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Assessing the Anatomical and Functional Efficacy of Aflibercept on Wet ARMD: An OCT and Mferg Recording

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Abstract

Objective: To investigate the therapeutic efficacy of aflibercept on sub-foveal choroidal neovascularization due to Age-Related Macular Degeneration (ARMD).

Methods: Fifteen patients (15 eyes) with sub-foveal choroidal neovascularization due to ARMD were treated with intravitreal aflibercept. The doses were monthly for the first three months, being repeated every three months after for one year, using 2 mg of intravitreal aflibercept. A total of six aflibercept injections were, finally, performed during the 12-month study. All patients underwent a complete ophthalmic examination, including the measurement of best-corrected visual acuity, fundus examination, intraocular pressure measurement, fluorescein angiography, optical coherence tomography (OCT) scan and multifocal electroretinography (mfERG) recording, at the baseline and at the first, second, third, sixth, ninth and 12th month after the first injection of aflibercept. Two masked examiners evaluated the visual acuity based on standard Snellen charts.

Results: Fifteen patients (15 eyes), of mean age 69.2 ± 4.9 years old, with sub-foveal choroidal neovascularization due to ARMD were participated in this study. The mean BCVA was 0.12 ± 0.08, 0.20 ± 0.10, 0.25 ± 0.1, 0.28 ± 0.1, 0.34 ± 0.14, 0.36 ± 0.14 and 0.40 ± 0.14 decimal, at presentation, 1st, 2nd, 3rd, 6th, 9th and 12th month, respectively. Significant differences in amplitudes but not in latencies of three rings were observed over time. Significant reductions were noted among the central retinal thickness measurements before intravitreal aflibercept and at 1st, 2nd, 3rd, 6th, 9th and 12th month (465.0 ± 161.4, 374.9 ± 139.5, 323.3 ± 113.8, 290.3 ± 85, 263.3 ± 69, 243.0 ± 60.6 and 226.9 ± 63.5, respectively).

Conclusion: This is the first time that anatomical and functional improvement of the macula in patients with sub-foveal choroidal neovascularization due to ARMD was shown objectively, based on OCT and mfERG recordings. In addition, the improvement of visual acuity was noted over time. Our study supports the fact that intravitreal use of aflibercept is safe and effective in treating sub-foveal choroidal neovascularization observed in patients with ARMD.

Keywords: Aflibercept; Age-related macular degeneration; Anatomical findings; Macular Function; Multifocal electroretinography; Optical coherence tomography; Sub-foveal choroidal neovascularization

Introduction

Age-Related Macular Degeneration (ARMD), which is the leading cause of blindness among Caucasian population (26% of blind people), is classified into two forms, the dry type, which includes the 90% of the cases, tends to progress more slowly than wet or exudative type, whose incidence ranges from 3.3% to 11.4% among people over 85 years old [1]. The involvement of vascular endothelial growth factor (VEGF) in the pathogenesis of exudative ARMD and its complications explain the widespread use of anti-VEGF agents in the treatment of the disease. These agents can inhibit the synthesis, the action or the binding of VEGF on its receptors. There are four basic anti-VEGF agents: (1) ranibizumab (Lucentis; Genentech, South San Francisco, CA/Roche, Basel, Switzerland, 2006), a humanized fragment of a monoclonal antibody, acting against all isoforms of VEGF-A, (2) bevacizumab (Avastin; Genentech, South San Francisco, CA / Roche, Basel, Switzerland, 2005), a humanized full-length antibody (3) pegaptanib sodium (Macugen; Eyetech Inc, Palm Beach Gardens, FL, 2004), a ribonucleic acid aptamer with high affinity for the isoform VEGF165 but less active than the previous agents, and (4) VEGF trap or Aflibercept (EYLEA; Regeneron Pharmaceutical Inc and Bayer Tarrytown, NY, 2011) [2].

The MARINA study demonstrated the beneficial effect of ranibizumab on visual acuity of patients suffering from choroidal neovascularization due to ARMD [3]. The most frequently observed ocular complications of intravitreal ranibizumab included intraocular inflammation, increase of intraocular pressure, cataract and subconjunctival hemorrhage, whereas endophthalmitis (1%), uveitis, intravitreal hemorrhage, retinal tear, lens injury and rhegmatogenous retinal detachment consisted severe and rare complications [4]. Non ocular hemorrhages (9%) and thromboembolic events have been referred as systemic side effects of intravitreal ranibizumab. The efficacy of bevacizumab in exudative ARMD was highlighted by ABC trial, which noted the absence of endophthalmitis or severe uveitis [5]. However, the incidence of thromboembolic events was 3-times higher in patients received bevacizumab compared to healthy individuals [6]. The maintenance therapy with pegaptanib was supported by the LEVEL study [7]. The cardiovascular events that mentioned included

cerebrovascular accidents (0.5%), myocardial infarction (0.7%) and ischaemia (0.2%) as well as non-ocular hemorrhages (0.4%). 62% of the patients received pegaptanib experienced an ocular adverse event, comprising conjunctival haemorrhage (6.3%), vitreous floaters (10%), vitreous haemorrhage (0.05%), endophthalmitis (0.05%), retinal haemorrhage (0.02%), and retinal tear (0.02%) [7].

