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An Objective Perimetry Study of Central Versus Peripheral Sensitivities and Delays in Age-Related Macular Degeneration

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Purpose: The purpose of this study was to compare central versus peripheral retinal sensitivities and delays in neovascular age-related macular degeneration (nAMD) using US Food and Drug Administration (FDA)-cleared multifocal pupillographic objective perimetry (mfPOP).

Methods: We recruited 18 patients with nAMD and commenced Pro re nata intravitreal anti- vascular endothelial growth factor (VEGF) injection. We compared macular (±15 degrees) and wide-field (±30 degrees) mfPOP variants. We examined temporal correlations between treated and untreated fellow eyes. We fitted linear models to selected treatment patterns, and compared the ability of central versus peripheral responses to predict the need for treatment.

Results: Central sensitivity decreased by -2.23 ± 0.051 dB/month ($P < 0.0002$) in treated eyes, and -0.17 ± 0.07 dB/month ($P = 0.033$) in untreated eyes. Treated eyes showed quicker central responses by 13.08 ± 3.77 ms than untreated eyes ($P = 0.001$). Based on peripheral responses, we identified two eye-types. Among positive-eyes peripheral sensitivity increased by 9.88 ± 4.41 dB ($P = 0.042$) before treatment; delays increased by 3.49 ± 1.75 ms/month ($P = 0.049$). For negative-eyes peripheral delays were shorter a month before treatment by 9.38 ± 3.59 ms ($P = 0.013$). Correlations between treatment and peripheral sensitivities or delays peaked at 1 to 2 months post-treatment. Peripheral data significantly determined treatment frequency and final acuity (all $P < 0.044$).

Conclusions: Peripheral macular function of treated and untreated eyes divided eyes into positive and negative groups. Those peripheral responses determined outcomes; changes preceding active disease by 1 to 3 months. Overall, mfPOP may provide potential biomarkers to assist nAMD management.

Translational Relevance: Objective perimetry may identify the requirement for treatment in nAMD that accords with the decision of a skilled clinician based on optical coherence tomography (OCT) and clinical findings.

Introduction

Age-related macular degeneration (AMD), accounting for 8.7% of blindness worldwide[1] and a global prevalence of more than 170 million,[2] is the leading cause of irreversible blindness in people over 49 years of age.[3,4] Its etiology includes genetic and environmental factors.[5] Modifiable risk factors include smoking and high-density lipoprotein cholesterol.[6,7] AMD causes losses of quality of life of between 17% and 60%[8]; losses of gross domestic product are

approximately $30 billion per annum.[8] The current global prevalence of AMD is 170 million,[2] and is projected to be 288 million by 2040.[1] A meta-analysis showed that the relative prevalence of AMD ranged from 7.3% in Asian populations to 12.3% in those with European ancestry.[1] One type of neovascular AMD (nAMD), polypoidal choroidal vasculopathy (PCV), is, however, more common among Asian populations.[9] In current practice, the precise diagnosis and grading of AMD is done with clinical evaluation supported by retinal photography and imaging with optical coherence tomography (OCT) to confirm to the size and location of drusen and retinal pigmentary changes, and to quantify exudation and neural degeneration.[2] OCT alone can be relied upon for detecting and monitoring choroidal neovascular activity, however, fluorescein angiogram (FA) has value in those with occult lesions that appear quiescent on OCT.[10] Moving forward from the era when nAMD was treated with photodynamic therapy[11] and later combined therapy,[12] the current mainstay of treatment is intravitreal injection of anti-vascular endothelial growth factors (anti-VEGFs).[13]

Clinical examination including best corrected visual acuity (BCVA), FA, and OCT are currently used to confirm whether anti-VEGF treatment is indicated.[14] FA and OCT findings are suggestive of only the structural integrity and changes. BCVA, which subserves only the foveal function, is the only functional test performed currently in the clinics to assess function. No functional test is performed to assess the extrafoveal function. Therefore, any loss of extrafoveal function is missed by the current clinical tools for informing treatment/retreatment indications. Multifocal pupillographic objective perimetry (mfPOP) as a multifocal functional test, checking both the foveal and extrafoveal function, could address these issues and better inform initial treatment or retreatment with anti-VEGF.

The mfPOP has the potential to indicate the severity of early-stage AMD,[15–17] as well as late-stage AMD,[16,18] and both the mfPOP methods used here have been shown to have reproducibility that is twice that of the standard automated perimetry.[19] Several hundred of tests of the second method, P129, done 5 minutes apart show no fatigue effects.[20] Additionally, mfPOP identifies ranibizumab-induced functional improvements in nAMD, and peripheral hypersensitivity indicates better outcomes for anti-VEGF treatment.[21] Taken together, these results suggest mfPOP might provide additional biomarkers indicting eyes-at-risk of active disease that would increase confidence about the decision to treat with anti-VEGF.

Two mfPOP studies reported that while patients with earlier stage AMD with large drusen showed broadly distributed minor sensitivity loss, those with nAMD can show both depressed central sensitivity and increased peripheral sensitivity.[15,18] Recently, another study reported that macular sensitivity (10-2 Matrix perimetry) and retinal thickness (OCT) from outside the central 8 degrees were significantly associated with visual acuity, among patients who had difficulty in facial recognition and social interaction due to neovascular AMD or geographic atrophy, indicating that peripheral macular measures may provide useful prognostic information in such cases.[22] We have also developed an early treatment diabetic retinopathy study (ETDRS) grid congruent version of mfPOP test, which takes only 80 seconds to test both eyes.[23] A rapid, noninvasive, noncontact, functional method evaluating extrafoveal function, further informing the need for retreatment would be useful. In the current study, we compared the central and peripheral functions of treated and untreated fellow eyes monthly for up to 28 months.

Methods

Subjects

Inclusion criteria were newly diagnosed unilateral or bilateral treatment-naïve nAMD cases, age ≥50 years, with indication for anti-VEGF treatment, and BCVA of ≥70 letters (6/12). Exclusion criteria included fellow eye nAMD or treatment with anti-VEGF within the last 6 months, anti-VEGF or retinal laser within 6 months in the study eye, cataract surgery within the last 6 months, or other ocular comorbidities affecting retinal and pupillary function. Cataracts were graded using the Lens Opacities Classification System III (LOCS III),[24] and patients with more than nuclear opacity 4, cortical cataract 2, and posterior subcapsular 2 were excluded. The study complied with the ANU Human Experimentation Ethics Committee (ETH.2010/194), and the ACT Health Human Research Ethics Committee (ETH.7.07.667), adhering to the tenets of the Declaration of Helsinki.

