Visual Function Tests as Potential Biomarkers in Age-Related Macular Degeneration

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PURPOSE. To evaluate the potential of psychophysical assessments of retinal function to provide diagnostic biomarkers of early age-related macular degeneration (AMD).

METHODS. Unilateral visual function was assessed in 221 participants (72.86 ± 9.94 years, 67% women) with early AMD (visual acuity better than 20/60) and 109 controls (73.07 ± 10.32 years, 65% women). Psychophysical assessment included steady state thresholds (4- and 14-Hz flicker and red and blue color) and dynamic tests (photostress recovery [PSR] and dark adaptation [DA]). All test parameters were compared in terms of their diagnostic capacity (sensitivity and specificity), reproducibility, and clinical applicability (test duration and participant’s perception of test difficulty). AMD status was determined by digital photography, according to the International Classification and Grading System.

RESULTS. All functional measurements were significantly worse, on average, in the AMD group than in the control group (P < 0.001). Static and dynamic parameters showed weak correlations (range, 0.003–0.225). Rod recovery in DA and cone recovery in PSR had the best diagnostic capacity (area under curve [AUC], receiver operating characteristic [ROC] analysis, 0.93 ± 0.016 and 0.85 ± 0.021, respectively). Considering diagnostic capacity together with test reproducibility and clinical applicability, the 14-Hz flicker gave the best outcome, followed by PSR. Combination of these two tests detected 71% of abnormal early AMD cases.

CONCLUSIONS. All the visual function tests had good diagnostic capacity. Combination of the 14-Hz flicker thresholds and dynamics of the PSR test provided optimal quantitative assessment of retinal function in early AMD, suggesting that this set is a potentially useful clinical tool for following progression of early AMD and assessing the efficacy of interventions. (Invest Ophthalmol Vis Sci. 2011;52: 9457–9469) DOI:10.1167/iovs.10-7043

The early clinical changes seen in age-related macular degeneration (AMD), such as drusen and pigmented abnormalities in the macula, are thought to be clinical markers of the risk of developing the late complications of AMD—geographic atrophy (GA) and choroidal neovascularization (CNV)—that threaten vision. Although studies have shown that the presence of these early fundus changes is a reasonably sensitive indicator of the risk of disease progression to the late stage,1,2 the specificity for the progression has been estimated to be only 55%.2 Although early AMD changes are common in persons older than 50 years and are present in approximately 15% of this population, only 1% to 2% of this age group goes on to develop GA and/or CNV.3–5 Thus, the presence of drusen and pigment alone is not sufficient to identify AMD patients who will develop the late stage of the disease. It is critical to identify, among the early disease group, those at greatest risk of developing severe visual loss. It is this group that should be targeted for inclusion in genetic and epidemiologic studies and clinical intervention trials designed to slow the progression of AMD.

With new interventions on the horizon, a significant impediment to their implementation is the lack of an outcome measure that can be monitored over time to determine a slowing in progression or reversal of the early AMD changes. Therefore, there is an urgent need for accurate markers for the various stages and forms of the disease. The main ocular structures that are involved in the early stage of AMD are the photoreceptors, RPE, Bruch’s membrane, and choriocapillaris.6–9 The integrity of these structures progressively declines, causing destruction of the RPE and photoreceptors, which results in the dry form of the disease (GA).10,11 Such evolution is currently thought to be a default pathway of AMD, whereas the wet form of the disease (CNV) is seen as the reactive outcome that targets a cluster of AMD patients who have a particular CNV genetic predisposition; CNV can occur at any stage of the default pathway.3–5 Therefore, AMD intervention should be aimed at the earliest possible stage of the disease, to prevent the development of GA and the potential for CNV. Such intervention requires diagnostic tools that can define AMD changes from as early as the preclinical stages of the disease toward the development of GA or CNV. Significant histopathologic changes occur long before the clinical manifestation of drusen and pigmentary changes are observable.7 Although assessments of the structural changes are within reach through such methods as autofluorescence imaging or OCT,12–14 these methods still lack the ability to accurately quantify the degenerative transition. Defuse deposits through Bruch’s membrane, cannot be differentiated by the imaging techniques, although they cause retinal stress and lead to functional disturbance. Hence, functional assessment of retinal changes should have a high potential in monitoring AMD and in conjunction with the imaging tech-
One common approach in assessing retinal function is measuring visual acuity (VA). Although acuity testing is simple to implement in a clinical setting, it does not give exclusive information on retinal function in early AMD when changes in VA are minimal (two letter loss, or logMAR 0.04)\textsuperscript{15} and are often within the range of variability of the test.\textsuperscript{16} VA therefore does not accurately reflect early AMD structural changes (photoreceptor, RPE, Bruch’s membrane [BM], and choriocapillary complex disintegration).\textsuperscript{6–9} As it also involves extensively higher order cortical processing,\textsuperscript{17} better targeted functional tests would allow more information to be gathered in the early disease stage and might act as more effective disease monitors, even providing further insight into disease mechanisms.

It is well recognized that function of the basic aspects of vision (color, contrast, and adaptation) decreases with age, with further losses in AMD.\textsuperscript{18–36} AMD functional abnormalities of both rods and cones have been established for static (photopic and scotopic thresholds, color, spatial contrast, and temporal sensitivity) and dynamic (dark adaptation [DA] and photostress recovery [PSR]) testing modalities.\textsuperscript{30} Given that AMD is a disease of central retina, affecting the RPE–photoreceptor complex,\textsuperscript{6–9} tests that target these anatomic structures should provide effective diagnostic specificity for this condition. Indeed, to assay the functional capacity of the central photoreceptors in AMD, cone abnormality was widely studied using steady state tests such as color thresholds and suprathreshold tests,\textsuperscript{25,57–40} spatial contrast sensitivity,\textsuperscript{39–42} and temporal sensitivity.\textsuperscript{33,34,43–44} However, in AMD, rod functional abnormalities appear to exceed cone abnormalities.\textsuperscript{35,36} These outcomes are in line with histologic findings that show reductions in the number of rods in the macular region, especially in a ring-shaped pattern 3° to 9° around the fovea.\textsuperscript{45,46} Such selective reduction in the rod population is consistent with the selective sensitivity loss in the same retinal region.\textsuperscript{51} To assay the integrity of the RPE–photoreceptor complex, photopigment regeneration is measured using dynamic functional tests such as DA and PSR.\textsuperscript{47,48} Accordingly, rod recovery on DA has been shown to be a good test for the detection of AMD\textsuperscript{27,52} as well as cone recovery, measured with either a DA\textsuperscript{27} or PSR test.\textsuperscript{54,49}

