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ORIGINAL RESEARCH ARTICLE

Single-session photodynamic therapy combined with intravitreal ranibizumab for neovascular age-related macular degeneration: a comprehensive functional retinal assessment

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Abstract
Purpose To explore functional retinal changes in neovascular AMD patients (nAMD) treated with ranibizumab 0.5 mg combined with photodynamic therapy (PDT) 3 days after the first injection in the long term.
Methods Patients with no prior treatment for nAMD were treated with 3 injections of ranibizumab 0.5 mg 1 month apart and a single session of standard PDT 3 days after the first injection. Best-corrected visual acuity and time-domain OCT at baseline and every 28 ± 2 days were performed; microperimetry at 3, 6, and 12 months and multifocal electroretinogramm (mfERG) at 3 and 12 months were repeated. Fluorescein angiography and vision-related quality-of-life questionnaire were performed at baseline and 12 months.
Results 12/15 nAMD patients completed the 12 months study and received an average of 3.4 ± 0.7 injections. Mean VA changed from 54.67 ± 15.72 to 59.0 ± 24.77 letters ($p = 0.371$), while mean retinal sensitivity from 5.5 ± 4.8 to 6.6 ± 6.0 dB ($p = 0.216$). MfERG N1–P1 response amplitude densities (RADs) were significantly different from baseline ($p < 0.01$) in the central 0°–2.5°, whereas in the peripheral retinal areas (2.5°–20°), not significant ($p > 0.01$) changes in N1–P1 RADs were detected. The "general vision" VFQ-25 subscale showed a statistically significant improvement at 3 and 12 months.
Conclusions Ranibizumab 0.5 mg combined with standard PDT 3 days after the first injection determines an improvement of mfERG values in the retinal central area in nAMD patients in long-term follow-up.

Keywords Age-related macular degeneration · Anti-VEGF · Choroidal neovascular membrane · Photodynamic therapy · mfERG · Ranibizumab

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Introduction

Age-related macular degeneration (AMD) is the leading cause of central vision loss in developed countries, and choroidal neovascularization (CNV) accounts for roughly 75 % of the cases of severe vision loss attributable to AMD [1].

The introduction of inhibitors of vascular endothelial growth factor (VEGF) has drastically improved the prognosis of this devastating disease, and ranibizumab, which targets VEGF-A, has become the gold standard treatment [2]. However, despite positive clinical benefits, several factors may limit the use of anti-VEGF monotherapy: Not all patients may benefit

from it; frequent retreatments appear to be necessary to maintain efficacy and may increase the potential for adverse effects. Whether such regular injections are required indefinitely is also unclear [3–5]. This intensive treatment schedule represents a significant burden to both patient and health-care system. This and the potential side effects of intraocular administration continue to drive research toward individualized dosing strategies and multimodal treatment combinations aiming to counteract the complex pathogenesis responsible for CNV and potentially to decrease the frequency of retreatment necessary for achieving optimal results [6].

The benefits of photodynamic therapy (PDT) with verteporfin (Visudyne; Novartis, Basel, Switzerland) for patients with subfoveal CNV associated with AMD have been established [7–9]. However, when compared head-to-head in a randomized, controlled trial, ranibizumab has been shown to be significantly more efficacious than PDT in monotherapy [10, 11]. PDT has therefore been used less systematically, despite its favorable safety profile. Moreover, the vaso-occlusive mechanism induced by PDT, which affects both CNV and the normal choroid, may produce an angiogenic response with enhanced expression of VEGF in human eyes [7, 8, 12–14].

Combination therapy has been explored as a way to decrease the number of intravitreal injections required in anti-VEGF monotherapy treatment regimens.

The aim of this study was to evaluate comprehensively patients with neovascular age-related macular degeneration (nAMD) treated with intravitreal ranibizumab 0.5 mg combined with a single-session standard PDT 3 days after the first injection by means of functional best-corrected visual acuity (BCVA), microperimetry and multifocal electroretinogram (mfERG), and morphological optical coherence tomography (OCT) retinal assessment.

