Diagnostic Sensitivity and Specificity of Dark Adaptometry for Detection of Age-Related Macular Degeneration

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PURPOSE. Difficulty with night vision is a common complaint of patients with age-related macular degeneration (AMD). Consistent with this complaint, dark adaptation (DA) is substantially impaired in these patients. Because of the severity of the deficit, measurement of DA has been suggested as a means for the diagnosis of AMD. Previous methods for measurement of DA were time intensive (>30 minutes), which made them unsuitable for clinical use. This study evaluated a rapid DA test ($\leq$6.5 minutes) for the detection of AMD.

METHODS. Dark adaptation was measured by using the AdaptDx dark adaptometer in two groups: subjects with normal retinal health and subjects with AMD. Subjects were assigned to their group by clinical examination and grading of fundus photographs. Subjects were classified as having DA consistent with normal retinal health (rod intercept $\leq$ 6.5 minutes) or having dark adaptation consistent with AMD (rod intercept > 6.5 minutes).

RESULTS. The eligible sample for analysis included 21 normal adults and 127 AMD patients. The rapid test was found to have a diagnostic sensitivity of 90.6% ($P < 0.001$) and specificity of 90.5% ($P < 0.027$). Thus, abnormal DA was detected in 115 of 127 AMD patients, and normal DA was found in 19 of 21 normal adults.

CONCLUSIONS. The high diagnostic sensitivity and specificity compared favorably to long-duration research methods for the measurement of DA, and slit lamp biomicroscopy performed by a retina specialist. These results suggest that a rapid DA test is useful for the detection of AMD.

Keywords: age-related macular degeneration, dark adaptation, human, diagnostic

Age-related macular degeneration (AMD) is the leading cause of irreversible legal blindness in developed countries. In the United States, one of every 10 adults older than 60 years is estimated to have AMD.[1,2] One study has found that 69% of patients are unaware that they have AMD until they are diagnosed with late-stage disease.[3] Up to 78% of patients when first diagnosed already have 20/50 or worse best corrected visual acuity, including 40% with 20/200 or worse.[4] For diagnosed patients, effective behavior modification, nutritional supplementation, and prompt anti-VEGF treatment reduce the incidence and progression of irreversible vision loss.

While patients with early to intermediate AMD typically have good best corrected visual acuity, impaired night vision is a prominent self-reported problem.[5–8] These symptoms are in concordance with the large impairment of dark adaptation measured in patients with early AMD.[9,10] The dramatic impact of AMD on dark adaptation speed appears to be caused by the lipid-rich cholesterol deposits within the RPE/Bruch's membrane layer, which form the basis of drusen and disturb the retinoid cycle, especially in the rod photoreceptors.[6,8] The impairment is substantial and suggests that dark adaptation may serve as a diagnostic indicator of AMD. Diagnostic sensitivity, the ability to detect AMD among confirmed cases, has been estimated to be greater than 80% in multiple independent studies.[10–13] Diagnostic specificity, the ability to detect normal retinal health in normal adults, has been estimated to be more than 90%.[10–13] These figures correspond to a false-negative rate of 20% and a false-positive rate of less than 10%.

Dark adaptometry's utility as a practical diagnostic aid has been hampered by long test duration, high patient burden, and lack of standardized dark adaptometers. Dark adaptation protocols used in prior research require up to 60 or more minutes, and typically more than 100 threshold estimates are made. The long duration and high number of threshold measurements may fatigue some patients and adversely affect reliability. We previously developed a short-duration ($\leq$12.5 minute) dark adaptation protocol that minimized patient fatigue, increased operator ease of use, and maintained the high sensitivity and specificity of research protocols.[11] From that study, we developed a rapid dark adaptation test ($\leq$6.5 minutes) to detect the dark adaptation impairment associated with AMD. The reduction in test duration was achieved by optimizing a reduced bleaching intensity. The primary aim of this study was to validate this rapid test by assessing whether it had an acceptably high (>70%) diagnostic sensitivity and specificity. The secondary aim of this study was to evaluate whether a 20-minute version of this protocol (extended test) could be used to determine whether the speed of dark

adaptation was related to AMD disease severity. To evaluate these aims, a multisite clinical study was conducted at Penn State Hershey Eye Center, Massachusetts Eye and Ear Infirmary (MEEI), and Wilmer Eye Institute to assess dark adaptation in subjects with normal retinal health and a range of AMD severities.