Overall, findings of previous studies indicate a broad range of functional abnormalities in eyes affected by AMD. Therefore, to ensure the detection of all functional defects due to early AMD, it is imperative to use tests that cover a wide array of functional measurements. In this study, we purposely selected a battery of psychophysical tests that we believe will capture the broad expression of AMD-related deficiencies. Tests of rod and cone adaptation were chosen to assay Bruch’s membrane-RPE-photoreceptor complex integrity.\textsuperscript{47} Photopic short- and long-wavelength color thresholds were used to assay distinct cone groupings.\textsuperscript{25,57–40} Finally, flicker thresholds were employed to consider whether retinal metabolism is compromised in early AMD.\textsuperscript{35,54,50,51} Visual function was recorded across a large cross section of AMD participants with diverse expression of early fundus changes and good VA. These results were compared with similar measurements acquired from an age- and sex-matched control group. All functional assays were contrasted in terms of their diagnostic capacity, reproducibility, and clinical applicability in identifying the most optimal test(s) with a potential to monitor visual function changes in early AMD, predict progression to the late stage, and evaluate the efficacy of an intervention.

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants after explanation of the nature of the study. Participants with early AMD were recruited from the Royal Victorian Eye and Ear Hospital clinics and from private practices. Control subjects were recruited from unrelated family members and friends of the cases.

Inclusion criteria for the study eye in AMD participants were the presence of soft (≥125 μm) or reticular drusen, with or without pigmentary changes (no late AMD),\textsuperscript{52} and VA better than 20/60. If both eyes satisfied the inclusion criteria, the eye with the better VA was designated the study eye. If the acuity was the same in both eyes, the eye with more advanced fundus changes was chosen. In cases in which the VA and the fundus status were the same, the right eye was assigned as the study eye. Clinical status of the fellow eye ranged from the presence of hard drusen as the worst fundus feature, through early AMD changes, to late stage AMD (GA or/and CNV). Visual acuity in fellow eyes ranged from 20/10 to light perception.

Exclusion criteria for all participants were significant cataract according to the Wilmer Grading System,\textsuperscript{53} glaucoma, diabetes, heterotropia, amblyopia, color blindness, high blood pressure uncontrolled by medication (systolic >150 and diastolic >90 mm Hg), and neurologic or systemic diseases, all of which could compromise vision; physical or mental impairment; medications that may affect retinal function; and inability to sign an informed consent form.

The control group was selected so that its distribution of age and sex was similar to the AMD group, being matched to cases in 5-year age groups. The difference between the average age of controls and cases for all age groups was less than 0.55 years, and the difference in proportions between the sexes for all age groups was less than 20%.

All participants underwent a standardized examination procedure. First, an interview regarding history and current symptoms of eye disease, medical history, and medication intake was conducted. This information was used to exclude cases of systemic disease and/or medication that could compromise vision, performance, or interpretation of the results. The interview was followed by best corrected VA assessment (using a 4-m logMAR chart), clinical eye examination, retinal photography, and a range of visual function assessments.

AMD Diagnosis

All participants underwent a dilated (0.5% tropicamide and 2.5% phenylephrine hydrochloride) fundoscopic examination conducted by an ophthalmologist. The examination was followed by digital fundus photography with a nonmydriatic retinal camera (CR6-45NM; Canon, Saitama, Japan). When needed, an additional examination in the retinal clinic, including fluorescein angiography, was undertaken to verify the presence of CNV. Fundus images were graded according to the International Classification and Grading System for AMD.\textsuperscript{52} The grading was conducted by two independent senior graders (KA, GM) using software from an image management system (OptoMize PRO; Digital Healthcare, Ltd., Cambridge, UK). When disagreement occurred between the two graders, the results were adjudicated by a senior retinal specialist (RG). Data on grading agreement between the two graders have been published.\textsuperscript{54}

Psychophysical Assessment of Visual Function

Vision assessment included VA, four tests of steady state thresholds to assess cone function in the central retina (isoluminant red and blue color and 4- and 14-Hz flicker tests) and two tests of dynamic measurements (PSR for cones and DA for both cones and rods). The testing procedure followed a standard protocol established before the study. All visual function tests were conducted monocularly, in the study eye, after pupil dilatation of more than 7 mm (0.5% tropicamide and 2.5% phenylephrine).
hydrochloride). Refractive correction for the test distance was worn over the study eye, and the fellow eye was patched.

A fixed sequence of testing was used, to ensure that the photopigment bleaching procedure did not compromise any subsequent measurements. Before any testing, a participant was preadapted for at least 30 minutes to ambient laboratory lighting (29.5 ± 2.7 cd m⁻²), then the steady state thresholds were determined in a fixed order (4- and 14-Hz flicker and red and blue) after a training run using the red threshold procedure. Duration of the steady state thresholds assessment ranged between 10 and 25 minutes. These were followed by DA which involved 30% of photopigment bleaching and 30 minutes of adaptation time. Finally, the PSR test with >98% pigment bleaching was conducted. The total duration of all psychophysical measurements including preadaptation time, test explanation, and training, and the two photopigment bleaching procedures (DA and PSR) took between 1.5 and 2.0 hours (see test duration component in Table 5A).

Most of the participants were tested in one session (96%); those requiring a second session returned within 1 to 9 days. Before the second session, the participant had standard pupil dilation (described above) and was preadapted for 30 minutes to ambient laboratory lighting (described above).

To ensure the accuracy of the psychophysical measurements, the testing procedure followed an automated computer-controlled protocol administered for all tests by the same three experienced operators using a fixed set of instructions and demonstrations. The measure-
mments of all tests were quality controlled by establishing the interexaminer reproducibility on groups of participants (n = 24, steady state thresholds; n = 21, DA and n = 22, PSR) who repeated the tests with another examiner after a break (1.5 hours to 2 days) (interclass correlation coefficient [ICC], 0.89–0.91).

