercept injection (Tn), resulting in a total of six examinations.

Results: At Tn, all subjects exhibited improved VA for both far and near distances compared to values detected at T0. Similarly, VEP amplitude and latency values at Tn showed a greater P100 improvement than those observed at T0. Additionally, the CS examination at Tn demonstrated improvement, particularly at high spatial stimulation frequencies. The Tn SD-OCT results highlighted a reduction in macular thickness compared to T0 values.

Conclusions: This exploratory research indicates that intravitreal injections of AFL, following a fixed-dosing regimen, represent a valuable therapeutic approach for enhancing visual performance. This conclusion is supported by comprehensive statistical analysis of psychophysical, electro-functional, and morphological examinations within the same group of patients with nAMD, as demonstrated for the first time.

KEYWORDS
anti-VEGF, contrast sensitivity (CS), optical coherence tomography (OCT), visual evoked potential (VEP), wet age-related macular degeneration (AMD)

1 | INTRODUCTION

Age-related macular degeneration (AMD) is a sight-threatening and progressive ocular disorder that affects the central region of the retina. It is characterized by a complex and multifactorial etiology, including environmental, metabolic, and genetic factors.[1] In today's context, AMD stands as a leading cause of central visual function loss and legal blindness in industrialized countries, particularly among individuals aged over 55 years.[1–4]

According to the International Age-Related Maculopathy (ARM) Epidemiological Study Group, AMD represents an advanced stage beyond ARM. Early ARM is defined as a degenerative disorder in subjects over 50 years of age, marked by the presence of soft drusen (≥ 63 μm) and retinal pigment epithelium (RPE) alterations, such as hyper/hypopigmentation. As the clinical presentation worsens, retinal degeneration becomes associated with RPE geographic atrophy in the absence of neovascular tissue or in the presence of retinal hemorrhages, neurosensory detachment, and subretinal or sub-RPE neovascular membranes. This constellation of features defines the condition as AMD.[3,4]

Subsequently, AMD encompasses two distinct morphological subtypes:

1. Non-exudative (dry or atrophic) AMD: This subtype is the most common and is characterized by the absence of neovascular alterations.
2. Exudative AMD or neovascular wet AMD (nAMD): Clinically, this subtype is defined by choroidal neovascularization (CNV) resulting from vascular endothelial growth factor (VEGF) overexpression by RPE cells. CNV originates from the choriocapillary and extends through a break in the Bruch's membrane. Specifically, an imbalance between VEGF-A (which promotes new vessel growth) and the signal from the pigment epithelial-derived factor (PEDF) contributes to CNV development. Notably, PEDF exhibits both anti-angiogenic and neuroprotective properties.[4]

Therefore, abnormal, dysfunctional, and immature neovascularization induces intraretinal hemorrhages and leakage, leading to progressive visual acuity (VA) reduction, color vision impairment, and decreased contrast and spatio-temporal sensitivity.[5] Currently, the gold standard for CNV diagnosis is fundus fluorescein angiography (FA), supported by optical coherence tomography angiography (OCTA).[6,7] OCTA serves as a valuable tool for both CNV diagnosis and, specifically, for monitoring lesion response to treatment. This noninvasive technology aids in detecting retinal and choroidal circulations associated with neovascular AMD (nAMD) and is increasingly utilized in clinical practice for noninvasive CNV detection.[6]

Several types of chorioretinal neovascularization can be defined based on FA and spectral domain-optical coherence tomography (SD-OCT) findings:

1. Type 1: CNV within the sub-RPE space, also known as occult CNV.
2. Type 2: CNV within the subretinal space, commonly referred to as classic CNV.
3. Type 3: CNV with intraretinal angiomatous proliferation.
4. Type 4: Mixed form of CNV.
5. Polypoidal choroidal vasculopathy: The branching vascular network appears as RPE elevations, whereas the polypoidal lesions manifest as sharper, dome-shaped protuberances, often associated with exudative findings.[8,9]

To date, antiangiogenic or anti-VEGF therapy, such as bevacizumab, ranibizumab, faricimab, or aflibercept (AFL), administered via intravitreal injection (IV) remains the most effective treatment for managing exudative nAMD. Numerous studies have demonstrated comparable treatment efficacy and safety of IV anti-angiogenic therapies.[10,11] Different anti-VEGF treatment strategies exist, including fixed monthly or bimonthly injections, pro-re-nata, and treat-and-extend protocols.[12] These approaches contribute to visual function improvement or stabilization by reducing neovascular exudation.[13]

AFL (Eylea, Regeneron) received US Food and Drug Administration (FDA) approval as an alternative anti-VEGF treatment for nAMD in late 2011. AFL is a recombinant fusion protein composed of VEGF-binding portions from the extracellular domains of human VEGF receptors. These protein domains are fused to the Fc portion of a human immunoglobulin to enhance their half-life.[11,14] AFL functions by mimicking VEGF target receptors and effectively trapping VEGF-A, VEGF-B, and placental growth factor, thereby reducing downstream effects of these chemokines. Multicenter clinical trials have also confirmed the clinical efficacy of AFL in nAMD treatment.[11,14]

The objective of this study was to investigate the retinal morpho-functional characteristics of patients diagnosed with type 1 nAMD and treated with intravitreal injection of AFL. The fixed-dosing regimen protocol spanned 12 months, allowing a careful assessment of visual abilities over time. Parameters measured included VA for both far and near distances, contrast sensitivity (CS), visual evoked potentials (VEP), and SD-OCT.[15,16]

2 | MATERIALS AND METHODS

A prospective longitudinal observational clinical study was conducted on 35 eyes of 35 patients previously diagnosed with nAMD. The study participants were enrolled between September 2019 and October 2021 at the "Retina and Electrophysiology Unit, Department of Sense Organs, Umberto I Policlinic, Sapienza University of Rome."

Inclusion criteria:

- Male and female participants aged > 68 years.
- White population.
- Increased central foveal thickness.
- Alterations of the RPE/choriocapillaris complex.
- Diagnosis of exudative AMD confirmed by SD-OCT and OCTA.

- Presence of neovascularization within the sub-RPE space (type 1 CNV) or occult CNV.
- Signed informed consent.

Exclusion criteria:

- Diagnosis of non-exudative AMD.
- Previous anti-VEGF treatment.
- Presence of other ocular diseases, such as amblyopia, glaucoma, optic neuritis, opacity of the dioptric elements, ocular trauma, or surgery.
- History of cardiac/brain disorders or systemic diseases affecting patient compliance (eg, Parkinson's disease, multiple sclerosis, epilepsy, diabetes mellitus, vascular disorders, dysthyroidism, cardiomyopathies, and malignant neoplasms).

At baseline (T0), all patients underwent a comprehensive ophthalmological examination, including medical history assessment, best-corrected visual acuity (BCVA) measurement for both far and near distances, slit-lamp biomicroscopy (with and without pupillary dilation), intraocular pressure measurement using Goldmann applanation tonometry after topical anesthetic drops application, CS assessment, VEP, dilated fundus examination, SD-OCT, and OCTA.