METHODS

Two groups of subjects were recruited: adults with normal retinal health (normal group) and participants with early to advanced AMD (AMD group). The inclusion criteria for the normal group were (1) age $\geq$ 50 years, (2) $\geq$20/25 best corrected distance acuity in both eyes, (3) comprehensive eye examination within the 6 months before enrollment, (4) refractive error $\leq \pm6$ diopters spherical equivalent, and (5) clinical diagnosis of normal consistent with fundus photography grade of normal. The inclusion criteria for the AMD group differed as follows: (1) $\geq$20/100 best corrected distance acuity in the study eye, and (2) a clinical diagnosis of AMD consistent with a fundus photography grade of AMD. Exclusion criteria for both groups were (1) any eye condition, disease, history of surgery, or trauma in either eye (other than cataract) that can impair vision and (2) neurologic conditions that can impair vision. The study eye was randomly selected.

The protocol adhered to the Declaration of Helsinki and was approved by the following institutional review boards: Western, Penn State Hershey, Johns Hopkins, and MEEI. The protocol was Health Insurance Portability and Accountability Act compliant. Written informed consent was obtained before participation. The visit consisted of the following assessments: (1) ocular and medical histories, (2) refraction, (3) visual acuity, (4) dark adaptation, (5) three-field stereo color fundus photography, and (6) fundus grading. Ocular and medical histories were assessed to insure the subject met the study entrance criteria. Participants were refracted using the Early Treatment of Diabetic Retinopathy Study (ETDRS) refraction protocol. Refraction was performed to determine the best optical correction for test distance. Visual acuity was measured with the Electronic Visual Acuity Tester (JAEB Center, Tampa, FL) using the E-EDTRS protocol.[14]

Dark adaptation was measured by using the AdaptDx dark adaptometer (MacuLogix, Hummelstown, PA). Each participant's eyes were dilated to $\geq$6 mm by using 1% tropicamide and 2.5% phenylephrine hydrochloride. Corrective lenses for the study eye were introduced to the AdaptDx as appropriate for the 30-cm viewing distance to correct for blur. The fellow eye was occluded with an eye patch. An infrared camera displayed an image of the eye on the operator control screen. The operator centered the subject's eye to a red (635 nm) fixation light with the help of a reticule displayed on the image of the eye. The subject's eye was bleached by exposure to a 505-nm photoflash (0.8-ms duration, 1.8×10^4 scot cd/m² s intensity), equivalent to 76% bleaching level for rods, while the subject was focused on the fixation light. The flash of light passed through a square aperture sized to bleach a 4° area of the retina centered at 5° on the inferior visual meridian. The bleaching flash provided a uniform, focal bleach surrounding the area to be tested during sensitivity recovery measurements. Sensitivity measurements began immediately after bleaching. The subject focused on the fixation light and indicated when a stimulus light was visible by pushing a hand-held button. The stimulus light was a 505-nm, 2° circular test spot located at 5° on the inferior visual meridian. Sensitivity was estimated by using a three-down/one-up modified staircase threshold estimate procedure. The initial stimulus intensity was 5 scot cd/m². The stimulus light was presented every 2 or 3 seconds

for a 200-ms duration. The patient was given 2 seconds to respond if the stimulus was detected by pushing a response button. If the subject indicated that the stimulus was visible, the intensity was decreased for each successive presentation in steps of 0.3 log units until the subject stopped responding that the stimulus was visible. If the subject indicated that the stimulus light was not visible, the intensity of the target was increased for each successive presentation in 0.1-log-unit steps until the subject responded that the stimulus light was once again visible. This intensity was defined as a threshold. Successive threshold measurements started with the stimulus intensity 0.2 log units brighter than the previous threshold measurement. The subject had a 15-second rest period between threshold measurements. However, if a threshold had a large deviation from prior thresholds in the dark adaptation function, the point was considered unreliable and a fixation error was recorded and immediately an additional threshold was measured. Threshold measurements were made approximately once a minute for the duration of the dark adaptation test. The test terminated when the subject's sensitivity was twice consecutively measured to be greater than 5×10^{-3} scot cd/m² or the test duration reached 20 minutes, whichever was shorter.