Study Design

A prospective longitudinal study was conducted with monthly patient follow-ups over 14 to 28 months. Ophthalmological tests and diagnostic procedures were performed on each visit (see below), except FA, which was done only once in the

beginning of the study to confirm diagnosis and classify nAMD/neovascularization. After making a diagnosis of nAMD and enrolling the patients, three monthly injections of anti-VEGF were administered. After this, we followed the pro re nata (PRN) protocol. Any of the following indicated active disease: intraretinal fluid, subretinal fluid, new retinal hemorrhage, or unstable retinal pigment epithelium detachment (rPED) relative to the previous visit. If none were present, the eye was graded inactive and treatment was withheld. If the clinician was uncertain regarding activity, eyes were graded borderline and treatment was withheld providing acuity was within five letters of the previous visit. One retinal specialist (co-author R.W.E.) was involved in evaluation of patients clinically and deciding if the treatment was indicated.

Ophthalmic Examinations

Other tests followed mfPOP testing on the same day: Matrix 10-2 perimetry (Carl Zeiss Meditec, Dublin, CA, USA) using Alternative Eccentric Fixation targets,[25] BCVA, low contrast visual acuity (LcVA), corneal curvature (ARK-1s NIDEK; AICHI Japan), pachymetry (DGH Technology, Exton, PA, USA), and intraocular pressure (IOP) with Goldmann tonometry. Pupils were dilated with 1% tropicamide drops for the remaining eye examinations, including slit-lamp of the anterior and posterior segments. An 8 × 8 grid macular thickness scan and peripapillary retinal nerve fiber layer analysis were performed with OCT (Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany). FA was used to classify eyes as: predominantly classic choroidal neovascularization (P), minimally classic choroidal neovascularization (M), or occult choroidal neovascularization (O).

Multifocal Pupillographic Objective Perimetry

Participants refrained from drinking caffeinated beverages within an hour before testing. Presentation of stimuli and monitoring of the pupillary diameter with infrared cameras were performed using an FDA-cleared ObjectiveFIELD (OFA) mfPOP device (Konan Medical USA, Irvine, CA, USA). The OFA concurrently presents independent multifocal stimuli to both eyes while measuring direct and consensual pupil responses.[26] Vergence deficits were corrected before the tests. In addition to a central fixation cross, there was a large thin cross spanning the whole visual field, and a faint starburst radial grating imposed on the background that aided fixation and binocular fusion. The stimuli were presented at optical infinity and any refractive error was corrected within 3 diopters of the optical prescription. The maximal luminance of the stimuli was 150 cd/m^2 presented on a 10 cd/m^2 background. Two stimulus protocols were used: P129, covering the central 60 degrees (Fig. 1A,B), and P131, covering the central 30 degrees of visual field (Fig. 1C,D), each with 44 stimuli/eye. The per-region luminances (see Fig. 1B, 1D) were adjusted (balanced[27]) to make response amplitudes homogeneous across the fields of normal subjects, and used the clustered-volleys pseudo-random presentation method.[18] Each protocol had one transiently presented (30 ms) stimulus that was presented on an average of every 4 seconds to each test region, totaling 22 stimuli per second. Each test was run as 9 segments, each segment of 40 seconds, delivered with a short break

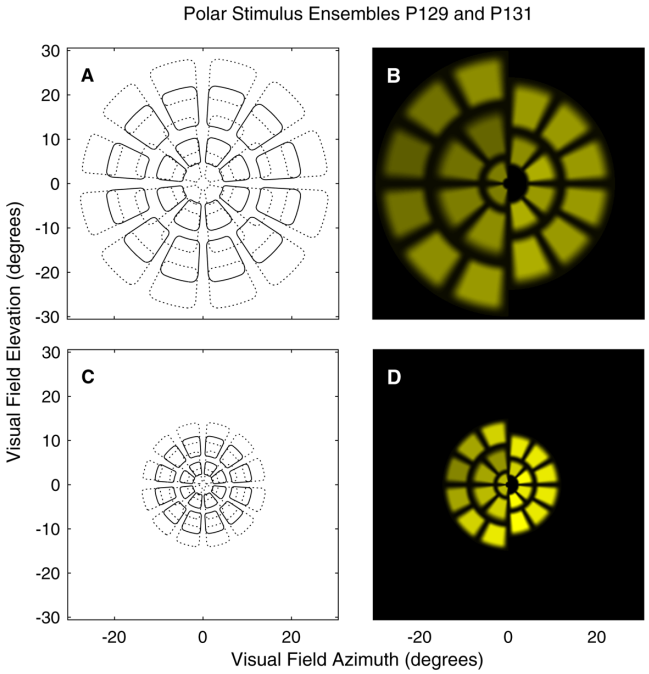

Figure 1. Multifocal stimuli arranged in a dartboard layout pattern presenting 44 stimulus regions per eye. (**A**) Protocol P129 is a wide-field stimulus covering the central 60 degrees (fixation to ±30 degrees eccentricity) (**C**) Protocol P131 covered the central 30 degrees (±15 degrees eccentricity). Both had a 44-region layout consisting of 5 interleaved rings of yellow stimuli. The stimuli were randomly presented over time at a total rate of 22/seconds, testing each location 90 times. Overlapping stimuli are never presented simultaneously. No stimuli encroached upon the horizontal and vertical midlines. (**B, D**) Illustrations of the per-region luminances of P129 and P131, respectively, with half the regions shown on the left and right sides to aid visibility (Methods). The pattern of luminances shown is for OS, the OD pattern was left-right mirror-imaged. The differences in luminance are slightly exaggerated to make them more visible. The stimuli contained no spatial frequencies above 2 cpd, minimizing the effects of mis-refraction.

in between them. Therefore, each test protocol ran for 6 minutes. Fixation losses and blinks were monitored online and data collected during these sections were removed.

Analysis

Data analysis was performed using MATLAB (2016b; The MathWorks, Natick, MA, USA). The response waveforms for each of the 44 test regions per eye were extracted from raw pupillary responses using multiple regression, with blinks removed from the pupil data.[28–30] Responses for each retinal region were fitted to a log-normal function,[31] allowing per-region estimation of constriction amplitude (sensitivity) and time-to-peak (delay).[26,32] Pupil diameter was standardized to 3.5 mm. Thus, we measured relative pupil diameter changes rather than absolute size,[33,34] and these were transformed to decibel sensitivity. The per-region sensitivities and delays of age- and sex-matched healthy subjects were subtracted from test values, as in standard perimetry. The normative model was based on an unpublished study (currently under review) of a cohort of 133 subjects each tested twice, 2 weeks apart.[35] The age range of normative cohort was 18 to 88 years.