Stimuli for all tests were generated on a calibrated high-resolution CRT monitor, as detailed previously. Color, flicker, and photostress stimuli were displayed on a 30 cm · m⁻² mean background. Color stimuli were foveal, red and blue equiluminous 2° diameter Gaussian blobs specified in the MacLeod-Boynton color space diagram (red, x = 0.49, y = 0.27; blue, x = 0.25, y = 0.12). Flicker thresholds were measured for foveal, 2° diameter Gaussian blobs at two temporal frequencies: 4 and 14 Hz. These frequencies were chosen, as they had been shown to be abnormal in early AMD. The stimulus was generated with a sinu- soid that had a time-averaged luminance of 30 cd m⁻² and a maximum Michelson contrast of 0.94. A staircase (4 dB up/2 dB down) was used to return threshold.

PSR was measured with a modified technique proposed by Phipps et al. The testing began by determining the average threshold of four measurements (2 dB up and 2 dB down method of limits) for a foveal 2° spot flickering at 5 Hz in the study eye. The eye was then exposed to a light source (12 × 10⁶ cd · m⁻²) for 45 seconds duration that bleached >95% of the photopigment, and recovery was tracked immediately after the light was turned off. Recovery time was recorded for five diminishing contrast levels that were above the subject’s threshold, estimated before bleaching (prebleach threshold [PT], ×3.5, ×3.0, ×2.5, ×2.0, and ×1.5; Fig. 1). The dynamics of the recovery were modeled by a single exponential decay, reported else-
where, and RT (recovery time to prebleach level) was solved by transposition of equation A5 of Dimitrov et al. The test returned three parameters: PT, recovery rate (RR), and an estimate of RT to prebleach level.

DA was measured by using methods described in Dimitrov et al. at three different locations: the fovea (stimulus size 4°) and two peripheral retinal locations, 3.5° and 10°, in the inferior retina along the vertical meridian (stimulus size 2°). Foveal DA was measured to provide direct comparison with color and flicker thresholds, and PSR, all of which were assessed at the same retinal location (foveal stimu-
lus). The 3.5° eccentric location was chosen to test an area that is most affected early in AMD, whereas the 10° location was chosen as a less affected region. Measurement of the DA dynamics commenced instantly after a 30% bleaching of the pigment. Recovery thresholds were measured for 0.2-second achromatic (1931 CIE; x = 0.267, y = 0.318) spots (4° foveal, two 2° peripheral at 3.5°, and 10° along the inferior meridian) randomly presented on a high-resolution, calibrated CRT monitor. A 1.0-second response window was followed by a 1.0-second interstimulus delay. The observers signaled stimulus detection with a response button. Recovery dynamics were modeled with a single exponential decay separately for the cone and rod components, described elsewhere. DA returned six parameters: cone RR (log₁₀ µm min⁻¹), cone absolute threshold (AT) (log₁₀ cd · m⁻²), rod RR (log₁₀ µm min⁻¹), rod AT (log₁₀ cd · m⁻²), the rod-cone break (minutes), and a rod criterion time (minutes). The rod criterion time was defined as the time point when rod recovery passed through a standardized sensitivity criterion designated at −2.2 log cd · m⁻² (Fig. 2), which was two standard deviations below the average cone AT in the control group. This parameter was employed as a faster method of evaluating the rate-limited second phase of rod recovery at its initial part (Fig. 2). Although the rod-cone break indicates the initial part of the second phase of the rod recovery too, this criterion depends on the level of cone AT (less sensitive cones will lift the plateau of the AT up, which would shorten the time of the rod-cone break, Fig. 2). As the rod-cone break time and rod RR have been shown to be good indicators of AMD functional abnormality, the assessment of the recovery time to a fixed rod recovery criterion could provide a faster and more accurate measurement.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933457/)

**Figure 1.** PSR for a patient with AMD. Recovery was established as the time needed to return to five fixed levels above PT (diamonds). The curve has been optimized to the data using a single exponential decay, as detailed elsewhere. The RT (downward arrow) represents the mathematical solution for this parameter derived from the exponential equation.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933457/)
Statistical Analyses

Group comparisons between AMD and age- and sex-matched control subjects were conducted with either the unpaired t-test for normally distributed data or with the nonparametric Mann-Whitney test, where the data were not normally distributed. The nominal level of statistical significance was set at $\alpha = 0.05$. No correction for multiple testing was applied.

The reproducibility of each of the psychophysical measurements was established from repeated testing, undertaken 1 to 6 weeks apart, and determination of the ICC. The ICC ranges between 1 and 0, where 1 indicates perfect reproducibility and 0 signifies that the reproducibility is no better than expected by chance. It has been suggested that a clinically reliable test should have an ICC of $>0.70$. Therefore, we used that value as the criterion that defines a reliable test.

The diagnostic value of each test was evaluated by the AUC of a receiver operating characteristics (ROC) analysis as a plot of the sensitivity for AMD visual function abnormality against the false-positive rate (1 − specificity). The number of abnormal cases was also considered for a fixed specificity of 97.5%, to allow a clinically meaningful comparison among the tests. We defined a test as valid when the AUC of the ROC is $>0.70$.

Correlations between the measured parameters were determined by using the Pearson coefficient returned from standardized values. The standardized deviate (z-score) was calculated for each AMD observer relative to the average value in the control group and expressed as a multiple of the spread in the control group distribution (SD). This normalization was undertaken to remove the influence of scale and range differences among the tests. To describe the strength of correlation, we used the Pearson correlation coefficient ($r$) as follows: nearly perfect, $>0.9$ or $<−0.9$; very strong, $0.9$ to $>0.7$ or $<−0.7$ to $−0.9$; strong, $0.7$ to $>0.5$ or $<−0.5$ to $−0.7$; moderate, $0.5$ to $>0.3$ or $<−0.5$ to $−0.7$; weak, $0.3$ to $>0.1$ or $<−0.1$ to $−0.3$; or trivial, $0.1$ to $>0$ or $<0$ to $−0.1$.