Aflibercept is a recombinant soluble decoy receptor that is composed of components of both VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2) fused to the Fc region of human IgG1. It exhibits high affinity against all forms of VEGF (80 times higher compared to bevacizumab and ranibizumab) and placental growth factor (PIGF). The intravitreal half-life of 7.1 days and the clinical duration of 2.5 months are strongly associated with the intermediate size of aflibercept (115 kDa compared to 48 kDa for ranibizumab and 148 kDa for bevacizumab) [8]. The mean maximal plasma concentration of unbound aflibercept, following the maximal intravitreal dose of 2 mg, is achieved in 1-3 days, and is estimated to be 200-fold lower than the concentration required for maximal systemic VEGF binding. The systemic half-life of unbound aflibercept is 1.5 days, whereas the corresponding values for bevacizumab and ranibizumab are 20 days and 6 hours, respectively [8]. Today, the guidelines of aflibercept for treating ARMD indicate that a 2mg (50 μl) injection should be initially performed monthly for three consecutive doses, followed by one 2mg injection every 2 months during the first year (total of seven injections in 1st year) [9].

Taking into account the wide use of aflibercept in the treatment of ARMD, we decided to investigate its therapeutic efficacy along with its possible anatomical and, for the first time in literature, functional impact on retinal tissue. The aim of this non-randomised prospective study was to record the electroretinographic changes of the foveal, parafoveal and perifoveal areas in eyes with sub-foveal choroidal neovascularization, using optical coherence tomography (OCT) and multifocal electroretinography (mfERG), before and after the intravitreal use of aflibercept.

Methods

Patients

In this non-randomised prospective study, fifteen patients (15 eyes) with sub-foveal choroidal neovascularization due to ARMD were treated with intravitreal aflibercept (EYLEA; Regeneron Pharmaceutical Inc and Bayer Tarrytown, NY, 2011). On presentation, all the patients underwent a complete ophthalmic examination, including the measurement of best-corrected visual acuity (BCVA), fundus examination, intraocular pressure measurement, fluorescein angiography, an OCT scan and a mfERG recording. The doses were monthly for the first three months, being repeated every three months after for one year, with 2 mg of intravitreal aflibercept. A total of six aflibercept injections were, finally, performed during the 12-month study. Two masked examiners evaluated the visual acuity at the baseline and at the first, second, third, sixth, ninth and 12th month after the first injection of aflibercept. BCVA was measured by means of standard Snellen charts and two masked evaluators were used to determine visual acuity.

All the eyes had predominantly classic, minimally classic or occult choroidal neovascularization, which involved at least the fovea and perifoveal areas, active leakage of the new choroidal blood vessels and decreased visual acuity. The exclusion criteria were any previous treatment for ARMD or ocular diseases which might influence the mfERG recording, such as high myopia, central or diffuse retinal degeneration, or cataract. The study was performed in accordance to the tenets of the Declaration of Helsinki and the protocol used was approved by the ethics committee of the University Hospital. Written informed consent was obtained from all participants.

MfERG recordings

The mfERG recordings were carried out according to the guidelines for a basic mfERG by the International Society for Clinical Electrophysiology of Vision (ISCEV) [10], based on the EP-1000 model (Tomey, Nagoya, Japan). The pupils of the patients were dilated with tropicamide 0.5% and phenylephrine 5% and the eyes were optically corrected for near vision so that the patients could see clearly the small fixation spot in the centre of the stimulus matrix. For signal acquisition, a bipolar contact lens was used, in which the active and the reference electrodes were incorporated in the contact lens. The ground electrode was attached to the ear lobe. The fellow eye was closed and the duration of the data acquisition was 8 minutes, divided into eight sessions of 60 seconds. Retinal irritation was performed by an array of 61 hexagonal elements that were displayed on a cathode ray tube color monitor, driven at a frame of 75 Hz. These hexagons were scaled in size (central hexagons were smaller than peripheral) to produce approximately the same signal intensity in all areas of retina and each of their elements had a 50% chance of being illuminated every time the frame changed. Each stimulus element flickered between black and white at a rate of 75 Hz, controlled by a predetermined m-sequence. The fixation point was located at the center (in a 20°-25° radius) and within a field of 40°-50° diameter, in order to include the blind spot.

