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Intravitreal bevacizumab therapy for choroidal neovascularization secondary to age-related macular degeneration: 6-month results of an open-label uncontrolled clinical study

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PURPOSE. *To investigate the 6-month safety and clinical outcomes of intravitreal injections of bevacizumab administered to treat choroidal neovascularization secondary to age-related macular degeneration.*

METHODS. *Twenty-seven patients underwent 1.25 mg intravitreal injections of bevacizumab at baseline. A similar intravitreal injection was administered to all eyes at 1 and 2 month follow-up visits. At baseline and at each follow-up visit (1, 2, 3, and 6 months), patients underwent best-corrected visual acuity (BCVA) measurement, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Laboratory testing, visual field analyses, and endothelial cell counts were performed at baseline and third and sixth months.*

RESULTS. *At 3 months, the mean BCVA remained substantially stable at 20/100. Mean central retinal thickness (CRT) decreased from 373 to 279 µm ($p<0.01$). Mean lesion greatest linear dimension (GLD) decreased from 4087 to 3782 microns ($p<0.01$). At 6 months, mean BCVA slightly decreased from $20/100^{-1}$ to $20/125^{-3}$ (not significant, $p=0.40$). Mean CRT was still inferior to baseline (305 µm, $p<0.01$). Mean lesion GLD was 4186 µm, not different from baseline values ($p=0.59$), but superior to 3-month mean GLD ($p<0.01$). Significant visual field defects or endothelial cell losses were not detected at 3 and 6 months. Laboratory testing did not reveal any clinically significant deviations compared to baseline values.*

CONCLUSIONS. *Intravitreal therapy using bevacizumab over 6 months showed stabilization of visual acuity and choroidal neovascularization activity; the safety data were convincing. (Eur J Ophthalmol 2007; 17: 230-7)*

KEY WORDS. *Age-related macular degeneration, Bevacizumab, Choroidal neovascularization, Intravitreal injection*

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INTRODUCTION

Intravitreal bevacizumab (Avastin®, Roche) is an appealing off-label therapy employed to treat ocular neovascular diseases. Recent studies have suggested that intravitreal bevacizumab is a well-tolerated therapy and it might be useful in treating choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) (1-6). Previous studies have reported an initial 3-month experience in relation to the safety and efficacy of intravitreal bevacizumab, but to date long-term results remain unidentified (2-6).

Our study investigated the 6-month safety and clinical outcomes of intravitreal injections of bevacizumab dispensed at monthly intervals for the first 3 months in patients affected by CNV secondary to AMD.

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METHODS

We evaluated prospectively 27 eyes of 27 patients with CNV secondary to AMD. Approval for the study was obtained from the University of Florence's institutional ethics committee, and participating patients signed consent agreements.

Study patient selection and follow-up

Patients not eligible for verteporfin photodynamic therapy (PDT), who had refused PDT, or who had not responded to PDT were included. Patients could have been treated for CNV by any other means (corticosteroids or pegaptanib sodium) at least 3 months prior to being included in the study.

Patients with uncontrolled hypertension (systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg), a history of thromboembolic events, recent or planned surgery, or coagulation abnormalities were eliminated from the study.

Baseline laboratory testing included electrocardiograms, complete blood counts, chemistry panels, prothrombin timings, partial thromboplastin timings, and urinalyses. Laboratory testing was repeated after 3 and 6 months. Blood pressure was measured at baseline, 3, and 6 months. Best-corrected visual acuity (BCVA) was evaluated using Early Treatment Diabetic Retinopathy Study charts at 2 m. Central retinal thickness was measured using optical coherence tomography (OCT) macular retinal mapping (Stratus OCT, Carl Zeiss Ophthalmic System, Dublin, CA, USA). Fluorescein and indocyanine green angiographies were performed using FF 450 plus IR (Carl Zeiss, Jena, Germany). We evaluated the greatest lesion linear dimension (GLD) and fluorescein leakage from CNV. Visual field examinations employed a Humphrey Field Analyzer (Humphrey Instruments, San Leandro, CA, USA) with threshold related screening strategy and full field 243 point test. Two repeated tests were performed before the treatment, and only the second test was considered baseline. If false positive errors, false negative errors, or fixation losses exceeded 30% after two consecutive baseline tests, the patient was excluded from the visual field examination. Central corneal endothelial cell counts were performed using a non-contact specular microscope (Cellcheck, Konan Medical, Inc.). All patients, at baseline and each follow-up visit (1, 2, 3, and 6 months after the first injection), underwent a BCVA, a slit-lamp examination of the anterior segment, a dilated fundus examination, a fluorescein angiography, an indocyanine green angiography, and OCT. Patients enrolled in the study underwent visual field testing and an endothelial cell count at baseline and at third and sixth month follow-up.