All 13 visual function test parameters were compared in terms of their diagnostic capacity and clinical applicability. For this purpose, we considered four test attributes: AUC in the ROC, number of abnormal cases for a specificity of 97.5%, test reproducibility (ICC), test time (minutes), and test difficulty. The test difficulty was measured on a scale of 0 to 10. A participant was asked to determine a score for each test according to his or her subjective impression of the testing procedure.

An interval scale was created to establish relative ranking between the test parameters. We assumed linearity and equal weighting for all test characteristics, with our purpose being test comparison, not quantification. For this purpose, we developed an interval scale that ranged linear from the span of the best to worst value: Given $(x_1, y_1) =$ minimum and $(x_2, y_2) =$ maximum, $y = mx + c$ can be determined; see the Results section for an example. The diagnostic characteristics were averaged to return the best diagnostic score for each test, and this was added to the scores of test variability, duration, and difficulty to yield a relative score indicating the preferred clinical test(s) for assessing visual function in AMD.

### Diagnostic Validity of the Visual Function Tests

#### Steady State Thresholds.

Color (red and blue) and flicker (4 and 14 Hz) thresholds were measured in 221 AMD participants and 109 age-matched controls (Table 2, Fig. 3). Group averages (±SD) across the four tests were significantly higher in the AMD group when compared with those in age-matched controls: red threshold ($0.026 \pm 0.033$ vs. $0.011 \pm 0.004$; $P < 0.001$), blue threshold ($0.270 \pm 0.240$ vs. $0.079 \pm 0.036$; $P < 0.001$), 4-Hz flicker ($0.037 \pm 0.067$ vs. $0.014 \pm 0.005$; $P = 0.001$), and 14-Hz flicker ($0.050 \pm 0.075$ vs. $0.018 \pm 0.007$; $P < 0.001$), respectively.

Although ROC analyses (Table 2) revealed that the AUC (±SE) for the blue threshold was greater than for the red, the difference was not statistically significant ($0.80 \pm 0.020$ vs. $0.77 \pm 0.026$, respectively $z = 1.61; P = 0.055$). Neither was there a significant difference between the two flicker tests ($0.026$ vs. $0.84 \pm 0.021$, and $4$ Hz, $0.82 \pm 0.023$; $z = 1.56; P = 0.059$). However, the 14-Hz flicker threshold yielded the highest AUC (±SE) value ($0.84 \pm 0.021$) among the four steady state tests (Table 2). The red threshold test had the weakest diagnostic capacity, with an AUC ± SE, $0.77 \pm 0.026$, and only 27% of cases were abnormal on this test (Table 2, Fig. 7). Comparison of the lowest AUC ± SE for the red threshold with the highest AUC for the 14-Hz flicker revealed a statistically significant difference in their diagnostic capacity (AUC ± SE, $0.77 \pm 0.026$ vs. $0.84 \pm 0.021$, respectively; $z = 4.07; P < 0.001$). The highest number of abnormal cases was detected with the blue threshold (41%; Table 2, Fig. 7) followed by the flicker thresholds (14 Hz, 34% and 4 Hz, 33%; Table 2, Fig. 7). The blue threshold had a significantly lower AUC than that of the 14-Hz flicker ($0.80 \pm 0.020$ vs. $0.84 \pm 0.021$, respectively; $z = 2.23; P = 0.013$), indicating a higher false-positive rate.

#### Photostress Recovery.

PSR, an estimate of cone adaptation (Fig. 1), showed significant abnormalities in the AMD participants ($n = 221$) when compared with age- and sex-matched control subjects ($n = 109$) across all three test components (PT, $0.016 \pm 0.011$ vs. $0.011 \pm 0.005$, respectively,

### Table 1. Fundus Status in AMD Participants

<table>
<thead>
<tr>
<th>Study Eye</th>
<th>Fellow Eye</th>
<th>Participants</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Any soft drusen (no pigmentary changes)</td>
<td>Any soft drusen with or without pigmentary changes</td>
<td>58</td>
</tr>
<tr>
<td>Any soft drusen and pigmentary changes</td>
<td>Any soft drusen with or without pigmentary changes</td>
<td>71</td>
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<tr>
<td>Reticular drusen with or without intermediate drusen</td>
<td>CNV</td>
<td>8</td>
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<tr>
<td>Any soft drusen (no pigmentary changes)</td>
<td>GA and/or CNV</td>
<td>13</td>
</tr>
<tr>
<td>Any soft drusen and pigmentary changes</td>
<td>GA and/or CNV</td>
<td>71</td>
</tr>
</tbody>
</table>
P \leq 0.001; RR, 0.010 ± 0.011 vs. 0.032 ± 0.013, P < 0.001; and RT, 25.98 ± 23.82 vs. 5.47 ± 4.99 P < 0.001; Table 2, Fig. 4). The two dynamic parameters (RR and RT) had similar diagnostic capacity, as is indicated by the comparable AUC values (RR, 0.83 ± 0.022 vs. RT, 0.85 ± 0.021, z = 1.09; P = 0.137; Table 2) and the number of abnormal cases (RR 63% and RT 64%; Table 2, Fig. 7). These parameters were also 2.4 times better at detecting abnormal cases than was the steady state PT (28% of abnormal cases; Table 2, Fig. 7).

Although the diagnostic capacity of the PT (the steady state parameter of PSR) was high, it was significantly lower than that for both dynamic parameters (PT, 0.74 ± 0.026 vs. RR, 0.83 ± 0.022, z = 2.23; P = 0.012; and PT, 0.74 ± 0.026 vs. RT, 0.85 ± 0.021, z = 2.65; P = 0.004). The PT is a steady state temporal contrast threshold for a 5-Hz 2° square-wave spot. As such, it may be expected to return outcomes similar to the other flicker thresholds (4 and 14 Hz sine wave blob). However, comparison of the diagnostic values for the PT and 4- and 14-Hz flicker (Table 2) reveals that the latter two had better diagnostic capacity (PT, 0.74 ± 0.026 vs. 4 Hz flicker, 0.82 ± 0.023, z = 2.27, P = 0.012; and PT, 0.74 ± 0.026 vs. 14-Hz flicker, 0.84 ± 0.021, z = 2.96; P = 0.002) possibly reflecting their better methodology. Accordingly, the PT gave a lower proportion of abnormal cases (PT, 27%; 4-Hz flicker, 33%; and 14-Hz flicker, 34%).