P129 and P131 contain 5 rings of stimuli (see Fig. 1). As in previous studies,[15,18] we sometimes found it useful to compute the median sensitivity or delay around these rings. Among other things, this allowed summary plots of central to peripheral responses across visits. We examined fluctuations in central and peripheral responses over visits, in treated and untreated fellow eyes.

Given the 28 months of data we found that many treatment patterns arose that could usefully be examined. We therefore needed to develop a method to succinctly describe different patterns. One example examined mfPOP characteristics among possible responders and nonresponders to anti-VEGF treatment, we examined different treatment patterns across consecutive months, where within a pattern "1" indicated a treatment, and "0" indicated no treatment. Thus, we identified patterns such as [1 1 1 1] and [0 0 0 1] within the sequences of 14 to 28 months of treatments. The pattern [1 1 1 1] would indicate a relative nonresponder, whereas [1 0 0 0] indicates a putative good responder. We have used mostly one instance per eye. In cases where an eye had the same pattern more than once, we only took the first three instances. Here, ring medians over the treatment pattern period were useful. Analysis of these data involved fitting linear models to compare a treatment day with other days across a particular treatment pattern, or fitting slopes indicating rates of change/month. We also split the eyes between those which received more or less than the median 0.7 treatments per visit into low-treatment and high-treatment eyes.

We performed two forms of correlation analysis across time. Then, first we did correlation over time between responses of treated eyes versus nontreated fellow eyes, analyzed separately for each ring. This allowed us to examine whether peripheral and central responses of treated eyes correlated with those of the fellow untreated eyes. The second correlation analysis addressed the question of whether any changes over time at any particular ring or rings was correlated with the history of treatment. We used the MATLAB function *xcorr* to examine these treatments versus response cross-correlations in 14 data sets of 15 months' duration. If these data sets contain serial correlation, then that can broaden the cross-correlation peak. We controlled for this by also fitting a more complex cross-correlation model using the MATLAB function *impulseest*, which adjusted for serial correlations. This required longer data sets. Fortunately, we had 5 data sets of 28 months' duration. The results indicated no significant broadening effect.

The cross-correlation analyses revealed two categories of eyes depending whether their peripheral region sensitives or delays are larger (positive) or smaller (negative) than normal. Next, we undertook separate treatment pattern analysis for positive and negative subgroups. We selected treatment patterns of interest and searched for them among our data and examined cases where we found at least eight of those patterns. We then created linear models where the treatment month was taken as the reference (constant) and two other months were fitted as contrasts to the treatment month. This analysis allowed us to assess the significance of the differences of those months relative to the treatment. There were two model versions: the first where data from ring 1 was fitted, and second, where the averaged data across rings 3 to 5 were fitted. We also fitted a slope across the months of the treatment patterns. See the Results section: Treatment patterns of subjects from positive and negative subgroups.

We next explored whether central and peripheral field responses were predictive of either visual acuity outcomes or treatment frequency. We took the median across the 15 visits and then the mean across selected central and peripheral rings of the differences from normal for the sensitivities and delays. For P129, the central rings were 1 and 2, and peripheral 3 to 5. To make the P131 rings more spatially comparable to P129, the central was set to 1 to 3, and peripheral 4 and 5. This created MeCentral and MePeripheral,

for sensitivities and delays, with one entry per subject. We also created similar measures for Matrix MD and PSD. Factors for age relative to the mean age, and sex, were included. We used stepwise linear models where we regressed on the mean frequency of treatment (TreatFreq), or one of three visual acuity measures: (1) the mean of the first 3 visits, visual acuity (VA)beg; (2) the mean of the last 3 visits, VAend; and (3) the difference VAend - VAbeg indicating the gain in letters (VAgain). See the Results section: Acuity and treatment frequency effects.

Results

The study included 18 patients (14 women, 77.8%; Table 1), 13 of whom received anti-VEGF injections in one eye, and 5 in both eyes. Of those, three cases received injections bilaterally from the beginning, and the other two cases began bilateral injection on later visits. Nine patients had smoked for 12.8 ± 15.9 years, but had quit 18.4 ± 16.3 years before the study.

Figure 2 represents data from the treatment pattern [0 0 0 1] representing 7 instances where anti-VEGF was not required for 3 monthly visits but was required on a fourth visit (see Figs. 2A, 2C). The pattern [1 1 1 1] included 44 instances where treatment was required on every month (see Figs. 2B, 2D). Each region of each panel represents the median sensitivity/delay deviation in decibels (dBs) at that region across the instances (see Figs. 2A, 2B). It is important to point out that the injection was administered after the mfPOP test on each day. Similar findings were observed with the wide-field stimulus protocol P129 (not shown).

The initial findings described above strongly indicate that the central and peripheral regions can behave differently both in terms of sensitivity and delay. We next simplified our analysis by taking the medians by rings. Figure 3 shows the data of Figure 2 replotted.

The comparison among 14 pairs of treated and untreated fellow eyes that each had 15 months of data (Fig. 4 for P131) showed significant central decline in untreated eyes and slower peripheral delays than treated eyes, and central sensitivity loss and shorter delays than untreated eyes.

Temporal Correlations

A feature of Figures 4C and 4D were months where changes occurring in treated and untreated eyes seemed to coincide (e.g. months 4 and 12). We therefore decided to examine the ring-to-ring correlations

Table 1. Subject Demographics for HT and the FA data (FAos and FAod)

Subject	Age	Sex	HT	VAos	VAod	LcVAos	LcVAod	FAos	FAod
85700	67	F	.	85	76	78	64	.	O
85701	82	F	.	81	80	74	61	O	O (22)
85703	69	M	.	89	69	79	68	.	O
85704	81	F	Y	48	79	37	67	P	O
85705	87	M	.	84	73	76	62	.	P
85706	79	F	Y	78	60	69	52	O (16)	O
85707	87	F	.	82	29	77	18	.	P
85708	81	F	Y	78	80	64	70	O	.
85711	73	F	Y	56	83	42	73	O	.
85712	83	M	.	77	88	65	78	O	.
85713	78	F	Y	79	87	62	76	O	.
85714	96	F	Y	19	43	10	25	O	P
85716	82	F	Y	76	77	55	61	M	.
85717	75	M	Y	78	59	59	28	M	M
85718	75	F	.	73	69	60	49	P	P
85719	66	F	Y	77	28	56	17	O	.
85721	83	F	Y	86	79	78	66	.	P
85724	88	F	Y	70	79	77	64	.	O

a'.' indicates none. For FA the definitions of P, O, and M are given in the Methods. The (16) and (22) indicate the study month in which that fellow eye developed new nAMD. The high (VAos, VAod) and low contrast (LcVAos, LcVAod) best corrected acuities are given in letter scores. For reference 85 is 6/6, 76 is 6/9, and 59 is 6/20.