Following clinical practice guidelines, all patients received a total of seven intravitreal anti-VEGF injections of AFL. The treatment regimen included a fixed monthly schedule for the initial 3 months (loading phase), followed by injections at 2-month intervals until the twelfth month from the start of treatment. Monthly follow-up visits were conducted, and various tests were performed.

The same psychophysical, electro-functional, and morphological tests conducted at T0 were subsequently repeated 4 to 8 weeks after the last AFL injection, resulting in a total of six examinations. Specifically, all participants underwent the following assessments:

- BCVA was measured using Early Treatment Diabetic Retinopathy Study charts at a distance of 4 meters. Results were expressed in the logarithm of the minimum angle of resolution (logMAR) units for distance vision and in points (pts) for near vision.
- CS test was evaluated at low, medium, and high spatial stimulation frequencies (SSFs) following the International Society for Clinical Electrophysiology of Vision (ISCEV) standard CS protocol.[15]
- VEP responses were elicited by stimuli with large, medium, and small SSF, adhering to ISCEV standard VEP protocols.[15]
- SD-OCT and OCTA.

The CS test assesses an individual's ability to discriminate between light and dark. It was performed at a distance of 200 cm using a specific testing program called "Static Contrast Photopic" on the opto-electronic stimulator (Vision Monitor MonPack 120 by Metrovision, Pérenchies, France). The grating pattern was initially presented to the patient as unseen, and the contrast level was gradually increased until a response was obtained, with 0.25 dB (decibel) increments of contrast. Testing was monocular, and optical correction for distance was applied if necessary. Multiple measurements were taken to evaluate response reproducibility, resulting in five sets of six measurements. Three mean values were calculated for six SSF in cycles per degree (cpd) at low (0.8–1.6 cpd), medium (3.2 to 6.4 cpd), and high (12.8–25.6 cpd) SSF. The final graph curve, shown in green, depicted all responses obtained for each SSF in cpd and at different contrast levels in dB.

TABLE 1 Descriptive analysis of demographic and instrumental variables values at baseline and last follow-up time collected in 35 patients (one eye per patient)

Variables			T0		Tn
Age, y			73.00 [68.50, 80.50]		
Sex	Female	Male	18 (51.4)	17 (48.6)	
Eye	Left	Right	16 (45.7)	19 (54.3)	
Visual acuity (far)			6.00 [5.00, 7.00]		8.00 [7.00, 10.00]
Visual acuity (near)			3.00 [2.00, 4.00]		1.00 [1.00, 2.50]
VEP amplitude 120′			6.70 [6.00, 8.00]		7.90 [6.95, 8.25]
VEP amplitude 60′			7.00 [6.00, 8.00]		8.30 [7.20, 9.00]
VEP amplitude 15′			5.00 [4.15, 6.00]		6.30 [5.30, 7.50]
VEP latency 120′			125.00 [116.50, 131.00]		116.00 [110.00, 121.50]
VEP latency 60′			127.00 [120.00, 131.00]		118.00 [112.00, 124.00]
VEP latency 15′			140.00 [135.00, 144.00]		133.00 [127.00, 138.00]
SSF low			19.00 [13.50, 21.15]		20.00 [15.75, 24.00]
SSF medium			18.00 [15.15, 22.65]		20.00 [17.75, 24.50]
SSF high			13.00 [9.00, 16.00]		16.00 [9.50, 19.00]
SD-OCT foveal			435.00 [393.50, 545.50]		300.00 [269.00, 343.50]
SD-OCT perifoveal			478.00 [431.00, 554.50]		380.00 [357.00, 426.50]
SD-OCT parafoveal			498.00 [427.50, 567.00]		357.00 [320.50, 401.50]

Abbreviations: SD-OCT, spectral domain-optical coherence tomography; SSF, spatial stimulation frequencies; VEP, visual evoked potential.

TABLE 2 Median of the differences with respective 95% confidence intervals of all instrumental variables values between baseline (T0) and last follow-up time (Tn) and *P* values of the Wilcoxon signed-rank test adjusted according to Bonferroni correction.

Variables	Median (95% CI)	*P* values
Visual acuity (far)	2.000 (1.038 to 2.962)	<0.001
Visual acuity (near)	−1.000 (−1.361 to −0.639)	<0.001
VEP amplitude 120′	0.900 (0.493 to 1.307)	0.001
VEP amplitude 60′	1.200 (0.706 to 1.694)	0.002
VEP amplitude 15′	1.300 (0.622 to 1.978)	<0.001
VEP latency 120′	−6.000 (−8.709 to −3.291)	<0.001
VEP latency 60′	−6.000 (−8.837 to −3.163)	0.001
VEP latency 15′	−6.000 (− 8.009 to − 3.991)	0.001
SSF low	2.300 (1.176 to 3.424)	<0.001
SSF medium	1.700 (0.509 to 2.891)	0.009
SSF high	2.000 (0.865 to 3.135)	<0.001
SD-OCT foveal	−156.000 (−204.954 to −107.046)	<0.001
SD-OCT perifoveal	−89.000 (−123.977 to −54.023)	<0.001
SD-OCT parafoveal	−115.000 (−165.160 to −64.840)	<0.001

Abbreviations: SD-OCT, spectral domain-optical coherence tomography, foveal, perifoveal, and parafoveal thickness; SSF, spatial stimulation frequencies of contrast sensitivity (CS); VEP, visual evoked potential.

Transient VEP waveforms were recorded at a distance of 100 centimeters (cm) using an optoelectronic stimulator (Vision Monitor MonPack 120 by Metrovision, Pérenchies, France), by ISCEV standards.[15] Transient VEP were elicited using checkerboard stimuli with small (15.4 minutes of arc), medium (60 minutes of arc), and large (120 minutes of arc) checks. The examination took place in an isolated room under dark conditions, with the patient positioned 1 meter away from the monitor. Three cutaneous electrodes were applied: an exploration electrode positioned 3.5 cm. above the inion, a reference electrode on the forehead, and a neutral electrode on the earlobe. The patient wore corrective lenses for far vision and performed the examination monocularly.

All standard pattern stimuli consisted of high-contrast, black-and-white checkerboards with reversing squares. The stimulus had a spatial frequency of 2.02 cpd, a temporal frequency of 1.85 Hertz (Hz), an analysis time of 250 milliseconds, and a maximal resolution of 0.1 microvolts (μV). The P100 principal wave was evaluated at 120′, 60′, and 15′ for both amplitude and latency (time-to-peak). A series of N75, P100, and N135 peaks characterized by amplitude and latency were identified. If the amplitude was equal to or less than 2.0 μV, it was considered zero. Electrode contact impedance values were maintained below 5 Kilohms to achieve the best possible signal-to-noise ratio.

Patients underwent imaging using SD-OCT (Spectralis HRA/OCT, Heidelberg Engineering, Heidelberg, Germany) with Heidelberg Eye Explorer (version 1.6.2.0). The axial resolution was 3.5 micrometers (μm), and the transverse resolution was approximately 15 of 20 μm. Parameters evaluated for each scan included macular, foveal, peri-foveal, and para-foveal thickness.

The study protocol received approval from the local Ethics Committee of Sapienza University of Rome (E.C. Rif. 6502 Prot. 0920/2021), and the procedures adhered to the principles of the Declaration of Helsinki. All participants were informed about data usage and provided signed informed consent.