Methods

Patients with nAMD have been evaluated in this prospective interventional open-label 12 months follow-up study. Only one eye of each patient was included. If both eyes met the inclusion criteria, the eye with the best distance acuity was selected. The study was performed in accordance with the ethical standards stated in the Declaration of Helsinki, approved by the local institutional review board. Each patient signed an informed consent before the enrollment in the study.

The inclusion criteria were as follows: age >50 years, diagnosis of active subfoveal CNV, and a baseline best-corrected visual acuity between 73 and 24 letters (20/40–20/320 Snellen equivalent) assessed with the use of Early Treatment Diabetic Retinopathy Study (ETDRS) charts, no previous treatments for AMD.

The exclusion criteria were as follows: CNV caused by other diseases, pathological myopia (spherical equivalent of −8 diopters or more), and other confounding ocular diseases as diabetic retinopathy or retinal vein occlusion, ocular surgery within the past 3 months.

All patients were treated with 3 injections of ranibizumab 0.5 mg 1 month apart and re-treated according to predefined criteria. Three days after the first injection, all patients received a single session of standard PDT performed according to the "treatment of AMD with photodynamic therapy (TAP) investigation" criteria [7, 8, 14].

Intravitreal injection of 0.5 mg/0.05 ml of ranibizumab was administered following the instillation of topical anesthetic drops under sterile conditions and followed the national and international guidelines for intravitreal injections [15]. Retreatment criteria after the loading phase were either a decrease in BCVA >5 ETDRS letters or a 100 μm increase in macular thickness or the presence/persistence of intra-/subretinal fluid.

At baseline, a complete ophthalmological examination including BCVA evaluation by ETDRS charts, central retinal thickness (CRT) measurement by OCT, fluorescein and indocyanine green angiographies, MP1 microperimetry, and mfERG was performed. Visual function questionnaire (VFQ-25) was used to quantify vision-related quality of life (QoL).

BCVA by ETDRS charts was performed at a distance of 4 m. If the patient was unable to read at least 20 letters at 4 m, BCVA was measured at 1 m, adding 0.75 sf correction. BCVA was scored as the total number of letters read correctly [16].

Patient's CRT was studied in the central 1 mm with time-domain OCT (Stratus OCT, Zeiss-Humphrey Instruments, San Leandro, CA, USA, software version 4.0, fast macular thickness map scan protocol) that allows a depth imaging of the retinal anatomy with a

10-μm axial resolution [17]. If errors were observed in the automated retinal boundary detection and retinal thickness measurements, the average thickness in a semiautomatic manner, measuring with calipers the subfoveal thickness in the six radial scans was calculated. To better classify subtypes of fluorescein angiography (FA), lesions have been classified independently by two retinal specialists and in case of disagreement by a third specialist. FA types of lesions have been classified in classic, predominantly classic, minimally classic, and occult lesions according to the "TAP investigation" criteria [7, 8, 14].

Retinal sensitivity was tested in all patients using MP1 microperimeter (MP1 Nidek Technologies, Padua, Italy), for its potential of correlating exactly retinal pathologies and functional defects [18, 19]. In our study, the following testing parameters were used: a grid of 37 stimuli covering the central 12° (centered onto the fovea), time between stimuli equal to 1 s, stimulus size equivalent to Goldmann III, white background set at 4 asb, and a bright red cross of 4° in size was used as the fixation target. A 4–2 double staircase strategy was carried out, and the first stimulus was presented at the level of 15 dB. In addition, the stability of fixation, graded on the basis of the preferred retinal locus, was reported before and after treatment. If >75 % of the fixation points were located within a predetermined limit area of variation of a 2°-diameter circle centered in the gravitational center of all fixation points, regardless of the position of the foveal center, the fixation was classified as *stable*. If <75 % of the fixation points were located within a 2°-circle, but >75 % of the fixation points were located within a 4°-circle, the fixation was classified as *relatively unstable*. If <75 % were located within a 4°-circle, the fixation was classified as *unstable* [20]. In each patient, microperimetry was performed twice within 1 week before baseline testing. Moreover, patients underwent a brief training at the beginning of each microperimetric test during the follow-up.