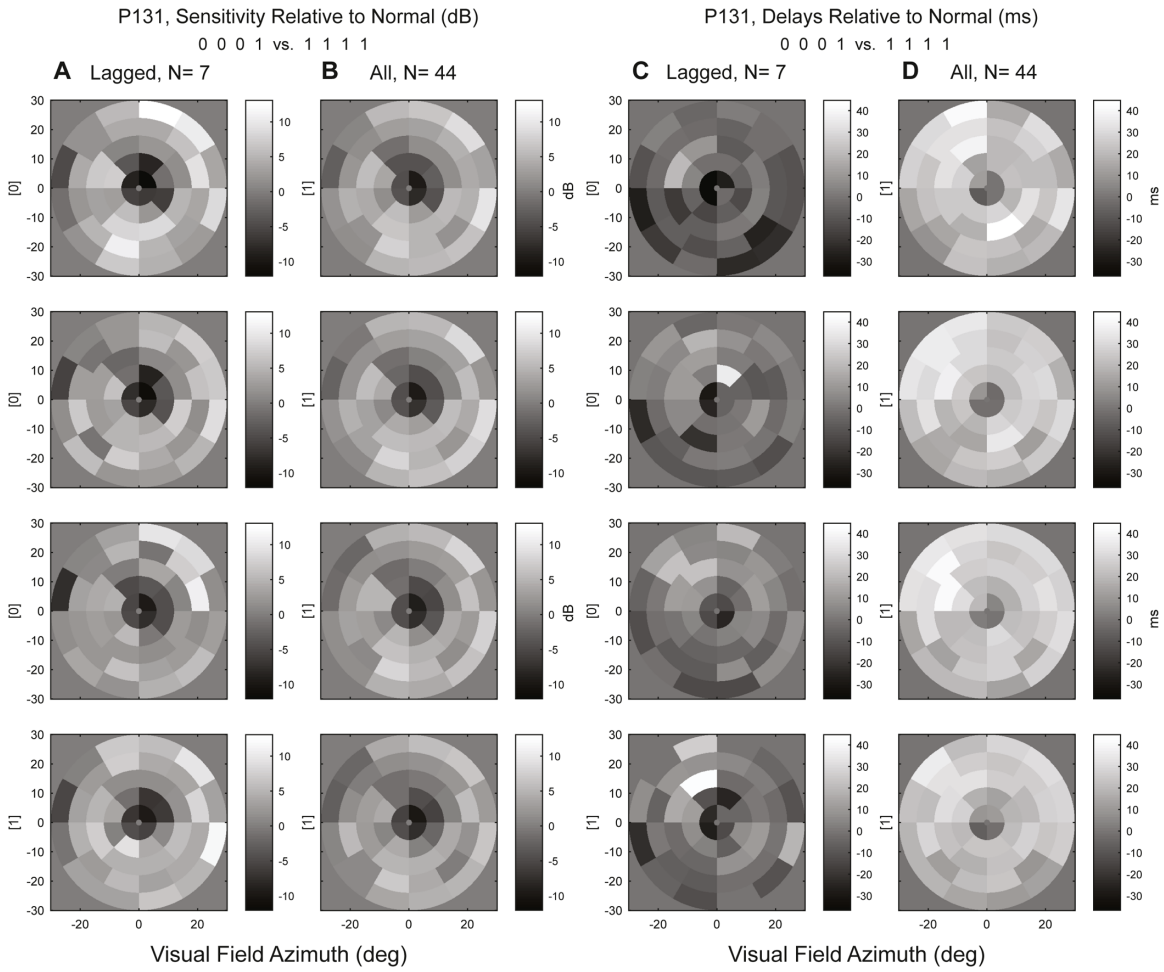

Figure 2. Regional sensitivity and delay deviations for two treatment patterns: [0 0 0 1], and [1 1 1 1]. "0" indicates treatment not given and "1" indicates treatment given. All data are for the macular P131 stimulus protocol. Each row is a consecutive month and the y-axis labels indicate treatment given or not given. No eye contributed more than their first three matching sequences. The data are median sensitivity (dB) and delay (ms) deviations relative to normal of that age and sex at each region. The calibration bars show that the background grey in each panel represents 0 sensitivity/delay difference from normal with darker shades representing reduced sensitivity or delay, and lighter shades increased sensitivity or delay. (**A**) Sensitivity for the two central rings of stimuli declines (rings 1 and 2), especially on the treatment day. Some peripheral rings (rings 3 to 5) show hypersensitivity of 2 to 5 dB relative to normal. The word "lagged" in the left column title was the name for the pattern [0 0 0 1], implying later treatment. (**B**) Central sensitivity is consistently low, peripheral sensitivity appears higher than for the less-treated eyes, with deviations up to +9 dB. The word "All" in the column title indicates treatment on all four visits. (**C**) Peripheral delays appear to become longer in the time leading up to treatment. (**D**) Central delays are consistently shorter than normal by around 10 to 20 ms, and peripheral delays longer by up to 40 ms.

over time between treated and untreated eyes. We first examined both sensitivity and delay deviations from normal corresponding to the P131 data of Figures 4A to 4D. The results are given in Figures 5A to 5F. The largest correlations were seen between per-ring delays in the periphery (see Figs. 5D to 5F). The correlations were all positive, so when sensitivity or delay rose (or fell) in a treated eye, the untreated eye did the same. We also split the eyes between those which received more or less than the median 0.7 treatments per visit. For P131, the resulting 7 low-treatment eyes had many high correlations (see Fig. 5E), but the 7 high-treatment P131 delays also showed several significant correlations (see Fig. 5F). The pattern for ring 1 was different to the peripheral rings. Untreated ring 1 was uncorrelated with any treated eye rings (see Fig. 5E), but ring 1 of the treated eyes was frequently significantly correlated with middle rings of untreated eyes, especially for low-treatment eyes (cf. ring 1 data of Figs. 5E, 5F). The correlations for P131 sensitivity were rarely significant (see Figs. 5A to 5C).