2.1 | Statistical methods

Demographic and instrumental examination variables were collected in 35 patients (one eye per patient) at six different follow-up times (ie, at the time of admission, and subsequently at 1, 2, 3, 5, and 7 months after admission). A descriptive analysis was performed for all the demographic and instrumental variables, both at admission and at the last follow-up time. Because of the nonsymmetrical distribution of the variables and the presence of outlier values, continuous variables were summarized using median and quartiles; categorical variables were reported using absolute frequencies and percentages. Variable distributions at both times were graphically presented through boxplots. Patients' trajectories across follow-up times were presented through spaghetti plots for each of the instrumental examination variables evaluated in the study; a Locally Weighted Scatterplot Smoothing (LOESS) smoothing regression line was added to the plot to graphically foresee a trend.[17] Friedman rank sum test was performed to assess potential changes over time of each of the instrumental variables across all the six follow-up time points for each patient. Subsequently, as the major clinical interest of the present study consisted in the difference between pre- and post-treatment measurements, a comparison between baseline (before starting intravitreal AFL treatment: T0) and last follow-up time (4–8 weeks after the last treatment: Tn) was performed through Wilcoxon signed-rank test for all the instrumental examination variables considered. Moreover, to evaluate the morphofunctional analysis of the macula before and after the treatment, we explored the correlations among pre- and post-treatment changes in the measurements of OCT vs CS and VEP measurements changes through Spearman's rank correlation coefficient. All hypothesis tests were two-tailed and the level of significance was set at $\alpha = 0.05$; Bonferroni correction was applied to account for the multiple comparisons. To assess the difference between baseline and last follow-up time, medians of the differences were estimated through quantile regression techniques.[18] All analyses were performed using the statistical software R (version 4.1.2).[19]

3 | RESULTS

The patients enrolled in this research underwent psychophysical, electro-functional, and morphological tests. The study included 35 eyes (19 right and 16 left, accounting for 54.3% and 45.7%,

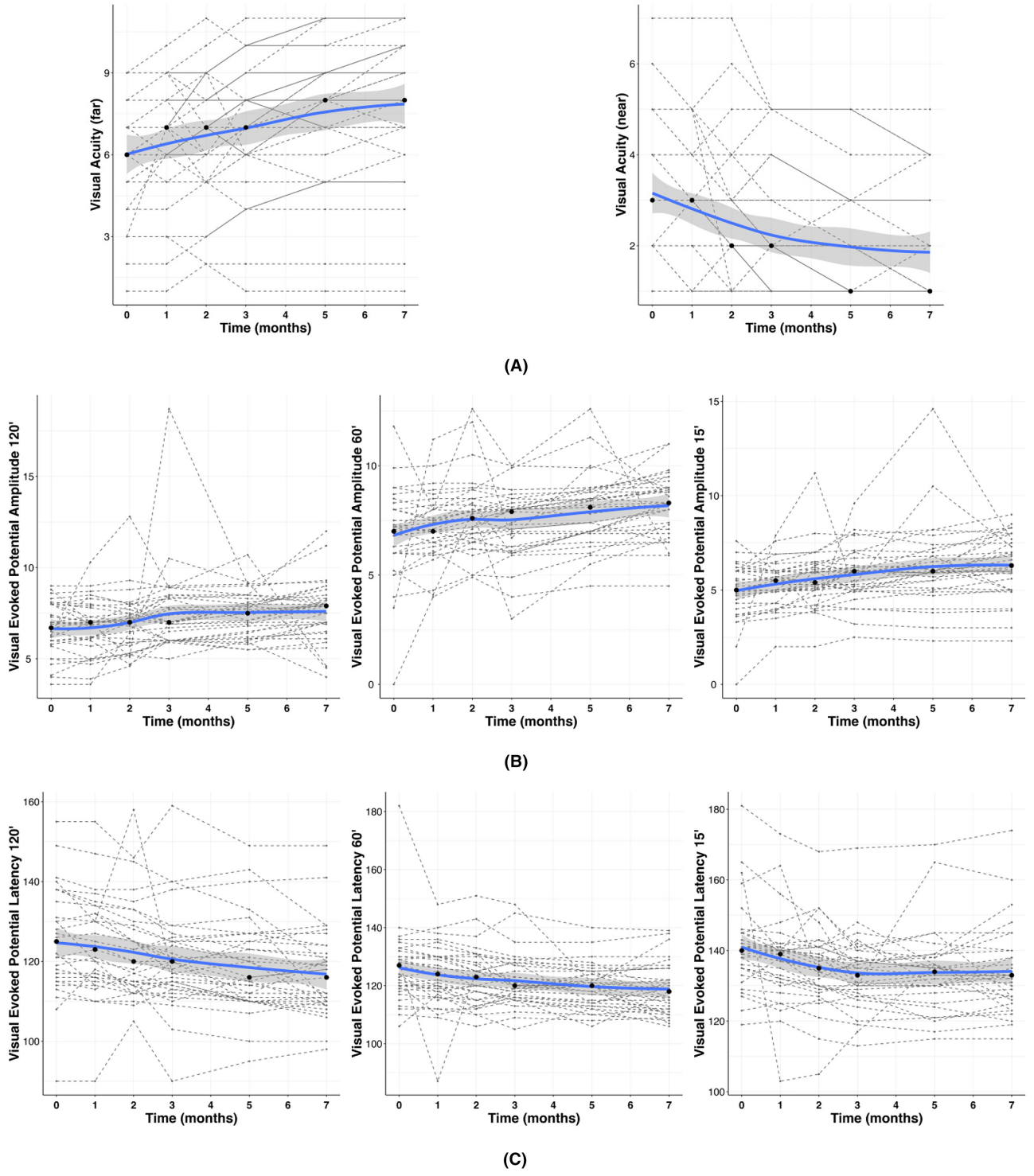


FIGURE 1 Spaghetti plots of the instrumental variables across follow-up times. Patients' trajectories are represented by gray dotted lines and LOESS regression is represented as a blue line; black dots represent the medians evaluated at each time point. No statistical test was associated with this data representation. (A) Visual acuity for far and near; (B) VEP amplitude to 120′, 60′, and 15′; (C) VEP latency to 120′, 60′, and 15′. LOESS, Locally Weighted Scatterplot Smoothing; VEP, visual evoked potential.

respectively) from 35 patients, equally representative of both sexes: 18 of 35 female patients (51.4%) and 17 of 35 male patients (48.6%). The median age was 73 years old, with the first and third quartiles ranging from 68.5 to 80.5 years (Table 1). Data were collected at T0 before initiating intravitreal AFL treatment and during the 12-month follow-up period, up to 4–8 weeks after the last intravitreal injection (Tn) (Tables 1 and 2). Spaghetti plots (Figures 1 and 2), generated using LOESS regression lines, demonstrated an overall trend of improvement across all instrumental examination variables throughout the study protocol.