**Dark Adaptation.** Table 3 compares the diagnostic capacity of DA at three retinal locations. The AMD group showed significantly abnormal results across all DA parameters when their average measurements were compared to age- and sex-matched control subjects (P < 0.001; Table 3). These abnormalities were of high diagnostic

### TABLE 2. Diagnostic Capacity for Color, Flicker, and Photostress Measurements

<table>
<thead>
<tr>
<th>Visual Function Tests</th>
<th>Psychophysical Measurement</th>
<th>ROC Analyses</th>
<th>Abnormal Cases</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control Group</td>
<td>AMD Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average ±SD</td>
<td>Average ±SD</td>
<td>AUC ±SE n %</td>
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<td><strong>Color Thresholds</strong></td>
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</tr>
<tr>
<td>Red, contrast</td>
<td>0.011 0.004</td>
<td>0.026 0.043</td>
<td>0.77 0.026 60 27</td>
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<tr>
<td>Blue, contrast</td>
<td>0.079 0.036</td>
<td>0.270 0.240</td>
<td>0.80 0.020 91 41</td>
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<tr>
<td><strong>Flicker Thresholds</strong></td>
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<td></td>
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<tr>
<td>4-Hz flicker, contrast</td>
<td>0.014 0.005</td>
<td>0.037 0.067</td>
<td>0.82 0.025 73 33</td>
</tr>
<tr>
<td>14-Hz flicker, contrast</td>
<td>0.018 0.007</td>
<td>0.050 0.075</td>
<td>0.84 0.021 75 34</td>
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<tr>
<td><strong>PSR</strong></td>
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<tr>
<td>Prebleach threshold, contrast</td>
<td>0.011 0.005</td>
<td>0.016 0.011</td>
<td>0.74 0.026 62 28</td>
</tr>
<tr>
<td>Recovery rate, ( \log_{10} s^{-1} )</td>
<td>0.032 0.013</td>
<td>0.010 0.011</td>
<td>0.85 0.022 117 63</td>
</tr>
<tr>
<td>Recovery time, min</td>
<td>5.47 4.99</td>
<td>25.98 23.82</td>
<td>0.85 0.021 119 64</td>
</tr>
</tbody>
</table>

All parameters were significantly abnormal in AMD group (n = 221) when compared with the control group (n = 109, P < 0.001).

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933457/) Comparison of steady state thresholds in the AMD group (n = 221) and age-matched controls (n = 109). Horizontal borders of the box indicate the upper and lower quartiles of the data, the *horizontal line* in the box is the median value and the *horizontal lines* at the ends of the whiskers are maximum and minimum values.
capacity as evident from the AUC (±SE) values provided alongside the group comparisons (Table 3).

The AUC values were estimated for the DA parameters that we were able to quantify with our DA technique, as some profoundly abnormal patients returned values outside the DA test criteria (range of the luminance output and/or 30-minute testing time frame) and although they were detected to be abnormal, we could not obtain a quantitative measurement for them. Figure 5 shows examples of DA in representative AMD participants. Proportions of patients who returned enough data to yield a sufficient value for the measured parameter are indicated in Table 3 (column “Assessed”). In our 221 AMD participants only the cone AT was detectable in all participants at all retinal locations.

Although cone recovery was quantified in most of the AMD cases across all three retinal locations (foveal DA, 96%; 3.5° DA, 95%; and 10° DA, 92%; Table 3), rod recovery was able to be quantified in only 69% of AMD cases in the foveal location compared with 93% at 3.5° and 96% at 10°. However, rod RR at 10°, a common region for DA assessment and the location of the highest rod population, returned the lowest diagnostic value, which was significantly lower than the 3.5° DA (AUC; 10° DA, 0.89 ± 0.021 vs. 3.5° DA, 0.93 ± 0.016; z = 1.82; P = 0.034) but not significantly different from the foveal DA (0.91 ± 0.018; z = 0.94; P = 0.174). Cone RR also had the lowest AUC at this location (Table 3) which was significantly lower than for foveal DA (10° DA, 0.69 ± 0.026 vs. foveal DA, 0.95 ± 0.013; z = 8.03; P < 0.001) and for the 3.5° DA (10° DA, 0.69 ± 0.026 vs. 3.5° DA, 0.86 ± 0.023; z = 4.29; P < 0.001). Given that we were able to quantify rod RR at 3.5° eccentricity in a higher number of participants than in the fovea (93% vs. 69%), with similar diagnostic capacity for both locations (3.5° DA 0.93 ± 0.016 vs. fovea 0.91 ± 0.018; z = 1.21; P = 0.114), and that the cone recovery at 3.5° was of a high AUC value (AUC ± SE, 0.86 ± 0.023), the 3.5° eccentricity most likely represents the optimal location for adaptation testing (Fig. 6). Therefore, in this study, only the outcomes of the 3.5° DA location were used in further analyses.

Figure 7 compares the proportion of abnormal cases identified with each of the 3.5° DA parameters and the other tests when using a criterion that fails in 2.5% of normal cases (diagnostic capacity at the fixed specificity, 97.5%). It is evident that all dynamic parameters in DA have greater capacity to detect functional abnormality (RR for cones, 62%; rods, 86%) than do static parameters (AT for cones, 32% and for rods, 41%).

Furthermore, rod-specific tests exhibited greater functional loss than did cone outcomes. Rod RR and rod criterion time returned similar diagnostic capacity (86% and 87% of abnormal cases), which is not surprising, as both reflect regeneration of rhodopsin and are thereby co-dependent variables. The rod–cone break was slightly weaker (82% of abnormal cases) than the previous two (86% and 87%), possibly due to the dependence of this parameter on two different aspects of the DA—the AT of the cones and RR of the rods (Fig. 2). Cone RR in DA had a number of abnormal cases similar to that of the PSR (62% and 63%, Fig. 7), meaning that only one of these parameters would need to be assessed to yield a high diagnostic performance.

**Relationships between Test Parameters**

Table 4 shows the correlations between parameters determined from standardized values (see the Methods section). Correlations between static parameters, such as flicker and color thresholds were in the strong to very strong range as were correlations between dynamic parameters (Table 4). However, correlations between static and dynamic parameters were only in the moderate-to-trivial range (Table 4).