Applying the ring pattern, the mfERG stimuli location and anatomic areas corresponded roughly as follows: ring 1 to the fovea (0°–2°), ring 2 to the parafovea (2°–7°), ring 3 to the perifovea (7°–13°), ring 4 to the near periphery (3°–22°), and ring 5 to the central part of the middle periphery (22°–30.5°). The responses were summed with increased eccentricity from the fovea and the amplitude of the summed responses was divided by the total area of the hexagons in that ring. A cross-correlation between the m-sequence for a particular area and the single raw trace recording was performed. The stretch factor that was used was equal to 10.5, which is the most widely used figure. These averages gave a more accurate view of the relative response densities of each group. The response amplitude per unit area or response density (nV/deg2) is the measure of expression and it appears the maximum value in the fovea. The mfERG recordings, consisting of the retinal response density (nV/deg^2) and P1 latency (ms), were evaluated at the first, second, third, sixth, ninth and 12th month after the first injection of aflibercept.

OCT measurements

The OCT examination was performed by the same well-trained and experienced operator, using Heidelberg Spectralis (Spectralis HRA +OCT, Heidelberg Engineering Inc., München, Germany). The acquisition rate of the Spectralis OCT is 40,000 A-scans per second, its optical depth resolution is 7 μm, its digital depth resolution is 3.5 μm, and the transverse and axial resolutions are 20 μm and 5 μm, respectively. During the procedure on the Spectralis OCT, subjects were asked to fixate on an internal fixation target to increase the chance of a well-centered scan at the fovea. The subjects were not repositioned nor the instrument realigned during the whole scanning procedure, in order to keep the measurement conditions as constant as

possible. Before examination, the pupils were dilated with drops containing 0.5% tropicamide and 2.5% phenylephrine.

The central retinal thickness (CRT) in the Spectralis OCT was calculated as the distance between the first signal from the vitreoretinal interface and the signal from the outer border of the RPE. To analyze CRT, a software algorithm of the Spectralis OCT interpolating thickness of the area between the scans was used. This provides a circular map analysis in which the average thickness is displayed as a color code or numeric values in nine ETDRS (Early Treatment Diabetes Retinopathy Study). The ETDRS map consists of three concentric rings with diameters , 3 and 6 mm, known from Stratus OCT. The OCT measurements were performed at the first, second, third, sixth, ninth and 12th month after the first injection of aflibercept.

Statistical analysis

The statistical program IBM SPSS Statistics 22.0 was used for the analysis. Descriptive analysis of all parameters, including the age, the gender, the central macular thickness, the amplitude and the latency as recorded by mfERG was first carried out. Non-parametric analysis Kolmogorov-Smirnov was used to check if the variables had a normal distribution. The paired two-tailed t-test was used to calculate the significance of the mean differences between baseline values and 1st, 2nd, 3rd, 6th, 9th and 12th month values. If the data failed the normality test, the non-parametric Wilcoxon matched-pairs signed-rank test was used. Linear mixed-effects models were used to detect any relation among the visual acuity, the central retinal thickness and the mfERG recordings (amplitude and latency). The foveal thickness and visual acuity were measured only in the foveal area (ring 1). A p value less than 0.05 was considered to indicate significance.

Results

Demographics

Fifteen patients (15 eyes) with sub-foveal choroidal neovascularization due to ARMD were participated in this study, including 7 males (46.7%) and 8 females (53.3%). The mean age of patients was 69.2 ± 4.9 years old, ranging from 61 to 77 years old. The distributions of sex and age were normal among participants, according to Kolmogorov-Smirnov test.

Evaluation of visual acuity

The mean BCVA was 0.12 ± 0.08, 0.20 ± 0.10, 0.25 ± 0.1, 0.28 ± 0.1, 0.34 ± 0.14, 0.36 ± 0.14 and 0.40 ± 0.14 decimal, at presentation (before treatment), 1st, 2nd, 3rd, 6th, 9th and 12th month, respectively (Figure 1). BCVA was consecutively improved after intravitreal injections of aflibercept and there were statistically significant differences in means of BCVA between treatment intervals, resulting in increase of BCVA by 0.08 ($t(14)=-3.525$, $p=0.003$), 0.13 ($t(14)=-8.789$, $p<0.001$), 0.16 ($t(14)=-8.702$, $p<0.001$), 0.22 ($t(14)=-8.876$, $p<0.001$), 0.24 ($t(14)=-10.944$, $p<0.001$), and 0.28 ($t(14)=-10.512$, $p<0.001$) decimal at 1st, 2nd, 3rd, 6th, 9th and 12th month, respectively, compared to the one before treatment. The difference in means of BCVA was also significant between the 1st and 3rd (close of 3 consecutive injections) month ($t(14)=-4.799$, $p<0.001$) and between the 3rd and 6th (after the first quarterly injection) month ($t(14)=-3.154$, $p=0.007$). The subsequent period there were no statistical differences in BCVA between treatments (6th-9th month: ($t(14)=-1.146$, $p=0.27$, 9th-12th month: $t(14)=-1.702$, $p=0.111$).