Results for P129, whose stimuli extend to 30 degrees eccentricity (9 mm on the retina), are shown in Figures 5G to 5L. The pattern of correlations for delays

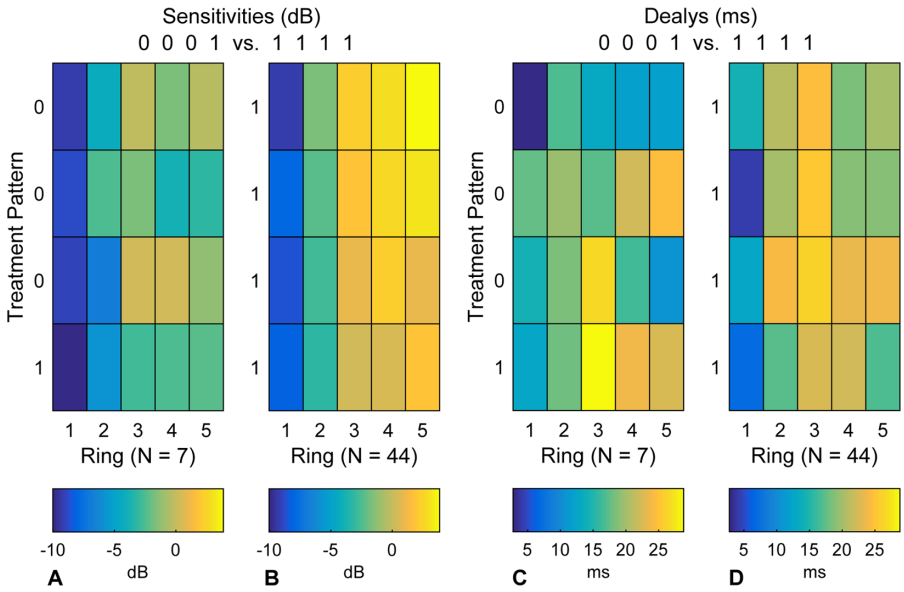

Figure 3. The ring-medians deviations for the same data as in Figure 2 above: ring 1 is the median result of the central 4 regions, ring 5 for the peripheral 12 regions, 0 dB, or ms indicating normal sensitivity or delay. (**A**) In the treatment-pattern [0 0 0 1], the sensitivity of the central regions decreased until the treatment, whereas some peripheral regions showed hypersensitivity. The slope of the sensitivity data in rings 3 to 5 was significant at -0.76 ± 0.18 dB/month, $P = 0.002$. (**B**) For the treatment pattern [1 1 1 1], the central sensitivity increased over visits but remained below normal, with peripheral regions showing consistent hypersensitivity. **C** to **D** correspond to Figures 2C and 2D.

was similar to P131, especially for eyes with lower treatment frequency (see Fig. 5K). Unlike P131, correlations between per-ring sensitivities of treated and untreated eyes were frequently significant, at least out to ring 4 (see Fig. 5H).

The results raise the possibility of correlations with treatment. We therefore examined the cross-correlation in time between treatments and responses from treated eyes. To look for treatment effects, we had to select eyes with a reasonable number of visits without treatment. By selecting eyes treated in <75% of visits, we obtained 9 eyes each with 15 months of data. Figures 6A and 6B shows the results for sensitivity and delay for treated versus untreated eyes. Across the nine data sets, the sign of the peak correlation was either positive or negative indicating that the treatment effects could be positive or negative. Figures 6A and 6B are therefore the means of the absolute values of the correlations. Figures 6C and 6D illustrate that the sign of the peak correlations was generally opposite for sensitivity and delay. The negative or positive correlations are easy to understand because a given measure can start lower than normal and then move up toward normal with treatment, or the same measure could be higher than normal and move down toward normal with treatment. Figure 6 was based upon the means of the values in the peripheral rings 3 to 5, which showed peaks at +1 to +2 months post-treatment. The peak means of central rings 1 and 2 were at 0-lag, indicating the main treatment effect was in the month of treatment, and then less later.

The Durban-Watson statistic found that most of the data sets used had significant serial correlation in Figure 6. The linear model kernels for peripheral rings had 2 significant ($P < 0.05$) months, with month +1 almost always being larger than month 0, in agreement with Figure 6, verifying that the major treatment affects for peripheral locations was a month later.

Treatment-Patterns of Subjects From Positive and Negative Subgroups

Data were segregated into positive or negative types based on the median values across rings 3 to 5 and all months for each eye (Methods). Figure 7 shows the results for sensitivity for positive and negative subgroups separately. The three examples show significant effects in the month(s) before treatment was deemed necessary.

Similarly, Figure 8 shows the peripheral delays analyzed separately for positive and negative subgroups. Again, significant pretreatment effects were seen. Perhaps most interestingly, for Figure 8A, we found that over the 3 pretreatment months,

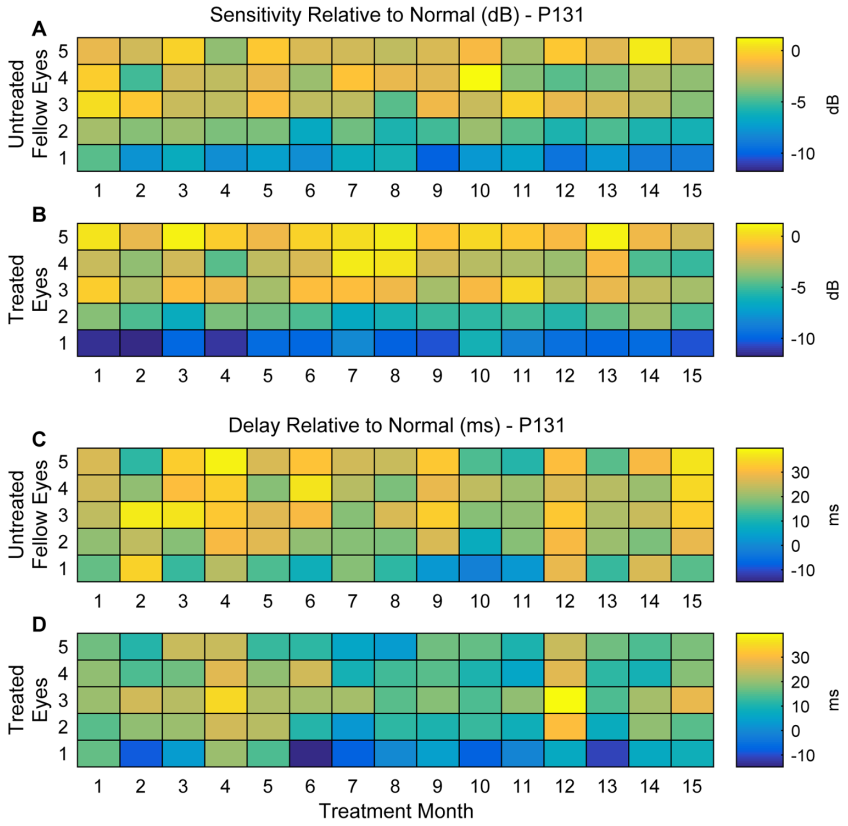

Figure 4. Ring-median data by month for 14 pairs of treated and untreated eyes assessed with the macular P131 protocol. (**A**) In the untreated eyes, the ring 1 median differences from normal sensitivity declined at -0.17 ± 0.07 dB/month ($P = 0.033$). (**B**) In the treated eyes, ring 1 sensitivity initially improved and then plateaued after about 5 to 6 months of treatment, being lower overall than untreated eyes by -2.23 ± 0.051 dB ($P < 0.0002$). (**C**) Treated eyes showed shorter than normal delays (-ve) than untreated eyes in ring 1 by 13.08 ± 3.77 ms ($P = 0.001$). (**D**) Untreated eyes rings 3 to 5 (averaged together) were also slower than normal, than by treated eyes by 7.81 ± 2.10 ms ($P = 0.001$).