Intravitreal bevacizumab injection

All patients were given 1.25 mg intravitreal bevacizumab injections at baseline. A similar intravitreal injection was administered to all eyes at 1 and 2 month follow-up visits even if total resolution of subretinal fluid and retinal pigment epithelium (RPE) detachment existed.

Bevacizumab is commercially available as a solution (100 mg; 25 mg/mL) and was not diluted, reconstituted, or altered whatsoever. The hospital pharmacy prepared 1.25 mg of bevacizumab in tuberculin syringes with a 30-gauge needle, and aseptic techniques and a laminar flow hood were used. The syringes were stored at 4–8 °C for a 48-hour time frame.

All intravitreal injections were administered using a standard procedure. Preinjection antibiotic drops were routinely applied 3 days before injection. Bevacizumab (1.25 mg; 0.05 mL) was injected into the vitreous cavity through the pars plana 3.5–4 mm from the limbus. Postinjection light perception was assessed, and intraocular pressure (IOP) was monitored for 30 minutes. All eyes underwent a similar procedure at 1- and 2-month follow-ups.

Safety was assessed by evaluating adverse events, blood pressures, laboratory tests, BCVA, fluorescein angiograms, indocyanine green angiograms, and OCT images. In addition, visual field analyses and endothelial cell counts were monitored regularly.

Continuous outcome variables were analyzed using generalized linear mixed models to replicate their change with time (7). LogMAR visual acuity was used in these analyses.

RESULTS

Twenty-seven eyes of 27 patients were evaluated. The mean age was 77.2 years, ranging from 67 to 86 years. There were 7 men and 20 women. Ten eyes had been previously treated with PDT, 3 eyes had had laser photocoagulation, and 1 eye had received intravitreal triamcinolone. Thirteen eyes received bevacizumab as primary therapy. Seventy-nine injections were given. Twenty-five patients received three injections. Two patients received

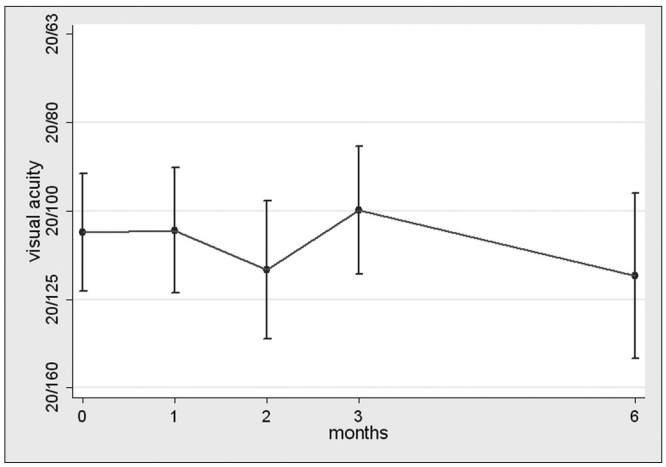

Fig. 1 - *Graphic representation of mean visual acuity during follow-up. Bar lines represent standard errors of the mean difference. There was no statistically significant difference in mean visual acuity during follow-up as compared to baseline.*