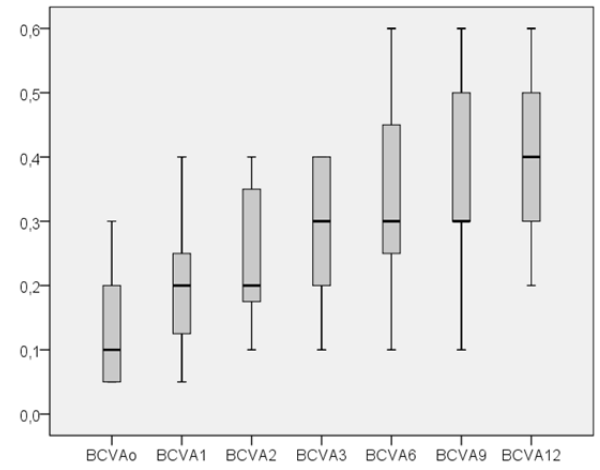

Figure 1: The participants' best corrected visual acuity (BCVA, in decimals) over time. (BCVAo= BCVA at presentation, BCVA1= BCVA at 1 month, BCVA2= BCVA at 2 months, BCVA3= BCVA at 3 months, BCVA6= BCVA at 6 months, BCVA9= BCVA at 9 months, BCVA12= BCVA at 12 months).

Assessing the mfERG

The recordings of amplitude (mfERGd) in three rings (Ring 1: mfERG1d, Ring 2: mfERG2d, Ring 3: mfERG3d) over time (at presentation, 1st, 2nd, 3rd, 6th, 9th and 12th month) are depicted in Figures 2-3.

The mean amplitudes in ring 1 (mfERG1do=103.5 ± 51.0, mfERG1d1=113.5 ± 46.3, mfERG1d2=118.2 ± 49.8, mfERG1d3=133.7 ± 47.9, mfERG1d6=145.8 ± 45.5, mfERG1d9=155.7 ± 45.4, mfERG1d12=161.4 ± 44.0 nV/deg2), in ring 2 (mfERG2do=68.1 ± 18.2, mfERG2d1= 73.3 ± 16.0, mfERG2d2= 78.1 ± 15.4, mfERG2d3=79.3 ± 14.9, mfERG2d6=85.9 ± 17.8, mfERG2d9=88.8 ± 18.7, mfERG2d12=91.9 ± 19.1 nV/deg2), and in ring 3 (mfERG3do=32.7 ± 4.7, mfERG3d1=39.3 ± 4.3,mfERG3d2=42.9 ± 4.0, mfERG3d3=45.3 ± 4.3, mfERG3d6=47.3 ± 4.6, mfERG3d9=48.9 ± 3.3, mfERG3d12=51.7 ± 3.1 nV/deg2), were increased over time. There were significant increases in the means of amplitudes in all three rings over time compared to the baseline amplitudes, as well as at the close of 3 consecutive injections and after each quarterly injection (Table 1).

The values of latencies (mfERGt) in three rings (Ring 1: mfERG1t, Ring 2: mfERG2t, Ring 3: mfERG3t) over time (at presentation, 1st, 2nd, 3rd, 6th, 9th and 12th month) are displayed. The mean latencies in ring 1 (mfERG1to=42.8 ± 4.9, mfERG1t1=42.9 ± 5.8, mfERG1t2=40.8 ± 3, mfERG1t3=42.7 ± 5.3, mfERG1t6=42.2 ± 2.8, mfERG1t9=42.4 ± 2.9, mfERG1t12=42.4 ± 2.8 ms), in ring 2 (mfERG2to=41.2 ± 4.3, mfERG2t1=40.6 ± 3.8, mfERG2t2= 40.7 ± 3.6, mfERG2t3= 40.5 ± 2.8, mfERG2t6=39.8 ± 3, mfERG2t9=39.9 ± 2.2, mfERG2t12=39.9 ± 2.7 ms), and in ring 3 (mfERG3to=41.5 ± 6, mfERG3t1=41.5 ± 6.5, mfERG3t2=41.2 ± 6, mfERG3t3=42.1 ± 7.3, mfERG3t6=40.9 ± 4.9, mfERG3t9=41.3 ± 4.9, mfERG3t12=40.8 ± 4.9ms) exhibited no significant differences over time (Table 2).