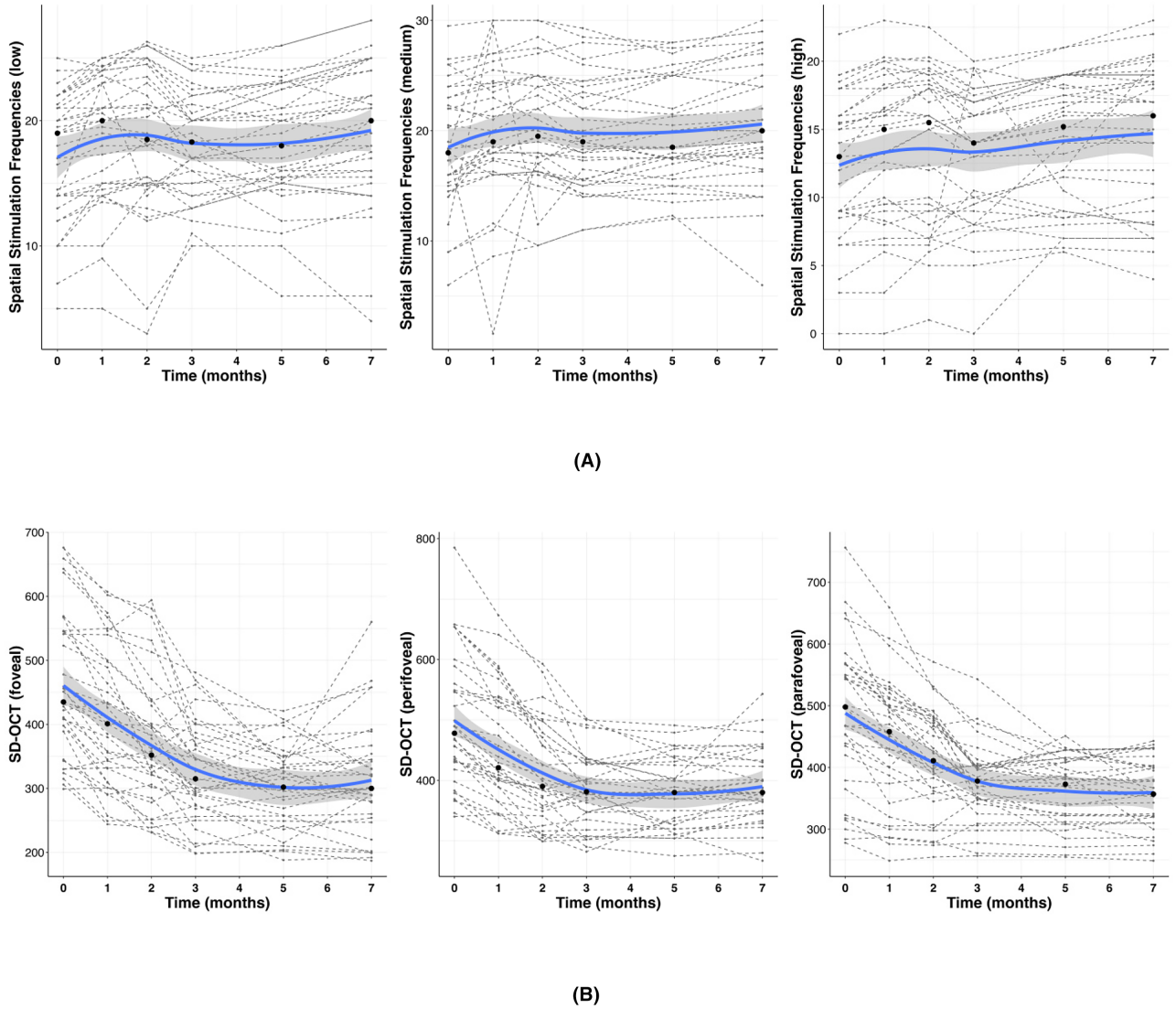


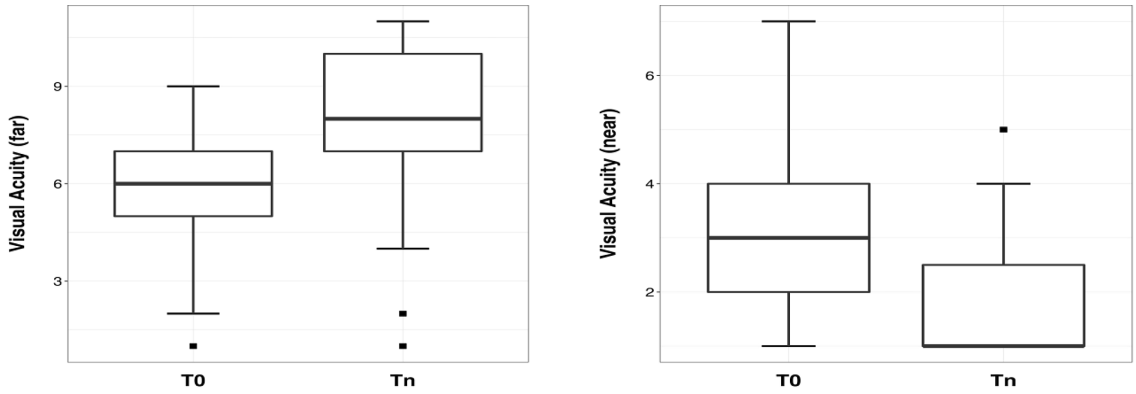
FIGURE 2 Spaghetti plots of the instrumental variables across follow-up times. Patients' trajectories are represented by gray dotted lines and LOESS regression is represented as a blue line; black dots represent the medians evaluated at each time point. No statistical test was associated with this data representation. (A) Low, medium, and high SSF; (B) foveal, perifoveal, and parafoveal SD-OCT. LOESS, Locally Weighted Scatterplot Smoothing; SD-OCT, spectral domain-optical coherence tomography; SSF, spatial stimulation frequency.

The Friedman rank sum test revealed statistical significance (P value <0.0001) for all instrumental variables, indicating differences across the evaluated time points for each patient. Specifically, the Wilcoxon signed-rank test compared T0 measurements with those at the last follow-up time (Tn), revealing statistically significant differences for each instrumental variable. A descriptive analysis of instrumental variable values at baseline and the last follow-up time is presented in Table 1 and Figures 3 and 4. Median differences (with respective 95% confidence intervals) and adjusted P values from the Wilcoxon signed-rank test are shown in Table 2. Additionally, Spearman's rank correlation coefficient was used to explore the relationship between pre- and post-treatment changes in OCT measurements and changes in CS and VEP measurements.