VERIS Clinic™ 4.9 (EDI, San Mateo, CA, USA) was used for mfERG assessment in the protocol session. The visual stimuli and the bioelectrical recording technique were the same as in previously published papers [21–25]. Briefly, the multifocal stimulus, consisting of 61-scaled hexagons, was displayed on a high-resolution, black-and-white monitor (size, 30 cm width and 30 cm height) with a frame rate of 75 Hz. The array of hexagons subtended 208 of the visual field. Each hexagon was independently alternated between black (1 cd/m^2) and white (200 cd/m^2) according to a binary m-sequence. This resulted in a contrast of 99 %. The luminance of the monitor screen and the central fixation cross (used as target) was 100 cd/m^2. The m-sequence had 2^{13}–1 elements, and total recording time was approximately 4 min. In order to maintain a stable fixation, a small red target (0.58), which was perceived by all subjects tested, was placed in the center of the stimulation field. At every examination, each patient positively reported clear perception of the cross-fixation target. The eye's position was monitored by a video system in the screen of the computer.

In the analysis of the mfERG biolectrical responses, obtained after automatic rejection of artifacts, the first-order kernel response, K1, was examined. We analyzed the averaged response amplitude densities (RADs) between the first negative peak, N1, and the first positive peak, P1, obtained in five concentric annular retinal regions (rings) centered on the fovea. Therefore, we analyzed the N1–P1 RADs derived from 0° to 2.5° (ring 1, R1), from 2.5° to 5° (ring 2, R2), from 5° to 10° (ring 3, R3), from 10° to 15° (ring 4, R4), and from 15° to 20° (ring 5, R5).

MfERGs were performed three times on three different days in each nAMD patient to check the repeatability of the obtained results. The recording with the highest signal-to-noise ratio (SNR) R1–R5 N1–P1 RADs was considered in the statistical analysis [21–25].

Vision-related QoL was measured by means of the extended version of the 25-items National Eye Institute Visual Function Questionnaire (NEI VFQ-39) at baseline and 12 months. The self-administered version, translated and validated in Italian language, was administered before clinical examination. The NEI VFQ-39 is composed by 39 items, the purpose of which is to assess eleven vision-related constructs and to ask a question regarding general health (published guidelines for the NEI VFQ-25. http://www.rand.org/health/surveys_tools/vfq/. Accessed September 1, 2006) [26].Vision-targeted subscales (domains) are as follows: general vision, difficulty with near-vision activities, difficulty with distance-vision activities, limitations in social functioning due to vision, role limitations due to vision, dependency on others due to vision, mental health symptoms due to vision, driving difficulties, limitations with peripheral and color vision, and ocular pain [26].

During the follow-up visits scheduled at day 1 and 7 day after each injection, only biomicroscopy was performed for safety reasons in order to detect any early signs of infection. At day 3, after the first injection, patients underwent standard PDT. While BCVA and OCT were repeated 28 ± 2 days after each injection, MP1 microperimetry and mfERG were repeated at 3, 6, and 12 months follow-up, and 3 and 12 months, respectively. FA was performed at baseline and at 12 months, and at the discretion of the investigator.

Primary outcome measure was the mean change from baseline in BCVA letters with ETDRS at month 12. Secondary outcome measures were as follows: number of retreatments; percentage of patients that gained ≥15 letters BCVA; percentage of patients that lost <15 letters; mean retinal thickness change from baseline to month 12; mean retinal sensitivity change at month 12.

Statistics

Data were described as mean ± SD and 95 % confidence intervals (95 % CI) for continuous variables and frequencies for categorical variables. Changes of functional and morphologic parameters from baseline were assessed by paired *t* test. The percentage of patients exceeding the threshold of 15 ETDRS read letters difference from baseline was calculated to better characterize the spectrum of functional changes after treatment. The analysis was performed with an intent-to-treat approach, and in case of patients lost during follow-up, the last observation available was carried forward. A $p < 0.05$ was considered statistically significant. For the comparison of mfERG changes from baseline, one-way analysis of variance (ANOVA) was applied.