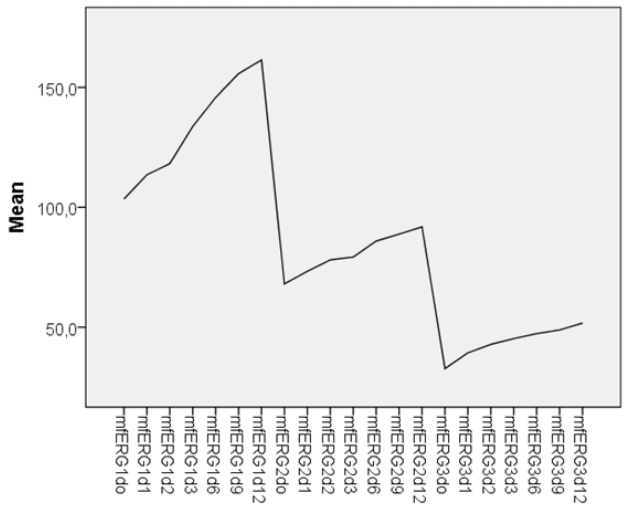

Figure 2: The amplitudes of retinal responses (in nV/deg2) in ring , 2 and 3 at presentation, 1st, 2nd, 3rd, 6th, 9th and 12th month. (mfERG1do=amplitude in ring 1 at presentation, mfERG1d1=amplitude in ring 1 at 1 month, mfERG1d2=amplitude in ring 1 at 2 months, mfERG1d3=amplitude in ring 1 at 3 months, mfERG1d6=amplitude in ring 1 at 6 months, mfERG1d9=amplitude in ring 1 at 9 months, mfERG1d12=amplitude in ring 1 at 12 months, mfERG2do=amplitude in ring 2 at presentation, mfERG2d1=amplitude in ring 2 at 1 month, mfERG2d2=amplitude in ring 2 at 2 months, mfERG2d3=amplitude in ring 2 at 3 months, mfERG2d6=amplitude in ring 2 at 6 months, mfERG2d9=amplitude in ring 2 at 9 months, mfERG2d12=amplitude in ring 2 at 12 months, mfERG3do=amplitude in ring 3 at presentation, mfERG3d1=amplitude in ring 3 at 1 month, mfERG3d2=amplitude in ring 3 at 2 months, mfERG3d3=amplitude in ring 3 at 3 months, mfERG3d6=amplitude in ring 3 at 6 months, mfERG3d9=amplitude in ring 3 at 9 months, mfERG3d12=amplitude in ring 3 at 12 months).

OCT recordings

CRT, as recorded by OCT, was found to be reduced over time (at presentation: 465.0 ± 161.4 μm, 1st month: 374.9 ± 139.5 μm, 2nd month: 323.3 ± 113.8 μm, 3rd month: 290.3 ± 85.1 μm, 6th month: 263.3 ± 69.1 μm, 9th month: 243.0 ± 60.6 μm and 12th month: 226.9 ± 63.5 μm). The abatements in CRT values over time were statistically significant compared to the baseline measurements (Wilcoxon matched-pairs signed-rank tests, 0-1st month: p=0.00, 0-2nd month: p=0.00, 0-3rd month: p=0.00, 0-6th month: p=0.00, 0-9th month: p=0.00, 0-12th month: p=0.001). Moreover, there were significant decreases in the means of CRT at the close of 3 consecutive injections (1st-3rd month: p=0.001) and after each quarterly injection (3rd-6th month: p= 0.010, 6th-9th month: p= 0.003, 9th-12th month: p=0.008).

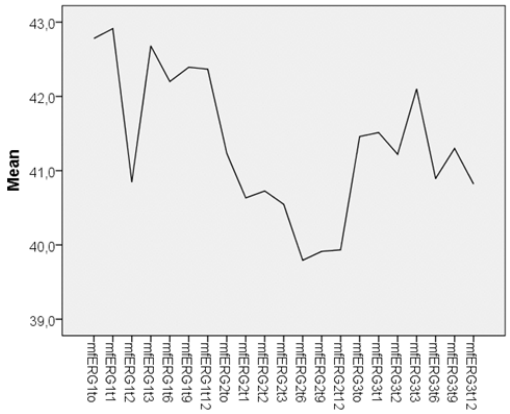

Figure 3: The latencies of retinal responses (in ms) in ring , 2 and 3 at presentation, 1st, 2nd, 3rd, 6th, 9th and 12th month. (mfERG1to=latency in ring 1 at presentation, mfERG1t1=latency in ring 1 at 1 month, mfERG1t2=latency in ring 1 at 2 months, mfERG1t3=latency in ring 1 at 3 months, mfERG1t6=latency in ring 1 at 6 months, mfERG1t9=latency in ring 1 at 9 months, mfERG1t12=latency in ring 1 at 12 months, mfERG2to=latency in ring 2 at presentation, mfERG2t1=latency in ring 2 at 1 month, mfERG2t2=latency in ring 2 at 2 months, mfERG2t3= latency in ring 2 at 3 months, mfERG2t6=latency in ring 2 at 6 months, mfERG2t9=latency in ring 2 at 9 months, mfERG2t12=latency in ring 2 at 12 months, mfERG3to=latency in ring 3 at presentation, mfERG3t1=latency in ring 3 at 1 month, mfERG3t2=latency in ring 3 at 2 months, mfERG3t3=latency in ring 3 at 3 months, mfERG3t6=latency in ring 3 at 6 months, mfERG3t9=latency in ring 3 at 9 months, mfERG3t12=latency in ring 3 at 12 months).