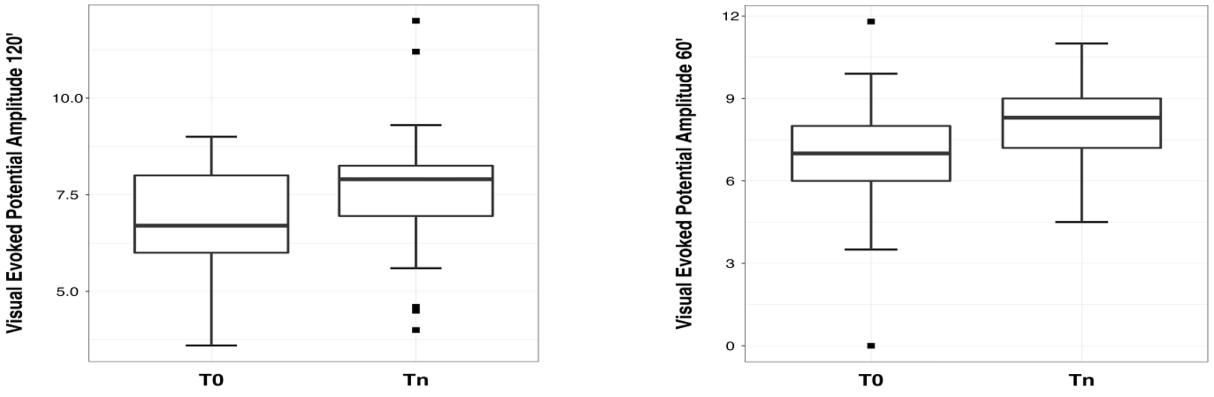
This correlation test was performed for all combinations of OCT measurements versus CS and VEP, respectively. Each change measure was evaluated as the difference between the last follow-up time value and baseline. However, none of the tests yielded statistically significant results, likely due to the small sample size.

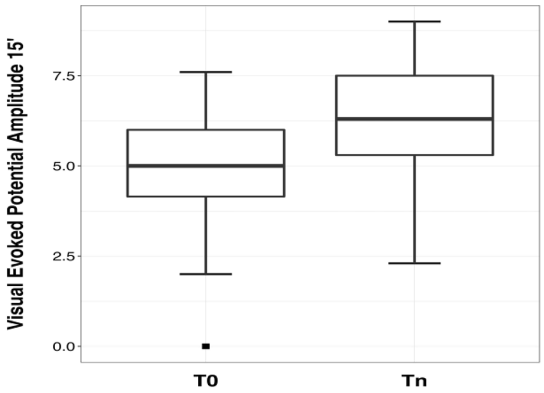
After the initial treatment with AFL, subjects exhibited a mean BCVA improvement of approximately 2 of 10 (equivalent to 19–23 letters) compared to T0. Similarly, corrected near visual acuity (CNVA) improved by approximately two characters relative to baseline values. VEP amplitude and latency values at low, median, and high SSF showed P100 improvement compared to both T0 and Tn. Notably, the CS examination at Tn demonstrated significant improvement at low and high SSF.

SD-OCT revealed reductions in foveal, perifoveal, and parafoveal thickness at Tn compared to T0 values (Table 2 and Figure 2). Importantly, no patients experienced drug-related side effects throughout the treatment period.



(A) VA far and near (SS ***)





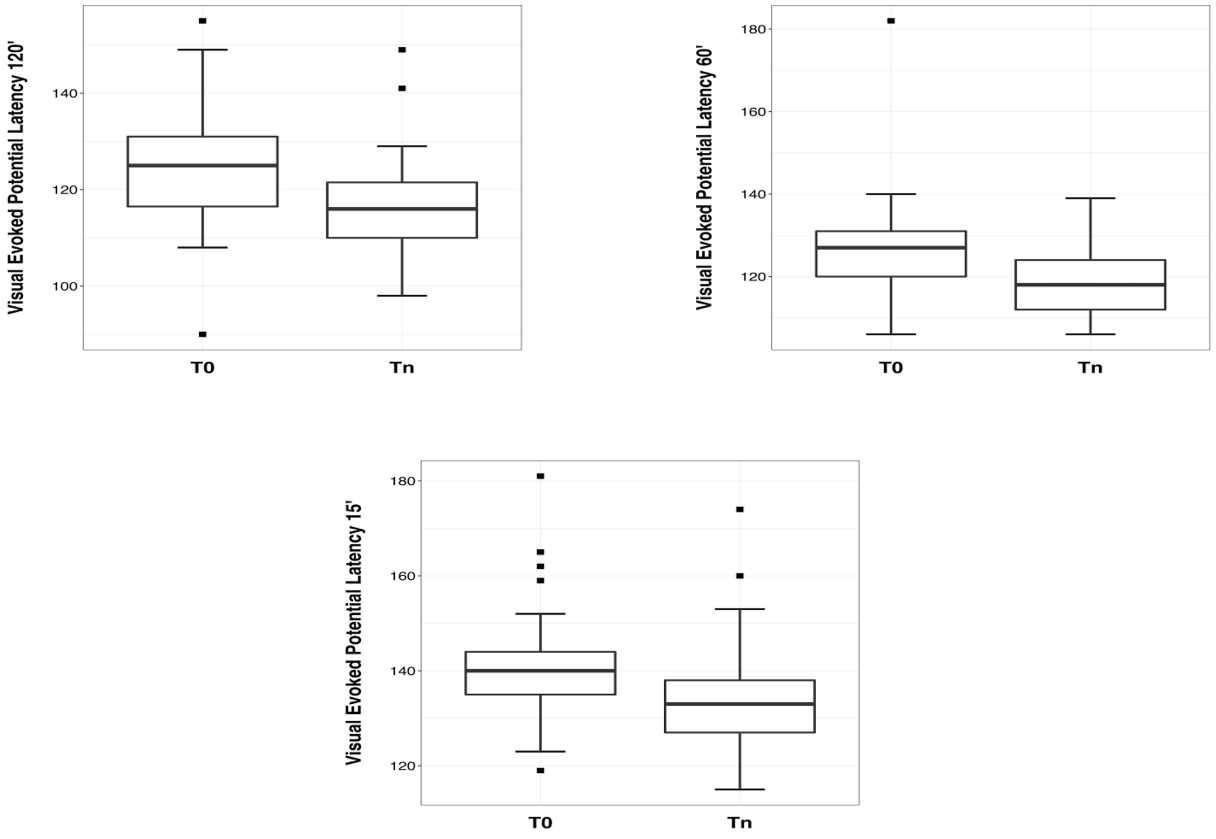
(B) VEP amplitude 120' and 60' (SS **) VEP amplitude 15' (SS ***)

FIGURE 3 (Continued)

Figures 5–7 report SD-OCT, OCTA, and retinography of a patient affected by type 1 CNV due to nAMD. These images show the reduction and subsequent disappearance of the epithelial neural detachment (END) before (Figure 5), during (Figure 6), and after (Figure 7) treatment with IV of AFL. However, mild cystoid macular edema (CME) and a spongy appearance of the neuroepithelium (EN) persisted in the area corresponding to the attenuated CNV (Figure 7).

4 | DISCUSSION

The purpose of this study was to establish a relationship between initial and final values related to the visual characteristics of patients with type 1 nAMD treated with IV of AFL. We demonstrated that IV of AFL constitutes a useful therapeutic approach for improving visual performance. This conclusion is supported by the first comprehensive study of psychophysical,



(C) VEP latency 120' and 60' (SS ***) VEP latency 15' (SS **)

FIGURE 3 Boxplots of the instrumental variables values at baseline (T0) and at last follow-up time (Tn); Statistically significant difference found at Wilcoxon signed-rank test adjusted according to Bonferroni correction for all the represented variables. (A) Visual acuity for far and near; (B) VEP amplitude to 120′, 60′, and 15′; (C) VEP latency to 120′, 60′, and 15′. SS, statistically significant; VA, visual acuity; VEP, visual evoked potential.

electro-functional, and morphological examinations within the same group of patients.