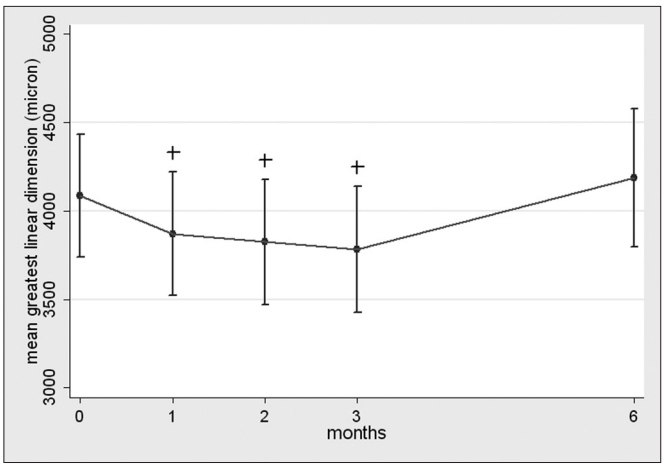

Fig. 2 - *Graphic representation of mean greatest linear dimension (μm) of the lesion during follow-up. Bar lines represent standard errors of the mean difference. Statistically significant comparisons (p<0.01) with respect to the baseline values are marked with a (+) symbol.*

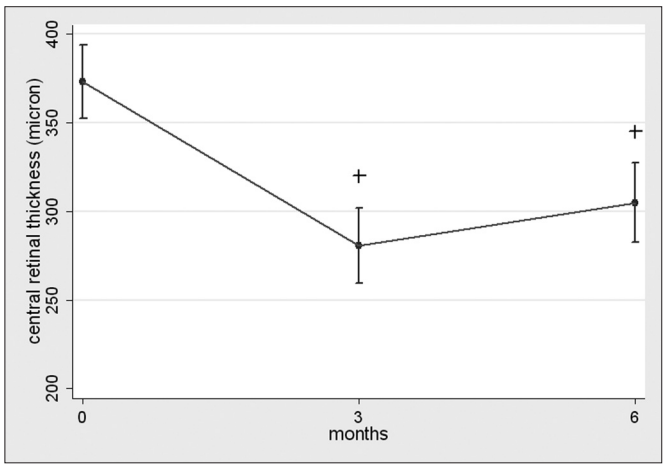

Fig. 3 - *Graphic representation of mean central retinal thickness (μm) during follow-up. Bar lines represent standard errors of the mean difference. Statistically significant comparisons (p<0.01) with respect to the baseline values are marked with a (+) symbol.*

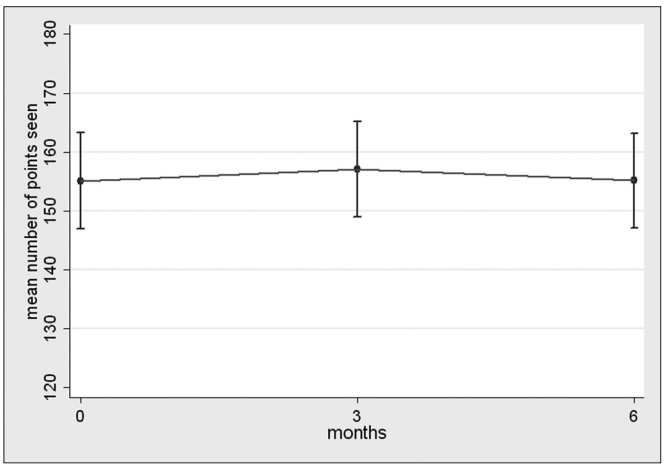

Fig. 4 - *Graphic representation of mean change in number of points seen at visual field examination during follow-up. Bar lines represent standard errors of the mean difference. There was no statistically significant difference in mean number of points seen during follow-up as compared to baseline.*

one and two injections respectively as a result of an adverse ocular event.

The baseline BCVA was a mean of 20/100^{-1}. The baseline lesion GLD was a mean of 4087 μm; baseline CRT was a mean of 373 μm; the mean number of points, seen at the baseline visual field examination, was 155; four patients were excluded from the visual field examination because of insufficient quality parameters detected at baseline; and the mean number of corneal endothelial cells at baseline was 2315 cells/mm^2.

At the 1-month follow-up, the mean BCVA was 20/100^{-2} and the mean lesion GLD decreased to 3816 μm.

The mean BCVA at the 2-month follow-up was 20/125. The mean lesion GLD decreased to 3825 μm.