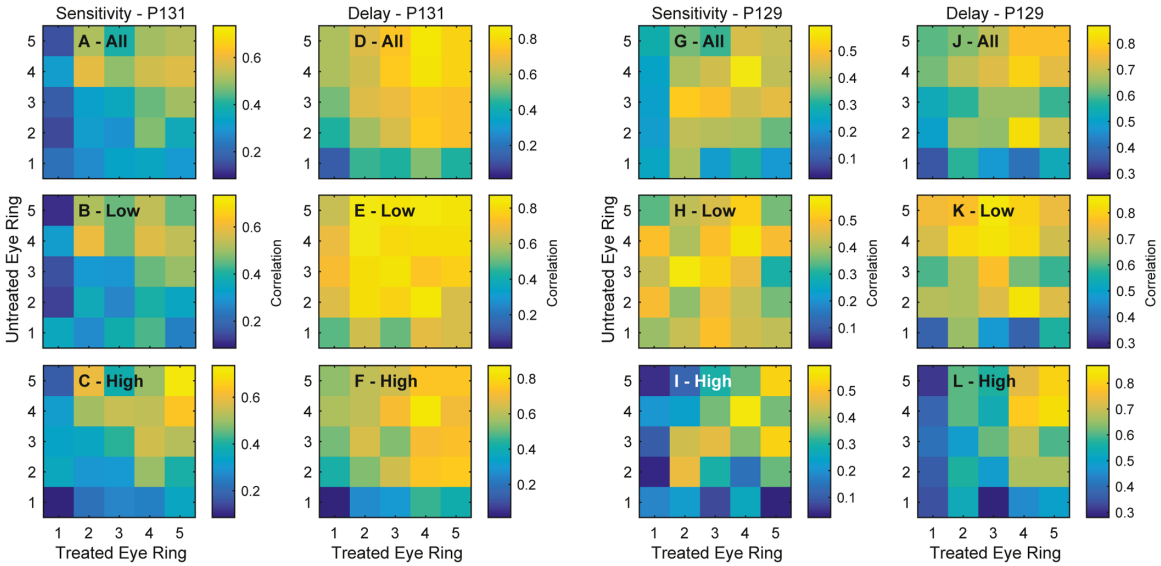

Figure 5. Correlations by ring of the results of treated versus untreated eyes for P131 (**A to F**, macular data), and P129 (**G to L**, wide-field data). Sensitivity data are shown in **A** to **C** and **G** to **I**; delays in **D** to **F** and **J** to **L**. The top row is for all 14 subjects (**A, D, G, J**); the middle row is for the seven low-treatment eyes (**B, E, H, K**); and the bottom row is for high-treatment eyes. The level of the correlation level that was significant varied a small amount across **A** to **L** but correlations above 0.55 were significant at $P < 0.05$ (i.e. *yellow-green* to *bright-yellow*).

Table 2. Linear Model Summaries

A. TreatFreq Sensitivity		Estimate	SE	t	P Value
P129	(Intercept)	0.48	0.10	4.66	0.001
	MeCentral	−0.03	0.01	−2.42	0.036
	MePeripheral	0.05	0.01	4.32	0.002
	Female	0.40	0.12	3.24	0.009
P131	(Intercept)	0.72	0.05	14.23	0.000
	MePeripheral	0.02	0.01	2.25	0.044
B. VAgain delay		Estimate	SE	t	*P* value
P131	(Intercept)	33.08	5.48	6.03	0.000
	MeCentral	1.89	0.38	5.03	0.001
	MePeripheral	−1.24	0.26	−4.83	0.001
	Female	−21.25	5.74	−3.70	0.005

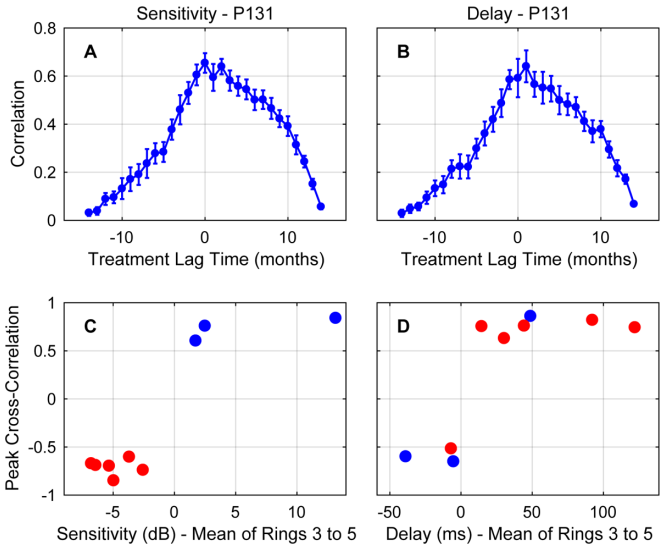

Figure 6. Cross-correlation results for P131 for the means of the values in the peripheral rings 3 to 5. Correlation with a lag of +1 or more months is likely to indicate a post-treatment effect. The data are for the 9 treated eyes treated on 70% or fewer visits for 15 months. (**A**) Sensitivity shows a secondary peak at +2 months. (**B**) Delay shows a secondary peak at +1 month. Error bars are SE. (**C**) Peak cross-correlations for sensitivity. (**D**) Peak cross-correlations for delay. Eyes where underlying per-patient curves for sensitivity had negative peaks are shown in red in both **C** and **D**. Thus, negative peak correlations for sensitivities generally corresponded to positive peak correlation for delay.

peripheral delay rose at 3.49 ± 1.75 ms/month ($P = 0.049$).

Acuity and Treatment-Frequency Effects

Treatment frequency (TreatFreq) produced significant models for P129 and P131 that included sensitivity measures (Table 2A). For P129, the largest effect was for MePeripheral (0.05 ± 0.01 treatment fraction/dB, $P < 0.002$), thus positive peripheral sensitivity increased the need for treatment. MeCentral and female subjects were also significant with positive central delays decreasing the need for treatment. The model adjusted $r^2 = 0.63$ and the model P value < 0.005. For P131, only MePeripheral was selected by the stepwise analysis (0.02 ± 0.01, $P = 0.044$).