After dark adaptation testing was completed, three-field stereo color photographs were taken of both eyes by using a Topcon TRC 50-EX fundus camera (Topcon USA, Paramus, NJ). An experienced grader and retina specialist (AI) graded the photographs by using the Age-Related Eye Disease Study (AREDS) AMD Severity System.[15] The grader was masked to the clinical and functional characteristics of the participants. Subjects having an AREDS severity step of 1 were classified as normal. Subjects having AREDS severity steps 3 to 5 were defined as having early AMD. Severity steps 6 to 8 were defined as intermediate AMD. Subjects having an AREDS severity step 9 or choroidal neovascularization or central geographic atrophy were classified as having advanced AMD.

For all patients, one dark adaptation function was measured with a maximum duration of 20 minutes. For evaluation of the rapid test, the dark adaptation functions were truncated to a maximum test time of 6.5 minutes. For evaluation of the extended test, the whole dark adaptation function was evaluated. This approach was used to minimize subject burden by requiring the measurement of only one dark adaptation function to address both study aims. The cut point of 6.5 minutes was determined in a prior range-finding experiment, which explored the effect of bleaching level and location on dark adaptation speed (Jackson GR, Edwards JG, unpublished data, 2009). The cut point was based on the normal reference range of old normal adults in that prior study. For each dark adaptation function, the fixation error rate was calculated as the number of invalid thresholds divided by the total number of thresholds. An invalid dark adaptation test was indicated by a fixation error rate of $\geq$30%. Valid thresholds were used to calculate the rod intercept, which is defined as the amount of time required for sensitivity recovery to reach a criterion sensitivity level of 5×10^{-3} scot cd/m². The criterion sensitivity level is located in the latter half of the second component of rod recovery and is completely mediated by rods. The rod intercept provides a uniform, objective parameter for characterizing dark adaptation speed. If the rod intercept does not occur within the maximum test duration (6.5 minutes for the rapid test or 20 minutes for the extended test), the algorithm attempts to extrapolate the intersection of the rod recovery with the criterion sensitivity level. If the rod intercept cannot be extrapolated, it is set at maximum test duration.

Statistics were calculated by using the SAS System software version 9.3 (SAS Institute, Inc., Cary, NC) and R version 2.15 (R Foundation for Statistical Computing, Vienna, Austria). Mann-

Whitney U and Kruskal-Wallis tests were used as appropriate to evaluate differences across groups for continuous variables. χ^2 tests were used to test associations between nominal variables. The statistical significance of the sensitivity and specificity estimates was evaluated with a one-sided binomial test. The binomial test evaluated whether the lower bound of the 95% CI of the estimate was greater than 70%, the minimally acceptable criterion level. To assess whether dark adaptation speed is related to disease severity, logistic regression was used to evaluate whether dark adaptation speed predicted classification as early AMD or intermediate AMD.

RESULTS

A total of 214 subjects were enrolled at the three investigational centers. The final sample for evaluation of the primary aim included 148 subjects (21 normal and 127 AMD). The attrition rate was high because there was no formal screening visit. Forty-four subjects were excluded because their retinal health did not meet the eligibility criteria. Additional causes of attrition included 14 subjects with invalid dark adaptation measurements because of high fixation error rates, three subjects with unreadable fundus photograph sets, and five subjects who were withdrawn because they could not complete the protocol (e.g., the study eye could not be dilated to ≥ 6 mm).

Participant characteristics are listed in Table 1. The AMD group was on average 8 years older than the normal group ($P = 0.0001$). The two groups had similar sex and racial distributions ($P = 0.37$, $P = 0.26$). The normal group had slightly better than 20/20 visual acuity in the study eye, compared with slightly worse than 20/25 for the AMD group ($P < 0.0001$). Based upon fundus grading, the AMD group consisted of 41 subjects with early AMD, 72 subjects with intermediate AMD, and 14 subjects with advanced AMD.