Three-month data

Three-month follow-up was available for all patients. Figures 1–5 summarize the functional outcomes after 3 months. Mean BCVA was similar to baseline (20/100^{+1};

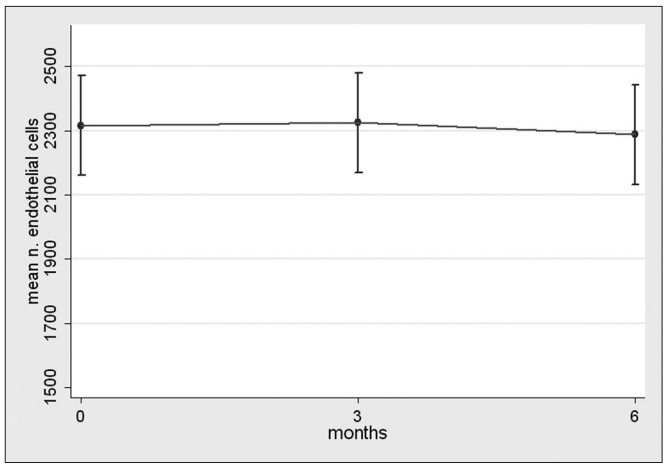

Fig. 5 - *Graphic representation of mean central corneal endothelial cell counts (cells/mm^2) observed during the follow-up. Bar lines represent standard errors of the mean difference. There was no statistically significant difference in mean number of endothelial cells during follow-up as compared to baseline.*

p=0.40); 7 patients (25%) had at least one line (five letters) improvement in BCVA, while 5 patients (18.5%) had less than five letters in worsening BCVAs; one patient had the same visual acuity. Mean CRT decreased to 279 µm (p<0.01), while the mean lesion GLD decreased to 3782 µm (p<0.01). Visual field defects or endothelial cell losses were undetected.

Six-month data

Six-month follow-up was available for 26 patients. Figures 1–5 summarize the functional outcomes after 6 months. Figures 6–8 show angiographic outcomes of three patients. Mean BCVA was 20/125^{-3}; one patient had at least one-line improvement in BCVA, while 8 patients (30.7%) had more than one-line worsening in BCVA. Mean lesion GLD increased to 4186 µm, which is similar to the baseline value (p=0.59), but more than at 3 months (p<0.01). Mean CRT was still lower than baseline (303 µm; p<0.01). Significant visual field defects or endothelial cell losses were not detectable. The intra-individual visual field and