Pairs	p value	Pairs	p value
mfERG1do - mfERG1d1	0.012	mfERG3do - mfERG3d6	< 0.001
mfERG1do - mfERG1d2	0.003	mfERG3do - mfERG3d9	< 0.001
mfERG1do - mfERG1d3	0.001	mfERG3do - mfERG3d12	< 0.001
mfERG1do - mfERG1d6	< 0.001	mfERG1d1 - mfERG1d3	0.005
mfERG1do - mfERG1d9	< 0.001	mfERG2d1 - mfERG2d3	0.007
mfERG1do - mfERG1d12	< 0.001	mfERG3d1 - mfERG3d3	< 0.001

mfERG2do - mfERG2d1	0.005	mfERG1d3 - mfERG1d6	0.006
mfERG2do - mfERG2d2	0.001	mfERG2d3 - mfERG2d6	0.012
mfERG2do - mfERG2d3	0.001	mfERG3d3 - mfERG3d6	0.071
mfERG2do - mfERG2d6	0.001	mfERG1d6 - mfERG1d9	< 0.001
mfERG2do - mfERG2d9	0.001	mfERG2d6 - mfERG2d9	0.004
mfERG2do - mfERG2d12	0.000	mfERG3d6 - mfERG3d9	0.112
mfERG3do - mfERG3d1	< 0.001	mfERG1d9 - mfERG1d12	< 0.001
mfERG3do - mfERG3d2	< 0.001	mfERG2d9 - mfERG2d12	0.006
mfERG3do - mfERG3d3	< 0.001	mfERG3d9 - mfERG3d12	0.010

Table 1: Two-tailed paired t-tests of amplitudes in ring ,2 and 3 over time. mfERG1do=amplitude in ring 1 at presentation, mfERG1d1=amplitude in ring 1 at 1 month, mfERG1d2=amplitude in ring 1 at 2 months, mfERG1d3=amplitude in ring 1 at 3 months, mfERG1d6=amplitude in ring 1 at 6 months, mfERG1d9=amplitude in ring 1 at 9 months, mfERG1d12=amplitude in ring 1 at 12 months, mfERG2do=amplitude in ring 2 at presentation, mfERG2d1=amplitude in ring 2 at 1 month, mfERG2d2=amplitude in ring 2 at 2 months, mfERG2d3=amplitude in ring 2 at 3 months, mfERG2d6=amplitude in ring 2 at 6 months, mfERG2d9=amplitude in ring 2 at 9 months, mfERG2d12=amplitude in ring 2 at 12 months, mfERG3do=amplitude in ring 3 at presentation, mfERG3d1=amplitude in ring 3 at 1 month, mfERG3d2=amplitude in ring 3 at 2 months, mfERG3d3=amplitude in ring 3 at 3 months, mfERG3d6=amplitude in ring 3 at 6 months, mfERG3d9=amplitude in ring 3 at 9 months, mfERG3d12=amplitude in ring 3 at 12 months.

Pairs	p value	Pairs	p value
mfERG1to - mfERG1t1	0.933	mfERG3to - mfERG3t6	0.977
mfERG1to - mfERG1t2	0.113	mfERG3to - mfERG3t9	0.589
mfERG1to - mfERG1t3	0.961	mfERG3to - mfERG3t12	0.807
mfERG1to - mfERG1t6	0.584	mfERG1t1 - mfERG1t3	0.915
mfERG1to - mfERG1t9	0.731	mfERG2t1 - mfERG2t3	0.871
mfERG1to - mfERG1t12	0.707	mfERG3t1 - mfERG3t3	0.570
mfERG2to - mfERG2t1	0.392	mfERG1t3 - mfERG1t6	0.766
mfERG2to - mfERG2t2	0.513	mfERG2t3 - mfERG2t6	0.303
mfERG2to - mfERG2t3	0.368	mfERG3t3 - mfERG3t6	0.297
mfERG2to - mfERG2t6	0.104	mfERG1t6 - mfERG1t9	0.549
mfERG2to - mfERG2t9	0.118	mfERG2t6 - mfERG2t9	0.824
mfERG2to - mfERG2t12	0.176	mfERG3t6 - mfERG3t9	0.361
mfERG3to - mfERG3t1	0.875	mfERG1t9 - mfERG1t12	0.901
mfERG3to - mfERG3t2	0.271	mfERG2t9 - mfERG2t12	0.954
mfERG3to - mfERG3t3	0.485	mfERG3t9 - mfERG3t12	0.378

Table 2: Two-tailed paired t-tests of latencies in ring ,2 and 3 over time. mfERG1to=latency in ring 1 at presentation, mfERG1t1=latency in ring 1 at 1 month, mfERG1t2=latency in ring 1 at 2 months, mfERG1t3=latency in ring 1 at 3 months, mfERG1t6=latency in ring 1 at 6 months, mfERG1t9=latency in ring 1 at 9 months, mfERG1t12=latency in ring 1 at 12 months, mfERG2to=latency in ring 2 at presentation, mfERG2t1=latency in ring 2 at 1 month, mfERG2t2=latency in ring 2 at 2 months, mfERG2t3=latency in ring 2 at 3 months, mfERG2t6=latency in ring 2 at 6 months, mfERG2t9=latency in ring 2 at 9 months, mfERG2t12=latency in ring 2 at 12 months, mfERG3to=latency in ring 3 at presentation, mfERG3t1=latency in ring 3 at 1 month, mfERG3t2=latency in ring 3 at 2 months, mfERG3t3= latency in ring 3 at 3 months, mfERG3t6=latency in ring 3 at 6 months, mfERG3t9= latency in ring 3 at 9 months, mfERG3t12=latency in ring 3 at 12 months.