Dark adaptation curves in response to a moderate bleaching intensity often lack features produced by a high bleaching intensity, such as the exponential cone sensitivity recovery, cone plateau, or a distinct rod–cone break. For the bleaching intensity used in this study, most normal subjects exhibited a linear sensitivity recovery, lacking distinct cone-mediated features (Fig. 1A). Subjects with early and intermediate AMD typically exhibited a cone plateau and rod–cone break (Figs. 1B, 1C). These features are apparent because of the large delay of rod-mediated sensitivity recovery. Subjects with advanced AMD often exhibit minimal or no rod recovery for 20 minutes (Fig. 1D). For the rapid test (6.5-minute maximum duration), the trend was for the rod intercept to increase with increasing disease severity ($P < 0.0001$; Table 2). The AMD group exhibited a 1-minute larger rod intercept than the normal group ($P < 0.0001$). Most of the rod intercepts for the AMD subjects were artificially limited to the maximum test duration of 6.5 minutes, limiting the ability of the rapid test to differentiate by disease severity. To assess whether aging affected dark adaptation speed, the mean rod intercept of normal adults older than 65 years was compared with that of younger normal adults. There was no effect of aging on the rod intercept, which suggests that aging is not a confounding factor for this protocol (mean difference = 0.6 minutes, $P = 0.32$). For the extended test (20-minute maximum duration), the rod intercept again increased with increased disease severity ($P < 0.0001$). The dark adaptation impairment measured for the AMD group was substantial. The mean rod intercept of the AMD group was 10 minutes greater than that of the normal group ($P < 0.0001$). For the typical AMD subject no rod recovery is exhibited at the time when most normal subjects have completed recovery (Figs. 2A, 2B).

TABLE 1. Participant Characteristics

Variable*	Normal Group, $N = 21$	AMD Group, $N = 127$	P Value
Age, y	65 (52, 81)	73 (51, 93)	0.0001
Sex, % female	52	65	0.37
Race, % white	95	99	0.26
Study eye acuity, letters correct	87 (81, 95)	78 (43, 95)	0.0001
Fellow eye acuity	89 (82, 95)	77 (30, 93)	0.0001
Disease severity, N			
Normal	21	0	
Early AMD	0	41	
Intermediate AMD	0	72	
Advanced AMD	0	14	

* Mean (minimum, maximum) for continuous variables.

The primary aim of the study was to estimate the diagnostic sensitivity and specificity of the AdaptDx rapid test. Sensitivity was defined as the percentage of AMD subjects who exhibited a rod intercept > 6.5 minutes. Specificity was defined as the percentage of normal subjects who exhibited a rod intercept $\leq$ 6.5 minutes. Diagnostic test sensitivity was calculated to be 90.6% (115/127, $P < 0.001$). The 95% CI for diagnostic sensitivity had a lower bound of 85.1% and an upper bound of 100%. Diagnostic test specificity was calculated to be 90.5% (19/21, $P = 0.0271$). The 95% CI for diagnostic specificity had a lower bound of 72.9% and an upper bound of 100%.

The AdaptDx measured normal dark adaptation in 12 confirmed AMD cases. To evaluate whether these false-negative cases were associated with a specific AMD phenotype, diagnostic sensitivity was calculated for each severity of AMD. The diagnostic sensitivities were 80.5% (33/41) for early AMD, 94.4% (68/72) for intermediate AMD, and 100% (14/14) for advanced AMD. The AdaptDx measured abnormal dark adaptation in two confirmed normal cases. The rod intercepts of the two false-positive cases were well beyond the diagnostic cut point of 6.5 minutes (7.7 and 7.8 minutes). Reviewing the subjects' medical histories found no likely causes for the abnormal dark adaptation. However, the magnitudes of the rod intercepts indicate some condition other than normal retinal health.

The secondary aim of the study was to assess whether the AdaptDx extended test could differentiate between early and intermediate AMD. The association between AMD severity and rod intercept was evaluated by using logistical regression on the extended test data. There was a positive relationship between the rod intercept and disease severity. The odds ratio for intermediate AMD versus early AMD was 1.19 (95% CI: 1.044–1.2, $P = 0.0015$). In other words, for every 1-minute increase in the rod intercept the odds of a subject having intermediate AMD increased 11.9%.