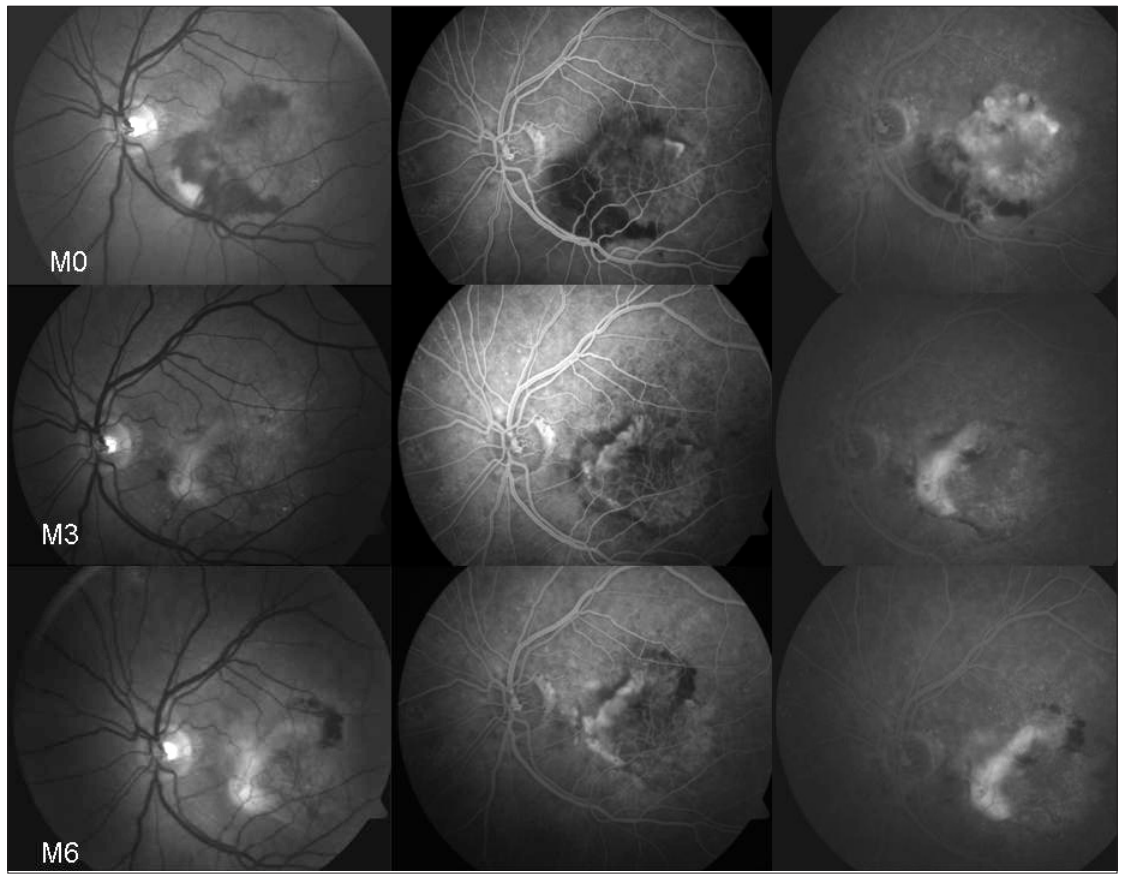

Fig. 6 - *At baseline (M0) red-free photograph revealing subretinal fluid and hemorrhage present at the margin of the subfoveal choroidal neovascularization (CNV); fluorescein angiography shows intense leakage from superior margin of CNV. After 3 months (M3) and three intravitreal injections, subretinal hemorrhage is reduced and with fluorescein angiography the lesion increased the staining and decreased the leakage. After 6 months (M6) a new relapse of CNV appeared at superior margin of the lesion.*