The strong correlation between the rod–cone break and the rod RR (−0.51; P < 0.001) indicated that the rod–cone break was determined by rod decay; however, this correlation was not as strong as between the rod RR and the rod criterion time (0.90; P < 0.001), suggesting that the rod–cone break depends on other factors as well. Indeed, the small but significant correlation between the rod–cone break and cone AT (−0.21; P < 0.001) indicates co-dependency between these parameters, such that an elevated cone AT would shorten the rod–cone break (Fig. 2).

Of note, cone recovery in the DA and PSR tests showed only a moderate correlation (0.28; P = 0.008). This could be due to the difference in the stimuli (flickering 2° foveal spot in PSR versus static, 2° spot at 3.5° inferior retina in DA) and/or the amount of pigment bleaching (30% vs. >95%).

Our visual function tests and VA gave trivial-to-moderate correlations. The correlations for steady state parameters (red and blue thresholds and both 4 and 14 Hz measures) were significant, consistent with the common cone substrate. Nevertheless, the low correlations suggest that these tests reflect components of dysfunction different from VA testing. Furthermore, the higher diagnostic capacity of all four steady state tests (Fig. 7) compared with the acuity test makes them better measures of visual function for AMD trials. As expected, the correlations of dynamic tests with acuity were not statistically significant.

**Comparison of Vision Function Parameters**

Table 5 lists various characteristics of the test parameters that allow comparison of them in terms of three main testing merits: diagnostic capacity (AUC of the ROC analyses and proportion of abnormal cases), reproducibility (ICC), and clinical ease of use (test duration and difficulty). Sorting the test parameters according to the rank scores (determined as detailed in the Methods section) revealed that all dynamic param-
TABLE 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AMD</th>
<th>ROC Assessed†</th>
<th>AUC ± SE</th>
<th>Group Average</th>
<th>SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone recovery rate, min−1</td>
<td>3.05 ± 0.70</td>
<td>1.12 ± 0.48</td>
<td>0.95 ± 0.13</td>
<td>66</td>
<td>1.34 ± 0.54</td>
<td>96</td>
</tr>
<tr>
<td>Cone absolute threshold, cd</td>
<td>20.4 ± 5.9</td>
<td>8.3 ± 2.1</td>
<td>6.0 ± 1.0</td>
<td>69</td>
<td>9.4 ± 3.2</td>
<td>98</td>
</tr>
<tr>
<td>Rod recovery rate, min−1</td>
<td>0.02 ± 0.01</td>
<td>0.15 ± 0.03</td>
<td>0.38 ± 0.06</td>
<td>69</td>
<td>0.25 ± 0.04</td>
<td>95</td>
</tr>
<tr>
<td>Rod absolute threshold, cd</td>
<td>16.4 ± 4.1</td>
<td>7.6 ± 1.8</td>
<td>5.8 ± 1.2</td>
<td>69</td>
<td>1.6 ± 0.4</td>
<td>95</td>
</tr>
<tr>
<td>Rod criterion time, min</td>
<td>3.65 ± 0.76</td>
<td>10.01 ± 0.4</td>
<td>3.14 ± 0.71</td>
<td>69</td>
<td>5.7 ± 0.74</td>
<td>73</td>
</tr>
</tbody>
</table>

* All DA parameters in AMD group (n = 221) were significantly abnormal when compared to age- and sex-matched control group (n = 1009, P < 0.001).

† Percentage of cases in which measured value was obtained.

DISCUSSION

Clinicians and researchers need a sensitive, specific, and clinically applicable tool to evaluate functional change in early AMD to monitor progression toward late complications, such as CNV or GA and to evaluate efficacy of novel treatments aimed at slowing this process. This treatment is especially important, as an intervention for the early stage of AMD is currently under extensive investigation. The progressive decline in ocular structures that are involved in the disease process (photoreceptors, RPE, Bruch’s membrane, and choriocapillaries) reduce the functional capacity of the retina, which in turn make functional measurements effective markers to track changes in AMD along the default pathway of the disease from early preclinical changes toward the degenerative stage of loss. The visual function tests used in our study showed good capacity to quantify functional abnormalities in the eyes affected by early AMD: AUC of the ROC (±SE) ranged from 0.74 ± 0.026 to 0.95 ± 0.013 (Table 5A) and the detection rate for abnormal cases ranged from 27% (red color threshold) to 87% (rod RR, Fig. 7). These significant functional abnormalities were present despite the majority of participants having age-matched normal VA (7% had VA outside the 95% CI, but not worse than 20/60, or logMAR 0.5). The differences in the diagnostic capacity between VA and all other functional tests, as well as low correlations between them (Table 4), suggest that they depend on discrete mechanisms of visual perception that are affected to different degrees in early AMD. Indeed, VA is a complex component of vision that depends on multifaceted retinal–central processing of a visual stimulus, whereas testing more basic aspects of vision, such as color perception, contrast sensitivity, and visual adaptation, is more likely to reflect an alteration in mechanisms affected in early AMD, such as those reliant on the normal photoreceptor/RPE/BM/choroid complex.

VA is a poor assay of early AMD, with the Beaver Dam Eye Study showing only a two-letter VA loss in early AMD that can be masked by considerable variability of acuity testing of between one and two lines (logMAR, 0.1–0.2). Monitoring parameters such as those offered in our functional suite compares favorably to VA and is well supported by previous reports. Yet currently, VA is still the most commonly used test of visual function by clinicians and researchers. It is essential to find a visual function test or combination of tests that could be introduced to a clinical setting and that are easy, reliable, repeatable, and relatively quick to perform.