AFL, a soluble recombinant fusion protein (115 kDa), received FDA approval in 2011 in the United States and in 2012 in the European Union for nAMD treatment.[20] It effectively binds several isoforms of VEGF-A, VEGF-B, and placental growth factors 1 and 2 with high affinity, thereby inhibiting immature neovessel proliferation.[21] Notably, AFL exhibits greater VEGF affinity than ranibizumab and bevacizumab, allowing for longer intervals between injections compared to other anti-VEGF drugs.[22] The FDA-recommended AFL dose is 2 mg (0.05 mL) via IV every 4 weeks for the first 3 months, followed by 2 mg (0.05 mL) every 8 weeks thereafter.[23]

In our study, 35 eyes of 35 patients with type 1 nAMD were evaluated. They received monthly AFL injections during the initial 3 months (loading phase), followed by injections every 2 months according to a fixed-dosing regimen. Significant improvements were observed in both BCVA and CNVA, consistent with other studies. VA gains were evident in both short-term (12 months) and long-term (96 weeks) follow-up (Figures 5–7).[24,25] Almuhtaseb et al[26] reported a mean letter gain of up to eight 1 year after baseline in patients receiving fixed-dosing AFL for nAMD.

Similar results were observed by Park et al,[27] who found no significant differences between ranibizumab and AFL treatments in terms of patients' VA outcomes. In line with the notion that electro-functional examinations, such as VEP, can serve as useful markers to identify abnormal responses in macular diseases, Jiang et al aimed to correlate BCVA and VEP. Photoreceptor damage in macular diseases can lead to alterations in the transmission of visual signals from these cells to retinal ganglion cells (RGCs).[28,29] However, only a few studies in the literature have analyzed the correlation between macular diseases and VEP,[30–33] particularly in patients with nAMD treated with anti-VEGF therapy.[34]

Previously, Vottonen et al observed improvements in VEP markers in patients with nAMD. Post-treatment VEP at low, median, and high SSF showed increased P100 amplitude.[35] Notably, P100 latency remained stable compared to results reported by the same authors. Specifically, Vottonen et al demonstrated a correlation between VA, changes in central retinal thickness, and VEP parameters. They also speculated that VEP could serve as a potential

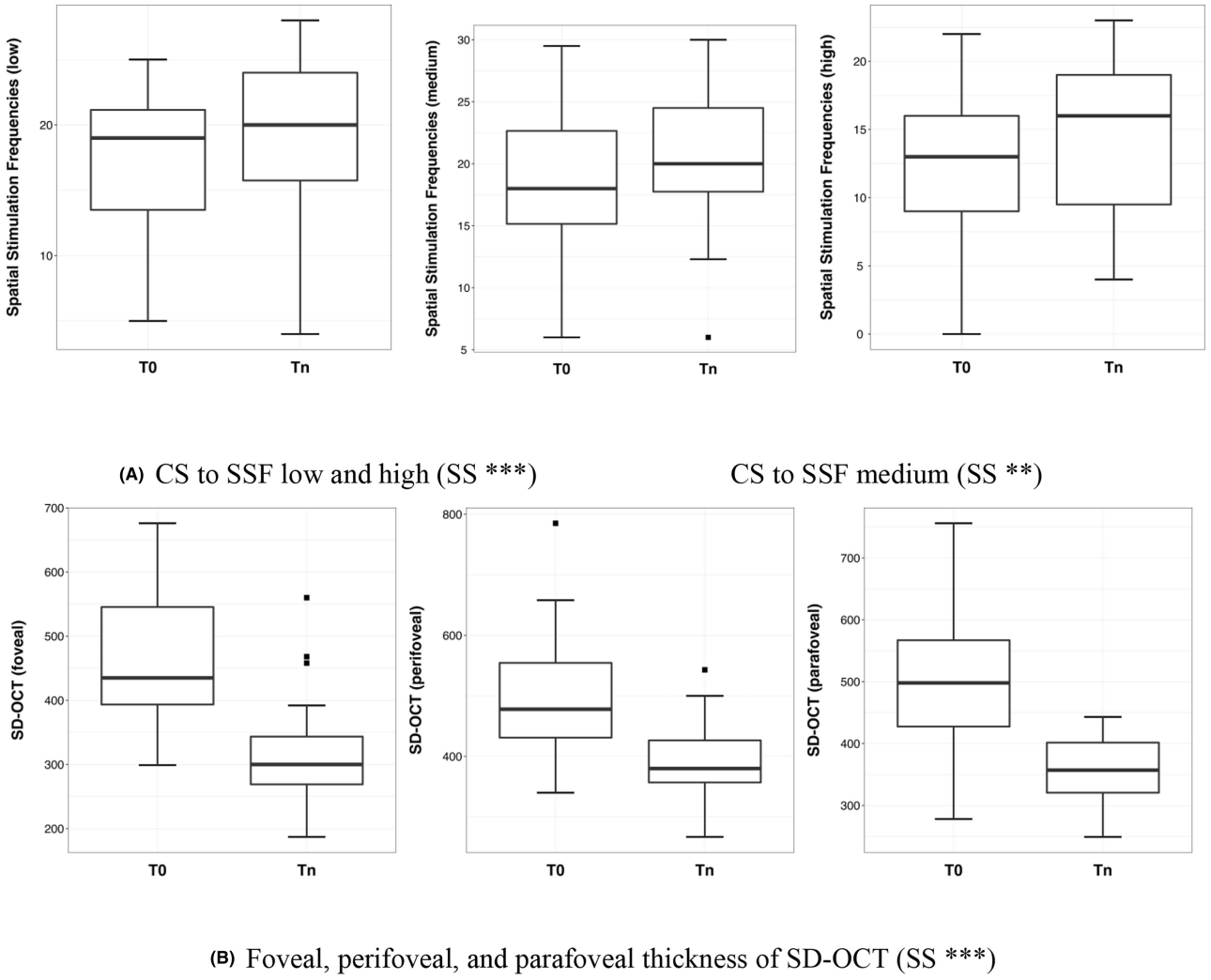


FIGURE 4 Boxplots of the values of the instrumental variables at baseline (T0) and at last follow-up time (Tn); Statistically significant difference found at Wilcoxon signed-rank test adjusted according to Bonferroni correction for all the represented variables. (A) Contrast sensitivity to low, medium, and high SSF; (B) foveal, perifoveal, and parafoveal SD-OCT. CS, contrast sensitivity; SD-OCT, spectral domain-optical coherence tomography; SS, statistically significant; SSF, spatial stimulation frequency.

new monitoring tool for patients with nAMD undergoing anti-VEGF therapy. The study revealed that both increased VEP amplitude and improved VA were associated with decreased retinal thickness in patients treated with intravitreal injections. However, the results did not highlight the additional utility of VEP in the diagnosis or monitoring of nAMD.[35]

Furthermore, patients with nAMD often experience decreased CS, making CS testing a crucial examination for detecting visual function and clinical abnormalities.[36]

Previous studies have suggested an influence of baseline CS on the final CS recorded. Additionally, younger individuals tend to exhibit better CS responses than older subjects.[37,38] According to Roh et al,[38] VA and CS do not follow the same trend during progressive visual loss, although they appear to have a moderate correlation.