Results

Twelve patients (7 females and 5 males, mean age 79 ± 4 years) out of 15 were enrolled and completed the 12 months of follow-up. One screened patient was not enrolled because previously treated with PDT and 2 patients because of a BCVA score <24 letters. At the time of initial diagnosis, nAMD lesions were classified as 33.3 % predominantly classic, 16.7 % minimally classic, and 50 % occult. Patients received an average of 3.4 ± 0.7 intravitreal injections during the follow-up. After the loading phase and the standard PDT at baseline, 33.3 % (3/12) were retreated once, and one patient (1/12) was retreated twice. The mean time of injection after the loading phase was 152 ± 43 days.

Mean BCVA, expressed as the number of ETDRS letters, changed from 54.67 ± 15.72 at baseline to 59.00 ± 24.77 at 12 months of follow-up with a mean change of 4.33 letters (95 % CI −5.90; 14.56; $p = 0.371$). About 25 % (3/12) of patients gained ≥15 letters in BCVA, 50 % (6/12) of patients gained <15 letters, and 2 patients (16.6 %) lost <15 letters. One patient showed no change in BCVA at 12 months follow-up (Table 1).

Mean retinal sensitivity by MP1 showed no statistically significant improvement from baseline changing from 5.5 ± 4.8 dB at baseline to 6.6 ± 6.0 dB at 12 months with a mean change of 1.1 dB (95 % CI −0.8; 3.0 $p = 0.220$). At baseline, 33.4 % of patients presented stable fixation (4/12), 50 % of patients had relative unstable fixation (6/12), and only 17 % had unstable fixation (2/12). After 12 months, the percentage of patients with stable fixation increased to 50 % (6/12). The initial mean retinal sensitivity was higher in the occult lesions with a better improvement during the follow-up (Table 1). Individual BCVA and MP1 values are reported in Table 2.

At 12 months, mfERG N1–P1 RADs were significantly different from baseline ($p < 0.01$) in the central 0°–2.5°, whereas in the more peripheral retinal areas (2.5°–20°), no significant ($p > 0.01$) changes in N1–P1 RADs were detected. In this analysis, we observed a decreased value of statistic significance moving from the central to the peripheral rings as regards of mean N1–P1 amplitude values (Table 3). Individual values of mfERG R1–R3 RADs are reported in Table 4.

OCT CRT significantly decreased from 356 ± 143 μm at baseline to 219 ± 65 μm at 12 months follow-up with a mean change of −136 μm (95 % CI −197; −76; $p = <0.001$) (Table 1). Individual OCT CRT values are reported in Table 2.

The "general vision" VFQ-25 was the only subscale that showed a statistically significant improvement (42.5 ± 13.9) both at 3 (53.7 ± 16; 95 % CI 3.3; 19.2; $p = 0.01$) and 12 months (53.3 ± 23.6; 95 % CI 0.2; 21.4; $p = 0.046$) from baseline.

The treatment procedure was well tolerated. No clinical evidence of inflammation, uveitis, endophthalmitis, or ocular toxicity was observed.

Table 1 Mean values ± SD and paired *t* test. *p* values versus baseline of retinal sensitivity, BCVA letter score, and mean central retinal thickness (CRT) at baseline and at each follow-up visit

Parameters	Baseline Mean ± SD	3 months Mean ± SD (95 % CI)	6 months Mean ± SD (95 % CI)	12 months Mean ± SD (95 % CI)	Mean change baseline-3 months	*p* baseline-3 months	Mean change baseline–12 months	*p* baseline–12 months
MP1 retinal sensitivity (dB)	5.5 ± 4.8	6.3 ± 5.3 (−0.7; 2.3)	6.8 ± 5.6 (−0.2; 2.7)	6.6 ± 6.0 (−0.8; 3.0)	0.8	0.265	1.1	0.216
BCVA letter score (letters)	54.67 ± 15.72	62.67 ± 19.52 (2.02; 13.98)	62.92 ± 20.9 (0.89; 15.06)	59.00 ± 24.77 (−5.90; 14.56)	8.00	0.013	4.33	0.371
Central retinal thickness (μm)	356 ± 143	210 ± 50 (−215; −74)	239 ± 98 (−169; −64)	219 ± 65 (−197; −76)	−145	0.001	−136	<0.001

CI confidence interval

Discussion

The results of our prospective open-label study showed that in patients with nAMD, the combination therapy with intravitreal injections of ranibizumab 0.5 mg associated with one single session of standard PDT 3 days after the first injection helps to stabilize retinal functional and morphological parameters.