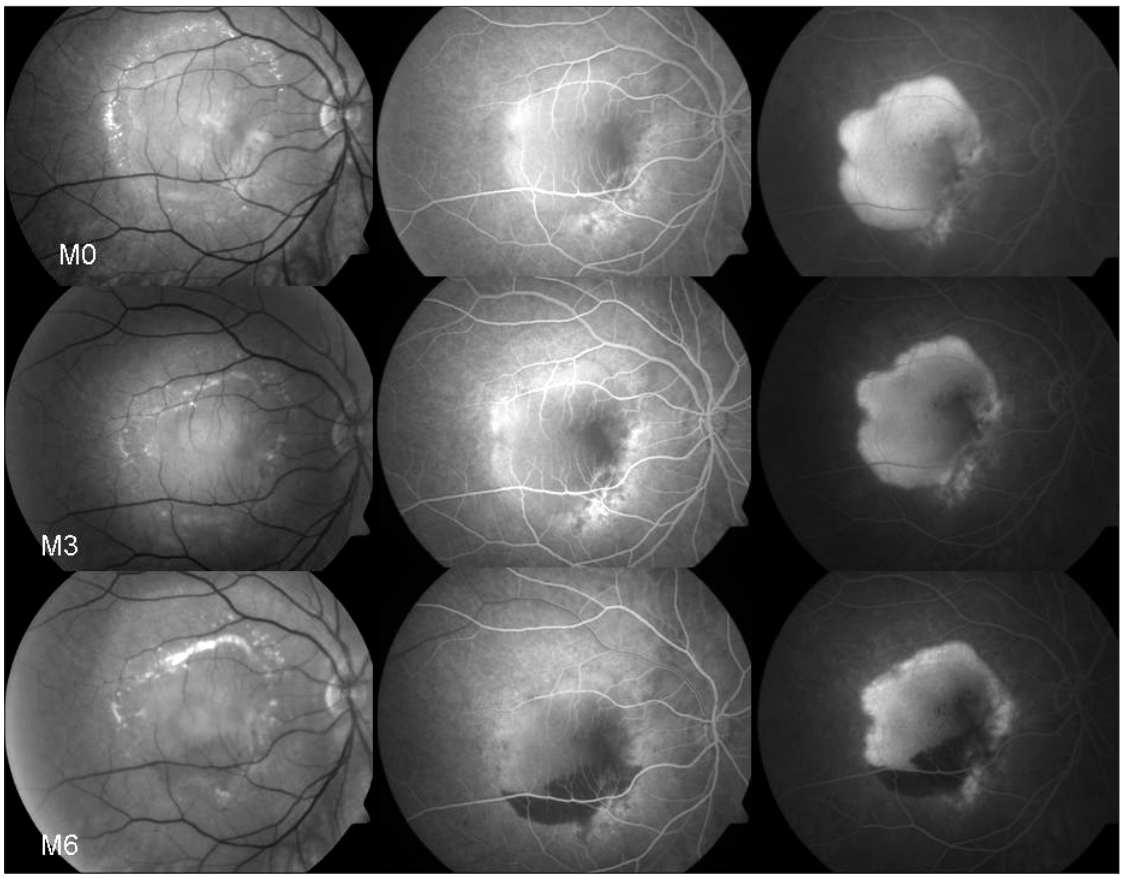

Fig. 7 - *At baseline (M0) red-free photograph and fluorescein angiography showed large retinal pigment epithelium detachment associated with occult CNV. After 3 months (M3) red-free photograph revealed a decrease of subretinal fluid and the dimension of the lesion was substantially similar when compared to baseline. After 6 months (M6) a new subretinal hemorrhage developed at the inferior margin of the lesion.*

endothelial cell count changes were not statistically remarkable.

Safety

No patient had experienced endophthalmitis at any point, nor was any progression of cataract noted. No cases of retinal detachment or episodes of ocular inflammation existed. Two patients experienced transient corneal epitheliopathy 1 day after the first intravitreal injection. Two patients discontinued the treatment because of the occurrence of an RPE tear in their study eye during the follow-up. They had received one and two intravitreal injections of bevacizumab instead of the three prescribed. Over the 6-month follow-up period, there were no thromboembolic events, which included cerebrovascular accidents, transient ischemic attacks, myocardial infarctions, or peripheral vascular diseases. All laboratory testing repeated at the 3- and 6-month follow-up visits did not indicate any clinically significant deviations compared to the baseline values. Blood pressures did not rise noticeably; no new antihypertensive medications were initiated during the follow-up.

DISCUSSION

Our data confirmed that repeated injections of bevacizumab are well tolerated immediately after being administrated and then over a 6-month period. No visual field defects and significant corneal endothelium cell losses were found during follow-up. After 3 months, lesion size and CRT decreased significantly; BCVA improved in 17 patients (62.9%) but the difference was not statistically significant, while 9 (33.3%) patients' vision worsened; 7 patients (25%) had at least one-line (5 letters) improvement in BCVA, but only one patient improved more than three lines; and, 5 patients (18.5%) had less than five letters in worsening BCVA. These 3-month BCVA results are positive. However, they are less impressive than those re-