Association between visual acuity, amplitude and central retinal thickness

Six models of linear mixed-effects analysis are presented in Tables 3 and 4. In Model 1, the outcome variable was the amplitude in ring 1, considering as explanatory variables the BCVA and the CRT. The CRT and the BCVA did not seem to be related significantly with the amplitude in ring 1 (Table 3). Similarly, no significant association was detected between the latency in ring 1 (outcome variable) and the CRT and BCVA (explanatory variables), according to the Model 2 (Table 3). Moreover, the amplitudes in ring 1 and the CRT (explanatory variables) were not found to have any effect on BCVA (outcome variable), using the Model 3 (Table 3). In Model 4, the outcome variable was CRT, considering as explanatory variables the BCVA and the amplitude in ring 1. The regression equation did not reveal again any significant relation among these variables (Table 3). Assessing the amplitude of responses according to the retinal area, a significant association was elicited between the amplitude and the ring area where it is recorded (Model 5, Table 4). Similarly, the latency of retinal responses was strongly related to the ring area (Model 6, Table 4).

Model 1: Amplitude in ring 1 (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
CRT	-0.261	0.099	-0.579-0.057
BCVA	-45.051	0.725	-317.634-227.532
Model 2: Latency in ring 1 (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
CRT	-0.003	0.734	-0.024-0.017
BCVA	0.172	0.983	-17.505-17.849
Model 3: BCVA (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
Amplitude in ring 1	0.000	0.725	-0.002-0.001
CRT	-0.001	0.060	-0.001-0.000
Model 4: CRT (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
Amplitude in ring 1	-0.807	0.099	-1.790-0.175
BCVA	-393.433	0.060	-806.820-19.954
BCVA: Best Corrected Visual Acuity; CRT: Central Retinal Thickness; CI: Confidence Interval			

Table 3: Linear mixed-effects analysis in the foveal area (ring 1) for the association among the amplitude (Model 1), latency (Model 2), the best corrected visual acuity (Model 3) and the central retinal thickness (Model 4).

Model 5: Amplitude (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
Ring 1	0,334	p<0.001	0.333-0.334
Ring 2	0,334	p<0.001	0.333-0.335
Ring 3	0,329	p<0.001	0.322-0.334
Model 6: Latency (outcome variable)			
Explanatory variables	b coefficients	p value	95% CI
Ring 1	0.332	p<0.001	0.325-0.339
Ring 2	0.334	p<0.001	0.321-0.346
Ring 3	0.332	p<0.001	0.326-0.338

CI: Confidence Interval

Table 4: Linear mixed-effects analysis for the association of amplitude (Model 5) and latency (Model 6) with the ring area.

Discussion

In this study, fifteen patients (15 eyes) with sub-foveal choroidal neovascularization due to ARMD were found to benefit from intravitreal aflibercept treatment. During the 12months period of treatment, the visual acuity and the amplitude as recorded by mfERG were both significantly increased, while the central retinal thickness was significant reduced. The BCVA was elevated between treatment intervals until 6th month (after the first quarterly injection), after which no statistical differences in BCVA between treatments were observed, although the visual acuity was significant higher at each visit (9th and 12th month) compared to the baseline measurements. The latencies of retinal responses displayed no statistical significant differences over time. However, both amplitude and latency were found to be associated with the ring area where they recorded. Despite the anatomical and functional improvement of patients underwent intravitreal injections of aflibercept, the visual acuity, the central retinal thickness, as well as mfERG recordings (amplitude and latency) in ring 1 were not found to be related. This may be explained by the fact that the macular edema is only a parameter that may affect visual acuity and the electrical activity of the macula. Consequently, increase or decrease of macular thickness does not necessarily reflect the course of the visual acuity as supported. Atrophy of the retina, particularly of the photoreceptors, atrophy of the pigment epithelium and scarring are all unmeasured parameters which influence vision.