At the end of the study, we recorded mean BCVA change from baseline of 4.33 letters (95 % CI −5.90/14.56; $p = 0.371$) with an improvement of ≥15 letters and <15 letters in 25 % (3/12) and in 50 % (6/12) of patients, respectively. Even though the mean retinal sensitivity showed a not statistically significant improvement with a mean change of 1.1 dB (95 % CI −0.8/3.0; $p = 0.216$) at 12 months from baseline, the percentage of patients with stable fixation increased from 33.4 % at baseline to 50 % (6/12) at 12 months. Although these functional results seem less impressive than those obtained with ranibizumab monotherapy [3–5], the percentage of patients that experienced BCVA improvement was high (75 %) with 33.3 % (3/12) of patients requiring retreatment only once, and one patient (1/12) who was retreated twice, calculating a mean time of reinjection after the loading phase of 152 ± 43 days.

In addition, the results of our study showed a functional macular improvement with an increase in mfERG N1–P1 RADs in the central area in the long-term follow-up.

The idea of combining anti-VEGF and PDT therapy, to address the angiogenic and vascular components of CNV, is supported by a strong rationale as they work through different mechanisms. The predominant effect of anti-VEGF agents in nAMD is to reduce vessel permeability, in addition to target angiogenesis by inhibiting the development of newly formed vessels. These agents have shown promising responses in terms of visual outcome, but their usage has some limitations. In fact, optimal visual outcomes require aggressive monitoring and may even require monthly treatments, causing a significant burden to both patients and physicians [3–5, 27]. Moreover, previous evidences suggest that anti-VEGF agents may become less effective in the later stages of exudative nAMD where more established and mature vasculature can be observed [6, 22, 28].

PDT has a different mechanism of action from anti-VEGF therapy. It targets the vascular component of CNV by occluding the newly formed vessels within the CNV lesion. For this reason, combining anti-VEGF agents that target the angiogenic component with PDT that targets the vascular component may be more beneficial than either therapy alone. The long-term clinical efficacy and safety of PDT have been proven in several multicentre, double-masked, randomized placebo-controlled studies [7–9]. However, several studies have also shown that PDT may affect the physiological choriocapillary bed surrounding the pathological CNV lesion, resulting indirectly in up-regulation of VEGF that may then further stimulate CNV growth. Up-regulation of VEGF shortly after PDT has been demonstrated detecting increase in lesion leakage and macular edema at FA [12, 13].

Table 2 Individual values for best-corrected visual acuity (BCVA), central retinal thickness (CRT) at OCT, and mean retinal sensitivity by means of MP1 at each follow-up visit

	BCVA (ETDRS letters)				CRT OCT (μm)				Mean *retinal sensitivity* MP1 (dB)			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
XXX #1	68	84	86	84	349	220	258	226	11.9	15.2	12.9	12.2
XXX #2	68	68	73	68	248	189	189	195	8.8	9.6	9.9	
XXX #3	46	45	38	37	729	308	506	383	0.2	0.3	0.1	0.2
XXX #4	73	81	78	83	213	162	168	175	7.2	8.7	7.6	13.8
XXX #5	33	33	35	36	208	197	194	195	4.8	4.4	6.3	4.9
XXX #6	53	81	84	84	315	161	165	164	2.3	2.2	3.5	3.9
XXX #7	67	86	88	84	365	169	181	171	8.8	12.8	16	16.4
XXX #8	65	64	58	53	369	197	302	184	0.1	2.7	0.3	1.9
XXX #9	43	53	54	53	509	311	261	317	5.1	2	5.4	5.9
XXX #10	69	74	78	80	362	186	176	181	13.8	13.5	15	12.9
XXX #11	44	51	52	16	323	208	297	221	1.7	5.2	4.7	0.1
XXX #12	27	32	31	30	282	223	171	218	0.8	0.2	0	0

To counterbalance the PDT-triggered increase in VEGF, all patients of our study received ranibizumab 0.5 mg intravitreal injection 3 days before the PDT treatment.