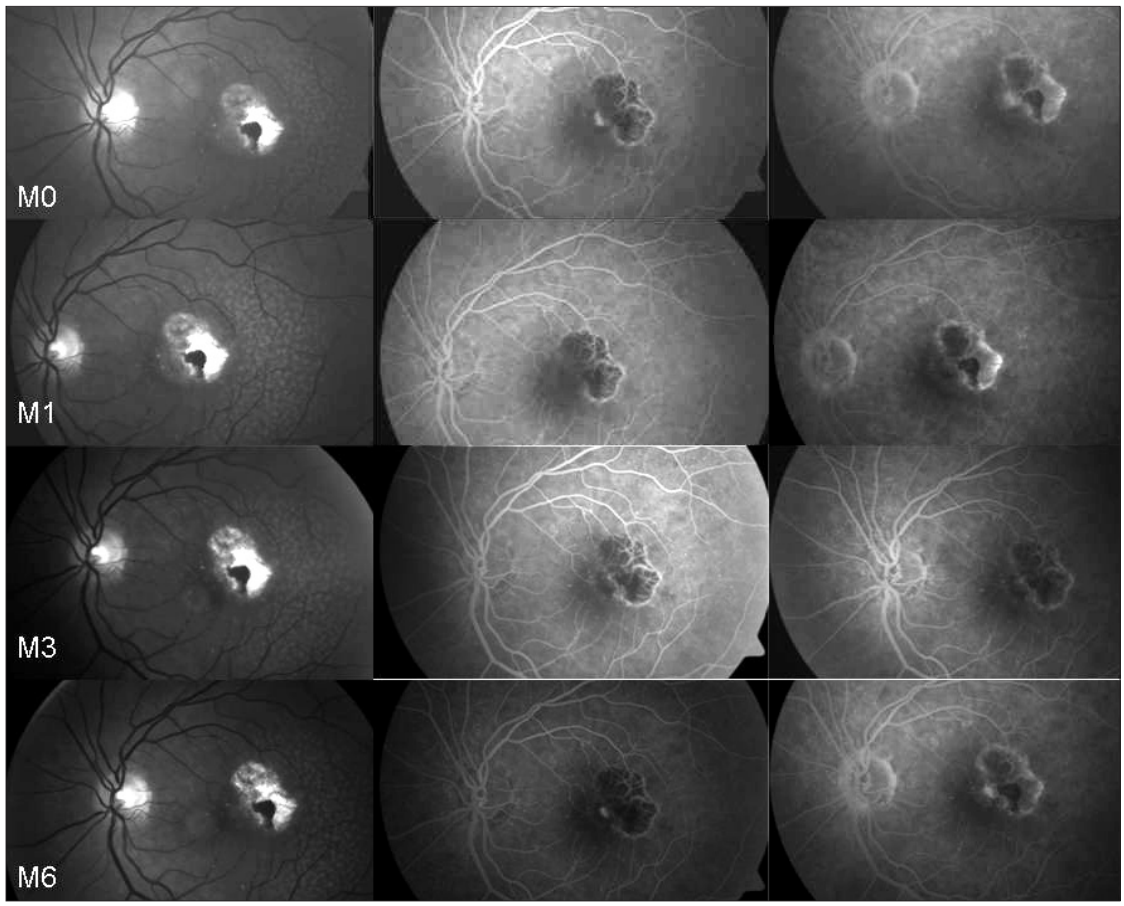

Fig. 8 - *Subfoveal choroidal neovascularization (CNV) arising from pigmented macular scar secondary to previous laser treatment. After 1 month from first injection the CNV regressed completely. After 3 months and three injections fluorescein angiography showed minimal leakage from the lesion. After 6 months fluorescein angiography detected new relapse of subfoveal CNV.*

ported by other authors. Rich et al reported that 44% of patients had at least a three-line improvement in visual acuity (4). Bashshur et al reported statistically significant improvement of mean and median BCVA (5). Spaide et al reported that 38.3% of patients had statistically significant VA improvement (3). Avery et al reported that median vision improved from 20/200 to 20/80 (2).

This variability in the extent of vision improvement may result from the types of neovascular lesion under study and the history of previous treatment. In our population, 10 eyes had been previously treated with PDT, 3 eyes with laser photocoagulation, and 1 eye with intravitreal triamcinolone; 13 eyes received intravitreal bevacizumab as primary therapy; 20 patients had occult lesions, 4 patients had classic lesions, 3 patients had minimally classic lesions; and 6 patients had lesions with clinical features of retinal angiomatous proliferation.

At the 6-month follow-ups, 4 months after the last injection of bevacizumab, the average VA was stable, even if many patients experienced slightly decreased VA.

At 6 months, the lesion GLD increased but CRT remained significantly lower compared to baseline.

With these results, we can speculate that bevacizumab is capable of eliminating leakage from CNV, can increase the staining of the lesion, and can stabilize vision, but the dosing interval used is not able to completely arrest lesion growth. A 4-month cessation of injections led to an increased relapse rate. About 60% of the eyes had new relapses of CNV located at the margin of the lesion at 6 months. The dimension of the lesions at 6 months increased when compared with 3-month values.