Among the acuity measures, the largest effects were seen for VAgain (Table 2B). VAgain and Vbeg were correlated: $r = -0.58$, $P = 0.03$, implying that lower initial VAs usually produced greater improvements. Only P131 delay differences produced a significant model for VAgain, with MePeripheral giving −1.24 ± 0.26 letters/ms ($P < 0.001$). Extra peripheral delay was common (see Fig. 4) and that reduced VAgain. MeCentral delay was often negative (see Fig. 4; i.e. quicker than normal), and this increased VAgain. The model adjusted $r^2 = 0.73$ and the model P value < 0.003. Matrix MD and PSD were included (Methods) but were not selected as being determinants of TreatFreq or VAgain.

Discussion

Several publications now support the diagnostic power of mfPOP in AMD.[15,16,36] It detects ranibizumab-induced changes in nAMD,[21] and is comparable to multifocal visual evoked potentials (mfVEP),[37] Humphrey Matrix, and short-wavelength automated perimetry.[38] Clinical guidelines for the use of mfPOP following mydriasis have been provided.[20] In the current study, we evaluated whether mfPOP could detect treatment-related changes in function peripherally or centrally (see Figs. 7, 8). We also noted correlations over time between treated and untreated eyes (see Fig. 5) and correlations with treatment 1 to 2 months

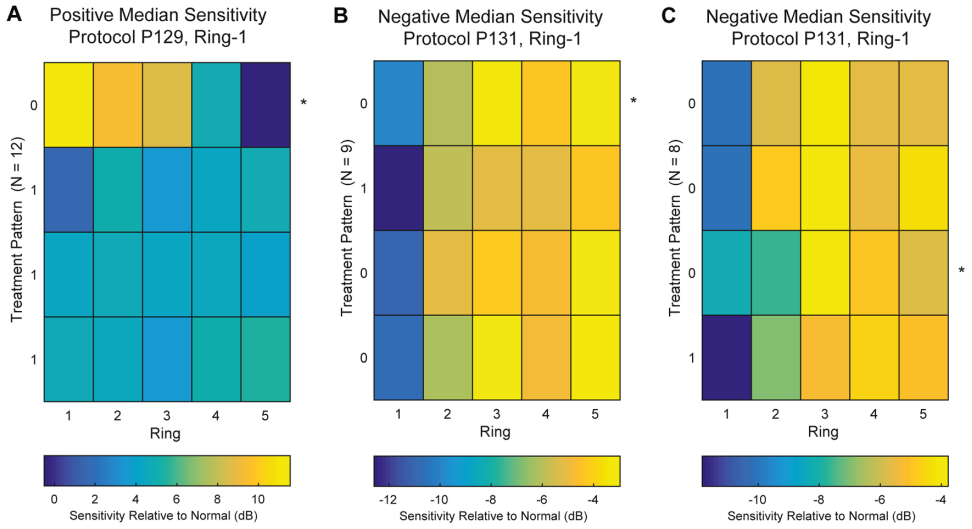

Figure 7. Sensitivity data. (**A**) Includes 12 peripherally hypersensitive (positive, see calibration bar at bottom) instances obtained with the wide-field P129 stimulus. These subjects responded relatively poorly having the treatment-pattern [0 1 1 1] for those 4 monthly epochs. The sensitivity in the first month for ring 1 is significantly higher than for the first treatment month by 9.88 ± 4.41 dB ($P = 0.042$). Thus, these 12 instances became significantly hypersensitive in the month before disease activity, potentially indicating need for treatment. (**B**) Includes 9 cases with peripheral P131 hyposensitive (negative, see calibration bar) responses for the treatment-pattern [0 1 0 0], apparent good responders. The fit shows ring 1 sensitivity of the first month was significantly higher than the treatment month by 2.69 ± 1.23 dB ($P = 0.040$). Significant hyposensitive changes occurred on the day treatment was indicated. (**C**) Includes cases with peripheral hyposensitive responses for a treatment-pattern examining the lead-up to a treatment after no treatments for 3 months: [0 0 0 1]. Here, the visits 1 and 2 months before the treatment are the fitted factors. In these eight cases, only the month before the treatment of ring 1 was significantly hypersensitive by 3.56 ± 1.61 dB ($P = 0.040$). The asterisk (*) on the right-hand side of each figure indicates significance ($P < 0.05$). Recall that the peripheral rings of P129 are at twice the eccentricity of those rings for P131.

post-treatment (see Fig. 6). The correlations with treatment highlighted that the retinas should be classified as positive or negative type according to whether their peripheral macular function was below normal or greater than normal. We had previously reported depressed central sensitivity and increased peripheral sensitivity[15,18] in nAMD and that was observed here again (see Figs. 2, 3, 4B). The idea that we should consider the less affected peripheral macular retina to understand nAMD is supported by a recent comparison of retinal thickness and Matrix 10-2 perimetry in the central and peripheral maculas.[22]

Sensitivity

The macular regions affected by the nAMD showed progressive deterioration in sensitivity, whereas the peripheral rings showed hypersensitivity, which was also reported by previous mfPOP studies.[37] Similar hypersensitive mfPOP regions peripheral to the affected retinal regions have also been reported using mfVEPs in the same subjects on the same day.[37] Standard perimeters do not flag hypersensitive regions as being abnormal, with the result that if they did occur they might be ignored. Another reason hypersensitivity may be recorded less often by standard perimetry is that hypersensitivity in perimetry is determined by recognizing tiny changes in contrast or luminance of the stimuli, whereas with mfPOP or mfVEPs, the hypersensitivity is defined by a larger than normal response obtained to a standard supra-threshold stimulus.

In eyes with peripheral hypersensitivity, even greater central hypersensitivity preceded the need for treatment in 12 cases (see Fig. 7A). By contrast, eyes with lower-than-normal peripheral sensitivity showed an increase in peripheral sensitivity before the need for treatment in nine instances (see Fig. 7B). In both groups, these were eyes that required treatment on the next three visits. In peripherally negative cases where no treatment had been given for 3 months, ring 1 became significantly hypersensitive before treatment (see Fig. 7C). Peripheral sensitivity was a significant determinant of the observed treatment frequency (see Table 2A). This seems to support earlier data[21] suggesting that those retinal regions affected by advanced AMD, or early AMD worsening to advanced AMD, show suppressed sensitivity, and that peripheral hypersensitivity is a potential biomarker in nAMD management.