The combination of both treatments may theoretically offer progressively longer treatment-free intervals, leading to a less-frequent dosing schedule of intravitreal injections, and may also make individualized treatment regimens possible for sustained efficacy and better compliance.

Mont Blanc study aimed to explore the efficacy and safety of the ranibizumab/PDT combination therapy performed in the same day, in patients with any type of subfoveal CNV secondary to nAMD. From Mont Blanc results, the combination pro re nata (PRN) treatment regimen with PDT and ranibizumab was effective in achieving BCVA gain comparable with ranibizumab monotherapy; however, the study did not show any benefit with respect to reducing the number of ranibizumab retreatment over 12 months [29].

On the other hand, the TORPEDO trial aimed to demonstrate long-term prevention of vision loss and improvement in BCVA after treatment with one-time reduced-fluence-rate PDT followed by administration of ranibizumab on a variable-dosing regimen over 24 months in patients with nAMD. Contrary to our schedule, in this trial, the combination therapy was performed the same day and reduced-fluence PDT was used. The BCVA mean improvement at 12 months follow-up was >1 obtained in our study (+7.2 vs +4.33 letters) even if the number of reinjections was higher (5.1 vs 3.4) [30].

In agreement with our results, Vallance et al. [31] found that no benefit was offered by the combination therapy in terms of visual acuity improvement, comparing the effect of standard-fluence PDT delivered on the first day of a ranibizumab regimen with that of ranibizumab monotherapy. The authors also concluded that the addition of PDT to ranibizumab does not influence the number of reinjections within 1 year.

Also, Bashshur et al. [32] used a single session of standard PDT combined with ranibizumab compared with ranibizumab monotherapy. The authors reported a greater improvement in BCVA observed in the cohort of patients treated with ranibizumab monotherapy (+12 vs +3.2 letters), but a lower number of reinjections were required in patients treated with the combination therapy (3.0 vs 6.0 injections).

Table 3 Statistical evaluation (one-way analysis of variance) in nAMD (12 eyes) with respect to baseline values

	R1 N1–P1 amplitude (nV/°^{2})	R2 N1–P1 amplitude (nV/°^{2})	R3 N1–P1 amplitude (nV/°^{2})	R4 N1–P1 amplitude (nV/°^{2})	R5 N1–P1 amplitude (nV/°^{2})
Baseline	30.4 ± 8.15	18.7 ± 4.90	13.9 ± 4.01	15.14 ± 6.90	13.00 ± 7.21
3 months	41.7 ± 14.95	25.8 ± 10.54	19.4 ± 8.54	13.08 ± 5.82	10.44 ± 5.15
ANOVA, $f(1, 23)$ versus baseline	$f = 5.22$, $p = 0.032$	$f = 4.55$, $p = 0.043$	$f = 4.64$, $p = 0.042$	$f = 0.05$, $p = 0.822$	$f = 0.08$, $p = 0.775$
12 months	52.7 ± 20.58	28.60 ± 13.10	21.2 ± 10.9	11.91 ± 4.52	10.54 ± 4.23
ANOVA, $f(1, 23)$ versus Baseline	$f = 12.2$, $p = 0.002$	$f = 6.12$, $p = 0.021$	$f = 4.67$, $p = 0.041$	$f = 0.15$, $p = 0.699$	$f = 0.09$, $p = 0.771$

R1–R5 refers to local multifocal parameters averaged in five retinal areas located at various eccentricity from the fovea: 0–2.5 (R1), 2.5–5 (R2), 5–10 (R3), 10–15 (R4), and 15–20 (R5)°