The dark adaptation results were similar across sites. To evaluate poolability, the sensitivity and specificity of the rapid test were evaluated between Penn State ($N = 99$) and the combined data from MEEI ($N = 45$) and Wilmer ($N = 4$). Diagnostic sensitivity at Penn State was 90.9% compared with 89.7% for MEEI/Wilmer. Diagnostic specificity at Penn State was 100% compared with 80% at MEEI/Wilmer. Both false positives in the overall data set were participants at MEEI, which accounts for the difference in specificity values between the sites.

DISCUSSION

This study found that a rapid dark adaptation test can be used to detect abnormal dark adaptation associated with AMD. The
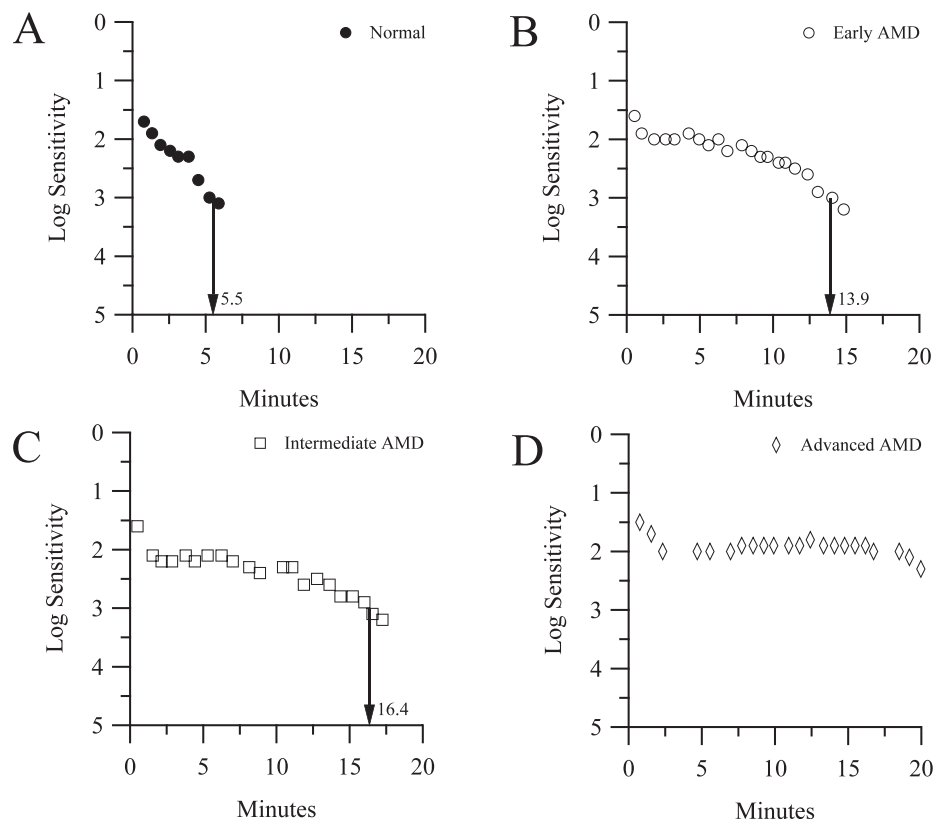
FIGURE 1. Representative dark adaptation curves for a normal adult (**A**), early AMD subject (**B**), intermediate AMD subject (**C**), and advanced AMD subject (**D**). Normal subjects typically exhibited a linear recovery of sensitivity. Early to intermediate AMD subjects typically exhibited minimal cone sensitivity recovery and a relatively long cone plateau before rod recovery. Advanced AMD subjects typically exhibited minimal or no rod recovery for the 20-minute test duration.

diagnostic sensitivity and specificity were both greater than 90%, comparable with longer-duration research protocols.[10,11] From a clinical perspective, the AdaptDx rapid test performance compares favorably to the ~82% sensitivity and ~91% specificity of retina specialists using slit lamp biomicroscopy.[16,17] The rapid test is amenable to the clinic because of its short duration and low patient burden. Furthermore, the rod intercept provides a simple, objective interpretation of dark adaptation speed. Use of dark adaptation testing in primary eye care practices would significantly increase the likelihood of diagnosing AMD in affected cases, similar to the way that visual field testing has increased the diagnosis of glaucoma.