In our study, we observed a modest improvement in CS at low and median SSFs, whereas a significant improvement was registered at high SSF. At baseline, CS responses were absent or reduced across all SSFs. These findings align with those reported by Nixon et al, who observed significant CS improvement in patients with nAMD resistant to IV ranibizumab, subsequently switched to intravitreal AFL.[39]

In a cross-sectional study conducted in 2019, Pondorfer et al measured VA and CS in patients with early, intermediate, and late AMD. The results emphasized the utility of these tests in examining patients with early AMD, rather than those with intermediate and/or late AMD. Overall, both CS and VA provide important parameters for assessing visual impairment and treatment outcomes.[16,40]

Notably, there are no studies in the literature linking VA, VEP, CS, and SD-OCT parameters in patients with nAMD treated with intravitreal AFL. However, our study demonstrated clinically significant improvements in VA, CS, VEP, and SD-OCT parameters between initial and final values. Additionally, a reduction in central foveal thickness was observed after the fifth AFL injection (Figures 1 and 2).

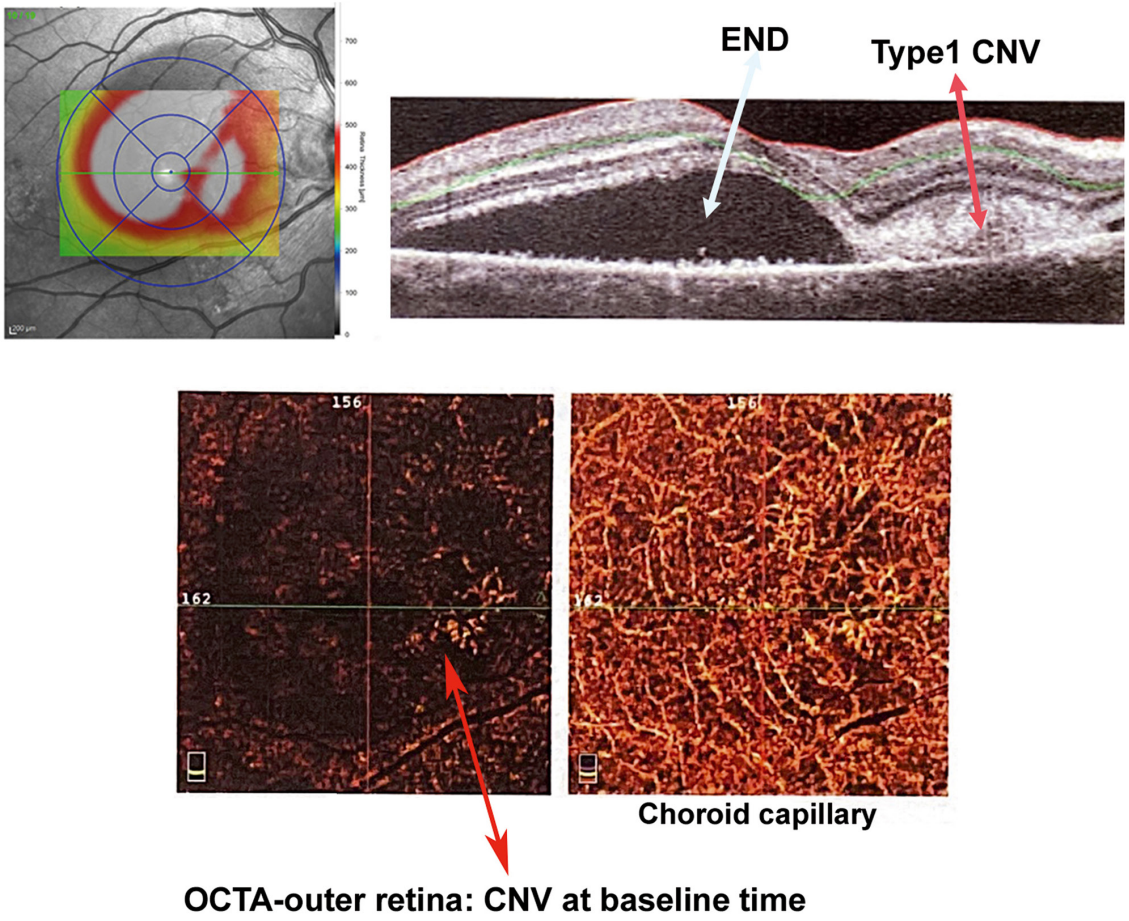


FIGURE 5 SD-OCT and OCTA examinations of a patient affected by nAMD before treatment with intravitreal injections (IV) of AFL. Type 1 neovascular membrane (CNV) at baseline (red arrow) and END can be observed. AFL, aflibercept; CNV, choroidal neovascularization; END, epithelial neural detachment; nAMD, neovascular age-related macular degeneration; OCTA, optical coherence tomography angiography; SD-OCT, spectral-domain optical coherence tomography.

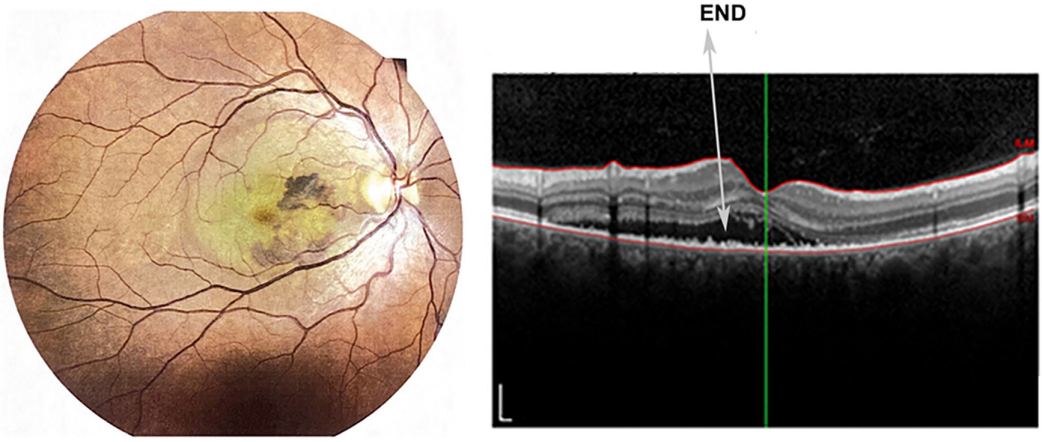


FIGURE 6 Retinography, SD-OCT, and OCTA examinations of the same patient (see Figure 5) affected by nAMD during treatment with AFL intravitreal injections (IV). Reduction of the END can be observed (white arrow). AFL, aflibercept; END, epithelial neural detachment; nAMD, neovascular age-related macular degeneration; OCTA, optical coherence tomography angiography; SD-OCT, spectral-domain optical coherence tomography.