In VIEW studies, 2475 patients with neovascular ARMD received 0.5 mg intravitreal ranibizumab every 4 weeks (Rq4 group), 0.5 mg aflibercept every 4 weeks (0.5q4 group), 2 mg aflibercept every 4 weeks (2q4 group), or 2 mg aflibercept every 8 weeks (2q8 group) after 3 monthly injections. Subsequently, the frequency of their original dosing was customized to the patients' needs from 52nd to 96th weeks, whereas there was a mandatory dosing at least every 12 weeks [11]. The BCVA remained stable for 94.4% to 96.1% of the patients at 52nd week, while 91.5% to 92.4% of the patients maintained their BCVA at 96th week. However, the percentage of patients with declined retinal fluid at 52nd and 96th weeks was higher at 2q4 group compared to Rq4 one. The outcome of the studies was that both ranibizumab and aflibercept groups improved and preserved their visual acuity until 96th week, but the number of injections from 52nd to 96th weeks was lower in the 2q4 and 2q8 groups than the Rq4 group (differences of -0.64 [95% CI, -0.89 to -0.40] and -0.55 [95% CI, -0.79 to -0.30]; P<0.000, post hoc analysis) [11]. Similarly, the conclusions of VIEW2 trial were that central retinal thickness and mean area of choroidal neovascularization decreased in all treatment groups with similar magnitude [12]. In addition, the ocular adverse events were balanced across treatment groups. Furthermore, the incidence of elevated IOP was estimated to be lower in patients treated with aflibercept than ranibizumab [13]. Specifically, the percentages of eyes with IOP>21 mmHg at week 96 were 20.2%, 14.2%, 12.1%, and 12.5% in Rq4, 2q4, 2q8, and 0.5q4, respectively.

De Oliveira Dias' study group investigated the short-term safety and efficacy of intravitreal ziv-aflibercept [14]. They noted subjective and objective improvement of visual acuity, decrease in intraretinal and subretinal fluid and microperimetric amelioration along with electroretinographic changes in a patient with exudative ARMD who received an intravitreal injection of ziv-aflibercept. Moreover, they did not mention any adverse effects. This was the only study that dealt with the mfERG findings of aflibercept before ours. Koizumi's study group looked into the efficacy of aflibercept in 46 eyes with wet ARMD and 56 eyes with polypoidal choroidal vasculopathy (PCV) [15]. All 102 eyes underwent 3 consecutive monthly injections of 2.0 mg intravitreal aflibercept. The mean subfoveal choroidal thickness was decreased by 86.5% from baseline to 3 months (from 252.0 ± 99.7 mm at baseline to 217.9 ± 95.6 mm at 3 months). The equal therapeutic efficacy of aflibercept in PCV and wet ARMD has been also highlighted by Oishi's study group, who additionally observed that the presence of external limiting membrane (ELM), the smaller greatest linear dimension, and the presence of polypoidal lesion were associated with better visual outcome [16].

On the other hand, Okuma's study group supported that the standard induction therapy (three monthly doses of 2 mg/0.05 ml aflibercept in weeks 0, 4th, and 8th) with intravitreal aflibercept is more beneficial in patients with PCV than in typical ARMD [17]. Furthermore, they noted that the absence of posterior vitreous detachment before treatment was associated with failure of treatment. The retinal sensitivity, as recorded by topographic microperimetry, was seemed to be improved after intravitreal aflibercept and this improvement was associated with the OCT features [18]. The best prognosis was noted for patients with subretinal fluid (SRF) and serous pigment epithelium detachment (PED) and to a lesser extent for intraretinal fluid (IRF), intraretinal cystoid space (IRC) and fibrovascular PED. The therapeutic effects of aflibercept, which were more pronounced at 3 months than after 12-months interval, were related to the anatomical recovery of the retinal pigment epithelial layer, the IS/OS line (inner and outer segment junction of photoreceptors) and the ELM [18]. The restoration of ellipsoid zone, which is observed after aflibercept injections, has not be correlated with the increase in BCVA, whereas ELM changes predict photoreceptors recovery and have directly associated with final BCVA [19].

Moreover, intravitreal injections of aflibercept seemed to be effective in rehabilitating central macular thickness as well as decreasing PED and macular volume in patients with ARMD resistant to ranibizumab or bevacizumab [20-22]. The central retinal thickness may be decreased in patients with wet ARMD refractory to ranibizumab, however patients with PCV appeared to be more benefited from aflibercept treatment [23]. Aflibercept can improve both macular thickness and visual acuity in PCV, prolonging the time of dry retina. Nomura's study group observed that intravitreal aflibercept in patients with wet ARMD resistant to ranibizumab, exhibits better results in absence of choroidal vascular hyperpermeability (CVH) than in presence of CVH [24]. However, subfoveal choroidal thickness and PED in patients with CVH can be better treated with aflibercept (2mg) than ranibizumab (0.5 mg) after three consecutive monthly injections of these drugs [25]. The decreased plasma concentrations of proangiogenic cytokine placental growth factor (PlGF) have been implicated in the resistance of ranibizumab and bevacizumab therapy [25]. The upregulation of PlGF after intravitreal aflibercept may be

responsible for the different response of patients to such treatment [26].

According to the results of this study, including small series of eyes with a one-year follow-up, intravitreal aflibercept seems to be safe and effective in treating sub-foveal choroidal neovascularization observed in patients with ARMD. For the first time in literature anatomical and functional assessment of the macula in patients with ARMD is shown objectively. The anatomical outcomes, as recorded by OCT and the mfERG responses, reflecting the functional level, were all significantly improved over the time of study. These observations encourage the use of intravitreal aflibercept in exudative ARMD.

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