A Functional Test for AMD

Although our dynamic parameters (rod and cone RRs) had good diagnostic capacity (DA rod RR, 87% and PSR cone RR, 64% of abnormal cases, Fig. 7), our steady state thresholds returned more modest detection rates of 27% (red) to 41%
There was also poor correlation between these two groups of tests ranging from moderate to trivial (Table 4). These differences imply that these two groups target different mechanisms. Indeed dynamic tests reflect regeneration of photopigment in the photoreceptors, whereas steady state thresholds for color and flickering stimuli assay the functional capacity of the photoreceptors and postreceptoral elements.4,3,4,65

Steady State Thresholds

We found that more AMD participants had abnormalities for blue (41%) color sensitivity than for red (27%). Although some reports show color deficiency in all three types of photoreceptors (red, green, and blue) in AMD, most find a preponderance of tritan (blue-yellow) defects, supporting our finding. Even though the blue stimulus had the highest detection rates of all steady state thresholds and had good reproducibility, its specificity was only moderate (Table 5A). This finding may reflect other abnormalities such as subtle lens opacification or diseases of the optic nerve. Although we excluded obvious changes in the lens and optic nerve, there are likely to be subtle defects in any elderly population, making a test reliant on detecting a blue threshold undesirable for clinical applications.

Both flicker thresholds (4 and 14 Hz) had a similar capacity for detecting abnormal cases of AMD (33% and 34%, respectively) and diagnosing the presence of the disease (AUC, 0.82 ± 0.023 vs. 0.84 ± 0.021, respectively, z = 1.56; P = 0.059). Similar findings have been reported in smaller clinical groups. Increasing frequency of flicker is thought to lead to an increase in the metabolic demand of retinal tissues, producing a compensatory retinal vasodilatation, to increased local blood flow and oxygen tension. In eyes with AMD these compensatory mechanisms may be stretched so that further compensation cannot occur, allowing flicker to uncover functional deficiencies. The higher diagnostic capacity for both flicker rates (4 Hz, 0.82 ± 0.023 and 14 Hz, 0.84 ± 0.021) than for both colors (blue AUC, 0.80 ± 0.020, and red AUC, 0.80 ± 0.020.)

![Figure 5](https://iovs.arvojournals.org/figure-access-ashx?url=/data/journals/iovs/933457/)

**Figure 5.** Example of DA curves in AMD participants compared with a model (gray line) for an age- and sex-matched control subject. (A) The recovery of a patient who demonstrated all the phases of DA. This case reveals slowed rod and cone recovery, elevated AT for both rods and cones and a delayed rod–cone break. (B) A case in which the second phase of rod recovery is too slow for the rod–cone break to be achieved within the 30-minute test time. Therefore, the rod parameters (RR, AT and rod–cone break) could not be quantified. (C) The cone AT is elevated to an extreme height, and the cone decay is therefore outside the luminance range of the DA stimulus. Slow recovery of rods in this case exposes only the early portion of the second phase of RR, missing the rod AT.

![Figure 6](https://iovs.arvojournals.org/figure-access-ashx?url=/data/journals/iovs/933457/)

**Figure 6.** Comparison of DA parameters (2° achromatic stimulus at 3.5° inferior retina) in the AMD group (n = 221) and age-matched controls (n = 109). Horizontal borders of the box indicate the upper and lower quartiles of the data, the horizontal line in the box is the median value and the horizontal lines at the ends of the whiskers are maximum and minimum values.
is consistent with the concept that the higher retinal metabolic demand induced by the flickering stimulus uncovers a functional defect before the static color thresholds. Thus, the 14-Hz flicker frequency may provide the optimal stimulus for AMD detection and tracking of progression.

**Dynamic Tests**

Recovery dynamics in both DA and PSR have been reported as abnormal in AMD participants.2,24,25,32,34,49,68–73 We found that rod RR was the most abnormal parameter among all dynamic tests (Fig. 7). This result is consistent with those of other reports.24,25,32,68 Of the three retinal locations assessed in the DA test, the 3.5° DA was the best to measure functional abnormalities in rods (Table 3), yielding good diagnostic capacity (AUC, 0.93 ± 0.016 and 86% of all AMD cases returning abnormal results). Although cone parameters were well assessed across all three DA eccentricities, there was a progressive decrease in the detection rate from the fovea toward the periphery (foveal DA, 3.5° DA, and 10° DA; Table 3), most likely due to a decrease in the cone population with eccentricity. Although the cone RR was significantly compromised in DA measures, it also was considerably abnormal in the PSR. The proportion of abnormal AMD cases was similar in both tests (62% in 3.5° DA and 63% in PSR) as were the results of ROC analyses (DA AUC, 0.86 ± 0.023, and PSR AUC, 0.83 ± 0.022; z = 1.11; P = 0.134). Therefore both DA and PSR were equally effective as assays of cone deficiency in early AMD. However, the correlation between these two measurements was only moderate (r = −0.28; P = 0.008) indicating that the outcomes of the two tests may have been influenced by either their testing paradigms or different mechanisms being isolated. Indeed, the level of bleach was significantly different between the tests (30% vs. >95%). Also the contrast modulating stimulus of the PSR reflects both receptoral and postreceptoral components of visual processing, whereas the luminous (static) spot used for DA biases toward receptoral elements alone.54–56,43,44 Therefore, although in our AMD cohort both tests exhibited similar diagnostic capacities, differences in tests should be considered when evaluating retinal function.

Consecutive application of two photopigment bleaching procedures in DA and PRS could potentially produce a carryover effect from the first bleach (DA 30% photopigment deactivation), which would influence the outcomes of the subsequent PSR test. This effect would presumably be more pronounced in at-risk retina in individuals with AMD, in whom the postbleach recovery is considerably delayed for both rods27,32,68 and cones.27,68,69 Although in this study DA cone recovery was delayed in 62% of the AMD participants (Fig. 7), the maximum time that was needed for the cones to reach their absolute threshold was below 14 minutes, which is well within 30 minutes of the DA test duration (examples are shown in Fig. 5). Accordingly, as is evident from Table 3 (column “Assessed”), all participants reached full cone recovery during the DA test for all test locations. Therefore, since the PSR test is an assay for cone dynamics only, we believe that the measurements of this test would not be affected by the bleaching in the preceding DA test.