N1–P1: Amplitude measurements refer to mean ± SD values

Table 4 MfERG responses in patients with nAMD at each follow-up visit

	MfERG R1 RADs (nV/°^{2})			MfERG R2 RADs (nV/°^{2})			MfERG R3 RADs (nV/°^{2})		
	Baseline	3 months	12 months	Baseline	3 months	12 months	Baseline	3 months	12 months
XXX #1	35.4	53.6	64.7	21.5	33.6	37.9	21.2	30.1	31.3
XXX #2	20.6	49.8	53.2	16.7	28.4	22.5	12.3	18.8	17.2
XXX #3	36.7	33.4	34.6	15.6	13.2	13.4	14.9	13.2	12.1
XXX #4	41.4	32.7	17.0	20.1	19.2	16.4	9.4	11.5	10.6
XXX #5	22.4	38.3	71.0	23.2	36.3	41.9	18.2	26.6	28.1
XXX #6	41.0	27.2	38.7	20.6	19.6	18.2	13.3	12.1	12.0
XXX #7	34.6	74.1	43.1	22.8	41.3	42.4	17.8	34.8	42.4
XXX #8	26.4	18.5	68.3	10.7	12.3	35.6	9.3	10.2	10.2
XXX #9	25.0	36.3	37.0	20.7	21.4	17.7	17.6	21.4	22.9
XXX #10	35.3	53.6	79.7	12.3	22.3	23.2	9.6	11.3	10.7
XXX #11	16.8	49.1	83.6	26.5	43.2	53.5	13.2	29.0	35.3
XXX #12	29.7	33.5	41.9	13.2	19.0	20.4	10.3	18.6	21.9

RAD refers to N1–P1 response amplitude densities. R1–R3 refers to local multifocal parameters averaged in three retinal areas located at various eccentricities from the fovea: 0–2.5 (R1), 2.5–5 (R2), and 5–10 (R3)°

Mataix et al. [33] instead evaluated the combined therapy of standard-fluence PDT and intravitreal ranibizumab in nAMD patients both repeated during the follow-up according to predefined criteria and found better visual acuity results compared to our study (+7.2 vs +4.33 letters) requiring 2.37 reinjections and 1.22 PDT treatments within 1 year.

In the present study, a lower psychophysical improvement both in BCVA and retinal sensitivity (as measured by microperimetry) was observed at 12 months compared to that obtained with ranibizumab monotherapy in a similar cohort of patients in a prospective study published by our group (gain of 4.33 vs 11.6 letters and 1.1 vs 2.48 dB) [34]. Although in a small sample size study and without a control group, we were able to add new data on macular function assessed by mfERG. We found that in the foveal region (0°–2.5° of eccentricity from the fovea), mfERG N1–P1 RADs at 12 months were significantly different from baseline ($p < 0.01$). This functional improvement over a 12 months span is not obvious. It means that there was a foveal rescue, with no significant photoreceptor toxicity from the combined use of intravitreal ranibizumab and PDT. Our findings support previous observations of early detection (3–7 days post-treatment) of absence of adverse

effects from PDT used in monotherapy for CNV in nAMD patients [35] and add consistent results over a 1-year follow-up to others previously reported [36]. Despite our findings, however, contradictory data [37] have shown impairment of foveal and parafoveal electrical activity 6 months after PDT applied alone on classic neovascularizations from AMD; this could mean that the amelioration of the electro-retinal function in the retinal locations overlying the CNV may be achieved by combining targeting both the vascular and the angiogenic component of the disease, as we describe in the present study.

The fact that the retinal sensitivity and the BCVA were almost stable within 1 year after the proposed therapeutic strategy can be ascribed to the functional improvement only the preganglionic elements, detectable by mfERG but not completely reflecting changes in the psychophysical measurements, as previously reported in a quite similar model of visual function in myopic CNV [25]. We already discussed that the functional improvement assessed by electrophysiological methods may be not accompanied necessarily by a significant amelioration of psychophysical responses, hypothizing that only a wide abnormality compromising photoreceptor density could determine changes detected by both techniques [23, 25].

In conclusion, in this open study, we observed that combination of ranibizumab 0.5 mg with one single session of standard PDT 3 days after the first intravitreal injection induced reduction in CRT together with stabilization of BCVA and macular sensitivity over a 12-month period, associated with rescue of the foveal function as demonstrated by the significant improvement of mfERG parameters in the central area. Compared with the results obtained with ranibizumab monotherapy [34], a smaller number of intravitreal injections (3.4 ± 0.7 vs 4.3 ± 1.64) have been required to stabilize visual function parameters, accompanied by an higher score of "general vision" subscale of VFQ-25, which corresponds to an improvement of the global subjective perception of vision.

Conflict of interest None.

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