It is useful to consider the clinical implications of missed cases of AMD (false negatives) and normal adults wrongly classified as having AMD (false positives). With regard to false negatives, no subjects with advanced AMD exhibited a normal dark adaptation curve on either the rapid test or extended test. Thus, none of the patients most in need of vision-saving

therapy were misclassified. Missed cases with less severe AMD are likely to be detected in future examinations and are at lower risk of immediate vision loss. The false-positive cases are of interest because their dark adaptation is clearly abnormal without an identifiable cause. One possible explanation for false positives is early stage lesions, which are not clinically detectable, such as basal linear/laminar deposits, or reticular pseudodrusen, which are not visible on standard color fundus photographs. There is an ongoing prospective study examining whether abnormal dark adaptation is predictive of incident early AMD. Results from such natural history studies may inform about the interpretation of dark adaptation impairment found in adults with apparent healthy retinas and no other medical cause for dark adaptation abnormalities.

In summary, impaired dark adaptation has been found in numerous cross-sectional studies of AMD. The impairment is substantial and may be used as an aid in the diagnosis and staging of AMD. In the future, it is possible that dark adaptation

TABLE 2. Summary of Rod Intercept Values by Disease Severity

| Measurement* | Normal | AMD | | | |
		Early	Intermediate	Advanced	Combined
Rapid test	21	41	72	14	127
Rod intercept, min	5.3 ± 1.3 (3.3, 7.2)	6.0 ± 1.0 (2.7, 6.7)	6.4 ± 0.5 (3.5, 7.1)	6.5 ± 0.0 (6.5, 6.5)	6.3 ± 0.7 (2.7, 7.1)
Extended test	23	41	71	15	127
Rod intercept, min	5.7 ± 1.9 (3.3, 11.8)	12.9 ± 6.1 (2.7, 20.3)	16.6 ± 5.2 (3.5, 24.2)	19.0 ± 4.5 (8.8, 27.6)	15.7 ± 5.8 (2.7, 27.6)

Eligibility for analysis based upon valid dark adaptation measurements assessed by fixation error rate.
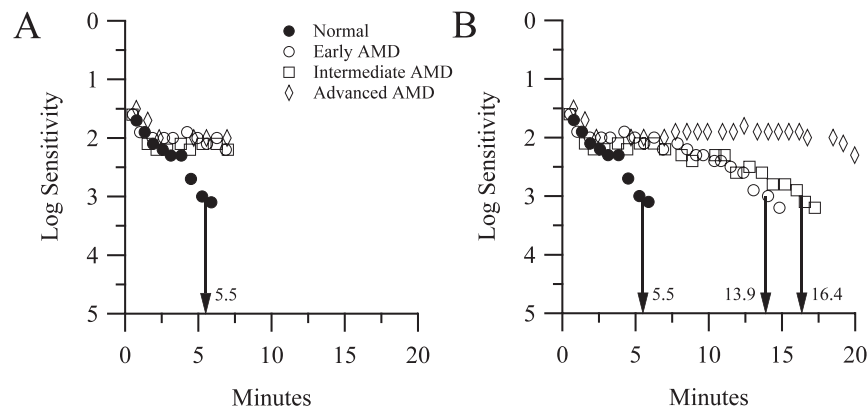
* N, mean ± 1 standard deviation, (minimum, maximum).

FIGURE 2. The rapid test (**A**) is useful for the detection of abnormal dark adaptation associated with AMD. Normal subjects typically exhibit a rod intercept within the 6.5-minute maximum test duration, while all severities of AMD typically exhibit no rod recovery. The extended test (**B**) is a useful aid for staging AMD severity. Dark adaptation speed is correlated with disease severity.

may be also useful for evaluating the risk of progression of AMD.

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