**Test Selection**

In assessing visual function in eyes with early AMD, dynamic parameters revealed more functionally abnormal cases than did steady state thresholds, with rod rather than cone outcomes being more often abnormal (Fig. 7). Rod RR in DA at 3.5° eccentricity was the best indicator of functional loss, detecting up to 87% of abnormal cases (Fig. 7). However, testing dynamics of rod function is an extremely arduous process, with an average difficulty score of 9.7 (of a highest possible score of 10) and 8% of AMD cases taking longer than 30 minutes to complete.
## Table 4. Correlation between Visual Function Measurements in AMD Participants

<table>
<thead>
<tr>
<th></th>
<th>Steady State Thresholds</th>
<th>Photo-Stress Recovery</th>
<th>Dark Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flicker</td>
<td>Color</td>
<td>Cone</td>
</tr>
<tr>
<td></td>
<td>4 Hz</td>
<td>14 Hz</td>
<td>PT</td>
</tr>
<tr>
<td>Flicker, 14 Hz</td>
<td>.834*</td>
<td></td>
<td>.745*</td>
</tr>
<tr>
<td>Threshold, red</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.566*</td>
</tr>
<tr>
<td>Threshold, blue</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.647*</td>
</tr>
<tr>
<td>PSR PT</td>
<td>.647*</td>
<td></td>
<td>.752*</td>
</tr>
<tr>
<td>PSR rate</td>
<td>.003</td>
<td>.032</td>
<td>.028</td>
</tr>
<tr>
<td>PSR RT</td>
<td>0.968</td>
<td>0.685</td>
<td>0.028</td>
</tr>
<tr>
<td>DA cone RR</td>
<td>-0.095</td>
<td>-0.124</td>
<td>-0.150</td>
</tr>
<tr>
<td>DA cone AT</td>
<td>0.190</td>
<td>0.087</td>
<td>0.038</td>
</tr>
<tr>
<td>DA rod-cone break</td>
<td>0.040</td>
<td>0.089</td>
<td>0.134</td>
</tr>
<tr>
<td>DA rod RR</td>
<td>0.594</td>
<td>0.227</td>
<td>0.070</td>
</tr>
<tr>
<td>DA rod criterion time</td>
<td>0.155</td>
<td>0.208*</td>
<td>0.215*</td>
</tr>
<tr>
<td>DA rod AT</td>
<td>0.108</td>
<td>0.013</td>
<td>0.01</td>
</tr>
<tr>
<td>VA</td>
<td>.267*</td>
<td>.326*</td>
<td>.272*</td>
</tr>
<tr>
<td></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Each cell represents $r$ (top statistic) and $P$ value (bottom statistic). Correlation category: nearly perfect, >0.9; very strong, 0.9 to >0.7; strong, 0.7 to >0.5; moderate, 0.5 to >0.3; small, 0.3 to >0.1; trivial, 0.1 to >0.

* $P < 0.05$.
expose rod recovery (Tables 3, 5A). The length and difficulty of the test compromised its reproducibility (Table 5C). Thus, testing the faster cone adaptation would provide a more clinically applicable, user-friendly test paradigm with adequate testing the faster cone adaptation would provide a more clinically applicable, user-friendly test paradigm with adequate diagnostic capacity (AUC, 0.85 ± 0.021 and 64% of abnormal cases; Table 5). Furthermore, although predominance of rod loss in AMD is consistent with previous psychophysical and histologic studies, there is also strong psychophysical and histologic evidence of prominent age-related rod loss but marginal cone loss in healthy population. Therefore, the predominance of rod loss in eyes affected by AMD could be amplified by the age-related rod vulnerability, whereas cone abnormality in an AMD cohort would be more disease specific, implying that tests involving measurements of cone dynamics in both DA and PSR were of similar diagnostic capacity (AUC, 0.85 ± 0.022; number of abnormal cases, 3.5° DA, 62% and 63%); therefore, either of these methods could be used for the functional assessment of AMD. However, the PSR would be our test of choice for the following reasons: First, it was shown that the flickering stimulus (as is used in PSR) provides more accurate detection of cone recovery than does a static stimulus. Second, the photopic background of PSR prevents rod–cone interactions influencing outcomes as reported for Sorsby’s fundus dystrophy (results not shown).

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In its present format, our PSR test uses a 5-Hz flicker stimulus. There is a strong argument, based on both metabolic demand and blood flow dynamics, to adopt a faster flicker rate such as 14 Hz for monitoring AMD progression. The 14-Hz flicker threshold assessment gave the best outcomes in our comparative analysis when considering test characteristics such as user friendliness (test duration, 5–8 minutes and test difficulty average score 3.2/10), as well as the diagnostic capacity and reproducibility (Table 5). Therefore, using a 14-Hz flicker rate for the PSR test would provide a fast assessment (5–20 minutes), which could be used to observe early AMD cases over time. We estimate that the number of abnormal cases detected with both PSR rate and 14-Hz flicker threshold was 71% of early AMD cases.

Given the computer controlled approach, our psychophysical tests are relatively user-friendly; however, just as with automated perimetry, these tests would need trained operators to ensure reliable and repeatable outcomes. Our laboratory-developed tests can be further improved for commercial use (e.g., our staircasing approach could be optimized by adopting Bayesian predictors, improving the ergonomics of the equipment, optimizing calibration techniques, and so on).

**Conclusion**

All visual function tests employed in this study are sensitive and specific in detecting an AMD-related functional abnormality and have significantly greater diagnostic capacity than does the universally used assessment of VA. Dynamic parameters in DA and PSR provide the best indicators of functional loss in AMD, whereas the steady state 14-Hz flicker threshold was the best test when the diagnostic capacity, together with reproducibility and clinical applicability, were considered. The lack of correlation between static and dynamic parameters means that they are likely to be measuring different aspects of the disease process and combining them provides a useful test suite for early AMD that enhances the detection of abnormalities. We propose that using a flickering stimulus (14 Hz) for the PSR test could provide a fast and user friendly diagnostic tool for tracking functional changes in early AMD. Whereas, rod RR in DA at 3° to 4° eccentricity may have a useful application in monitoring individuals at high risk of developing AMD due to a strong family history or known genetic risk, before clinical diagnosis is possible.

The present work provides valuable information on various characteristics of a potential functional biomarker in AMD. These testing strategies should be used in a prospective fashion to determine their utility in monitoring progression of early disease and ability to assess treatments to interfere with progression. Ultimately, the goal is to find a functional test for AMD that could detect pathologic changes very early in the disease process, before clinical signs are apparent, so that new intervention strategies can be implemented at these earliest stages.

**References**