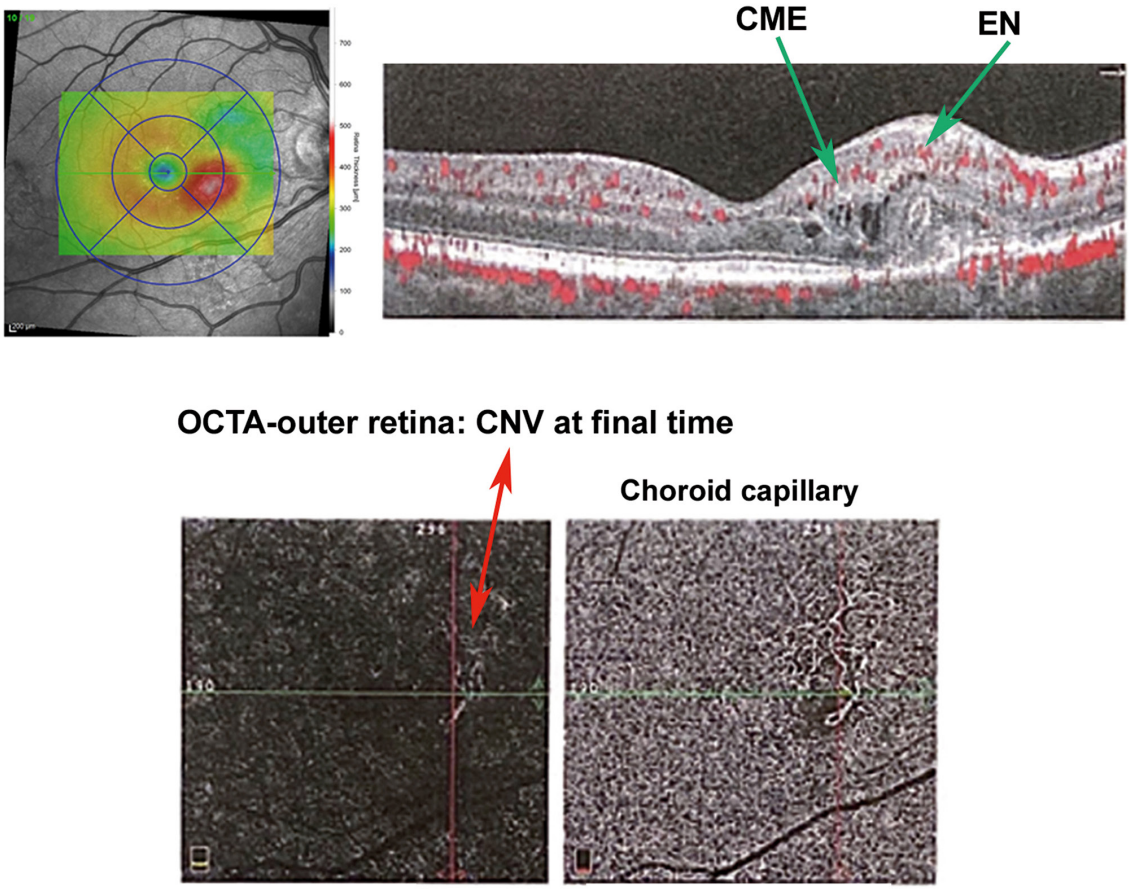


FIGURE 7 SD-OCT and OCTA examinations of the same patient (see Figures 5 and 6) affected by nAMD after treatment with AFL intravitreal injections (IV). The reduction of the type 1 neovascular membrane (CNV) on the OCTA examination can be observed after several IV injections of AFL and the disappearance of the END (red arrow). However, mild CME and a spongy appearance of the neuroepithelium (EN) persist in the area corresponding to the attenuated CNV. AFL, aflibercept; CME, cystoid macular edema; CNV, choroidal neovascularization; EN, neuroepithelium; END, epithelial neural detachment; nAMD, neovascular age-related macular degeneration; OCTA, optical coherence tomography angiography; SD-OCT, spectral-domain optical coherence tomography.

Hence, we can conclude that changes in VEP, VA for far and near, CS, and SD-OCT can be attributed to morphological improvements in macular anatomic structures, resulting in functional enhancement. Notably, these improvements were consistently observed after each IV injection of an AFL in our study. Although the pathological events driving the progression of AMD are still under investigation, we understand that serum-hemorrhagic exudative material plays a central role in causing visual damage. This condition arises from the presence of newly formed vascular tissue that affects various retinal layers, starting from the photoreceptors and extending to the ganglion cells.[1–5] Chorioretinal alterations due to dysmetabolic factors, cellular hypoxia, inflammatory biomarkers, oxidative stress, free radicals, and cell apoptosis represent the underlying triggers of senile maculopathy.[1–5] Once the pathological process begins, it tends to persist over time and only halts during tissue atrophy or gliosis phases.[1–5] Currently, repeated IV of anti-VEGF agents are the primary means to reverse early-stage disease progression or halt its advancement in intermediate stages.[13,20,25]

In our view, close monitoring of patients using multiple noninvasive and rapid tests is crucial. This approach provides valuable information about the health of the visual apparatus in treated subjects, allowing for timely adjustments and preserving as much vision as possible to maintain adequate autonomy. We believe that a careful follow-up of treatment-naive patients with nAMD after IV of AFL can provide valuable information to ophthalmologists who have a large patient load, limited time availability, and limited equipment for assessing drug benefits. Unfortunately, the limitation of our study lies in the short evaluation period for patients examined in this preliminary research. Based on the results obtained, we aim to validate these outcomes and continue the investigation by comparing various IV drugs, expanding the sample size, and extending the follow-up time to better evaluate disease progression in response to anti-VEGF treatment. Additionally, our next goal will be to explore correlations among changes in measurements obtained from OCT, CS, and VEP testing.

Furthermore, our future studies will evaluate an extension of the treatment interval to 8 weeks from the first injection. We will statistically demonstrate whether this regimen can effectively reduce the number of IV in elderly patients affected by nAMD.

5 | CONCLUSIONS

In summary, patients with nAMD exhibit significant morphofunctional alterations in the retina and macula, which profoundly impact visual function. IV injection of AFL represents a valuable therapeutic approach for improving visual performance and enhancing vision-related quality of life.[10,11,13] In this exploratory research on the CNV response to a fixed-dosing monthly regimen for 3 months (loading phase) followed by intervals every 2 months until the twelfth month from the start of anti-VEGF treatment, we found that CS, VEP, and SD-OCT, along with VA measurement, serve as valid and useful tools for follow-up and diagnosis of this ocular pathology.

AUTHOR CONTRIBUTIONS

Conceptualization: Nebbioso. *Methodology:* Franzone. *Software:* Vestri. *Validation:* Artico and Taurone. *Formal analysis:* La Cava and Livani. *Investigation:* Nebbioso. *Resources:* Franzone. *Data curation:* Nebbioso. *Writing original draft preparation:* Nebbioso. *Writing review and editing:* Franzone. *Visualization:* Bonfiglio. *Supervision:* Bonfiglio. *Project administration:* Nebbioso. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest related to this study.

ETHICS STATEMENT

This study is based on anonymous data obtained from the Department of Sense Organs, Policlinico Umberto I, Sapienza University of Rome. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Sapienza University of Rome (E.C. Rif. 6502 Prot. 0920/2021, dated October 13, 2021, Rome). Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients for the publication of this paper. Data are openly available upon reasonable request from the corresponding authors.

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