Recent studies have shown the localization of VEGF and its receptors on neurons and astrocytes suggesting a possible neuroprotective property and calling into question the long-term safety of anti-VEGF agents (8, 9). There is speculation that blocking VEGF may cause an increased apoptosis rate among ganglion cells and photoreceptors (10). Caution is required when ocular therapeutics that block all VEGF isoforms are administered (11). In experimental in vitro studies, bevacizumab showed no toxicity in human retinal pigment and neurosensory retinal cells (12). Different doses of bevacizum-

ab have been tested in rabbit eyes, and no toxicity has been detected by electroretinogram or histologic evaluations performed 2–4 weeks after injections (13, 14). Maturi et al evaluated the short-term electrophysiologic effects of intravitreal bevacizumab in 9 patients with AMD and the results suggested that no significant measurable photoreceptor toxicity over 3 months existed (15). Previous clinical studies reported 3-month safety of intravitreal bevacizumab (2-5). All these studies were retrospective except for the study of Bashshur et al, who conducted a prospective research with three 2.5 mg intravitreal injections administered at monthly intervals in 17 patients (5). In our prospective study, we performed fluoroangiography, indocyanine green angiography, and OCT at monthly intervals for the first 3 months and at 6-month follow-up to measure the anatomic outcomes of the lesions. To achieve a better evaluation of the ocular safety, we performed, in addition, visual field examinations and corneal endothelial cell analyses.

No serious systemic adverse events were reported through month 6. All laboratory and blood pressure testing were found to be substantially unchanged compared with baseline values. Two patients experienced RPE tear, respectfully, a week after the first and second injections, and they experienced severe vision loss. This ocular adverse event can be a consequence of the CNV's natural history. It is also described as a complication of other therapies such as intravitreal triamcinolone and PDT (16). Meyer et al and Nicolò et al reported acute RPE tear in occult CNV after intravitreal injection of bevacizumab (17, 18). Our two patients had occult lesions with RPE detachments. It is possible that the rapid absorption of the subretinal fluid after injection made RPE tear likely. Comparative trials will determine the frequency of this.

Many questions persist regarding the optimal dosage for intravitreal bevacizumab. We chose the 1.25 mg dose because nowadays it is used in most clinical practices and has been employed in previous studies. Bashshur et al used an intravitreal 2.5 mg dose assuming that a higher dose would have greater efficacy (5). However, after phase III clinical trials with anti-VEGF, pegaptanib sodium, the experimentation with a higher one did not result in any greater efficacy (19). We did not instigate trials with different doses of Avastin, and only future prospective and comparative studies will address these matters.

The optimum dosage sequence for intravitreal bevacizumab is undetermined. Intraocular pharmacokinetics of bevacizumab has not yet been clearly investigated in humans. In rabbit eyes, Shahar et al found full thickness retinal penetration present at 24 hours but essentially absent at 4 weeks (14). They found a strong, specific labeling in the bevacizumab-injected eyes for at least 7 days. We chose to treat 27 eyes at monthly intervals for the first three injections, and we checked the ocular outcome at 3 months, 4 weeks after the last injection, and at 6-month follow-ups. Our strategy was similar to the treatment strategy used in the phase III clinical trial with pegaptanib therapy (19) and ranibizumab therapy (PIER Study Group, unpublished data presented at ARVO, April 2006) for neovascular AMD. These trials used a fixed dosing regimen with injections every 6 weeks or every month. Bashshur et al reported a 12-week study using the same strategy based on monthly intervals for the first three injections of bevacizumab (5). In other previous retrospective studies the authors elected to defer re-injection into eyes when subretinal fluid, macular edema, and/or pigment epithelium detachment recurred (2-4).

In conclusion, our study shows potential for an ocular and systemic safety of intravitreal bevacizumab over 6 months, and points out that monthly injections might achieve short-term CNV control. In spite of this, a 4-month withdrawal of bevacizumab treatment appears to be too long to obtain a complete arrest of CNV even if subretinal fluids decrease over 6 months and VA remains substantially stable. Future investigations should examine carefully the effectiveness and most advantageous approach to be used when prescribing intravitreal bevacizumab therapy.

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