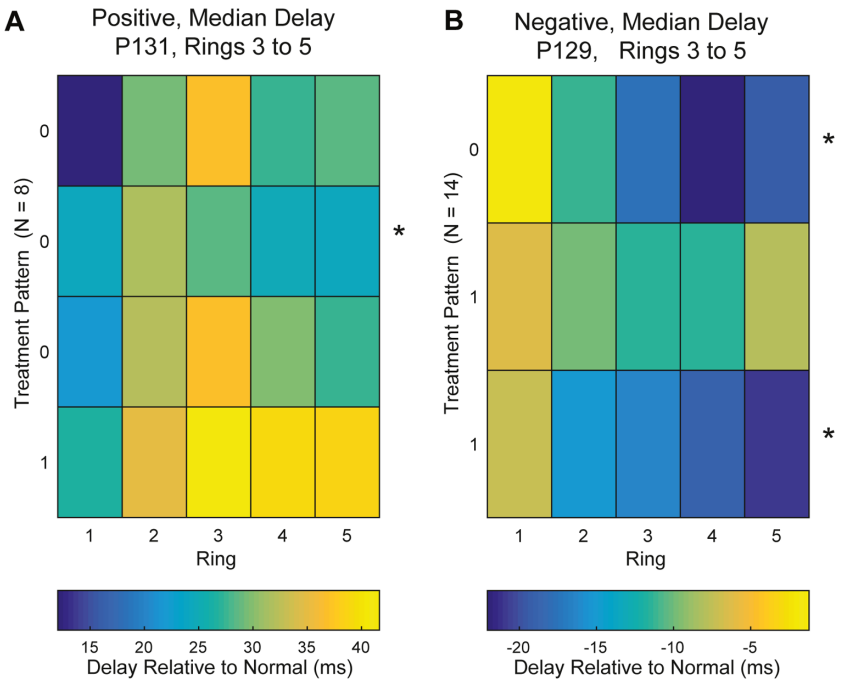

Figure 8. Time-to-peak. (**A**) includes eight treatment instances showing longer than normal peripheral delays (positive, see calibration bar) rings 3 to 5 for the same treatment-pattern as Figure 7A. The peripheral delay becomes longer as treatment day approaches, being 14.0 ± 5.23 ms quicker ($P = 0.009$; and so closer to normal) 2 months out from treatment. One month out, delays were also shorter by 8.52 ± 5.23 ms, but this was not significant ($P = 0.108$). Fitted as a slope, peripheral delay rose from month 1 at 3.49 ± 1.75 ms per month ($P = 0.049$). (**B**) Includes 14 treatment instances showing shorter than normal peripheral delays. The peripheral delays are thus negative relative to normal, see calibration bar). Here, the months before and after treatment are fitted for rings 3 to 5 of P129. Both are significantly quicker than the treatment month, which had become more delayed compared to normal. For months 1 and 3, the delays were shorter by 9.38 ± 3.59 and 8.50 ± 2.71 ms, ($P = 0.013$ and $P = 0.004$, respectively). Ring 1, month 1 responses are slower than the treatment day, but not significantly.

Among the treated eyes, sensitivity seemed to plateau after 5 to 6 months of treatment (see Fig. 4B). At the same time, sensitivity in ring 1 declined significantly in their untreated fellow eyes (see Fig. 4A). This may be due to poor ocular bio-availability of anti-VEGF in the untreated fellow eyes due to it being metabolized.[39] Interestingly, at least in less-treated eyes, there was strong correlation in fluctuations in peripheral sensitivity between treated and untreated eyes (see Fig. 5H). The peak correlations with treatment time occurred 1 to 2 months post-treatment in the peripheral 3 rings (see Fig. 6).

Sensitivity and delay behaved differently in the central and the peripheral regions. We observed that the central sensitivity among responders normalized with treatment, but among nonresponders it increased over visits but remained below normal, whereas peripheral regions showed hypersensitivity. From these findings, we infer that among the nonresponders, despite lack of clinical improvement like resorption of sub- or intra-retinal fluid or hemorrhage, or settling of a detached retinal pigment epithelium, retinal, and choroidal microvascular structure, and thus function may improve.[40] In our study, improved sensitivity may reflect reversal of the microvascular changes with anti-VEGF treatment before any structural improvements, as supported by the hemodynamic theory of AMD development.[40,41]

Delay

We observed that the central delay got progressively slower until anti-VEGF injection was needed (see Figs. 8A, 8B). This supports the previous mfPOP studies.[15,42,43] Like sensitivity, delay behaved differently in central and peripheral regions. The cross-correlations of responses flagged the possibility of two patterns of responses (see Fig. 6). In the positive type, there was an increase in peripheral delays up to 3 months before active disease was recognized (see Fig. 8A). For the negative type, peripheral delays were shorter 1 to 2 months before treatment was administered. These eyes showed increased macular delay (ring

1) in the month before treatment. Peripheral delays were related to the observed VAgain (see Table 2B).

Fellow Eye Effects

In bilateral diabetic macular oedema (DMO), intravitreal anti-VEGF injection in one eye was reported to improve macular thickness of untreated fellow eyes.[44] Similar effects have been reported in bilateral nAMD.[45] Systemic side effects and thromboembolic phenomena, such as cerebral vascular accidents or myocardial infarction, have been reported following anti-VEGF treatment.[39,46,47] It is reported that the systemic exposure following intravitreal injection was highest with bevacizumab, followed by aflibercept and least with ranibizumab.[48,49] We did not find obvious benefits in our untreated fellow eyes. Instead, their central sensitivity declined over 15 months (see Fig. 4A). The delay between the treated and the untreated fellow eyes correlated positively in rings 3 and 4, with the lowest correlation in ring 1.

Limitations of the Study

The study had only 18 subjects, however, data were collected for up to 28 visits, in which both eyes were assessed concurrently, for 2 stimulus methods. Structural changes that might influence retinal function, are not discussed here, but will be discussed in a separate article.

Conclusions

The mfPOP method detects localized functional changes at more locations than BCVA, which can remain largely unaffected in early to advanced AMD.[50] Peripheral responses suggested two categories of eyes. Positive eyes showing hypersensitivity and longer peripheral delays, and negative eyes showing hyposensitivity and shorter delays, all compared with healthy controls. Treatment appears to drive subsequent responses toward normality either from a positive or negative starting point. These changes in responses can predate treatment by 1 to 3 months. The frequency of treatment was significantly correlated with peripheral sensitivities and VAgain with peripheral delays. Matrix thresholds were not predictive. The findings indicate that mfPOP may provide potential biomarkers informing the need for anti-VEGF injection in nAMD.

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