IMPORTANCE  Clinical observations suggest that patients with age-related macular degeneration (AMD) have vision problems, particularly in dim light conditions. Previous studies on structural-functional analysis in patients with AMD with reticular drusen (RDR) have focused on photopic sensitivity testing but have not specifically assessed scotopic function.

OBJECTIVE  To evaluate retinal function by scotopic and photopic microperimetry in patients with AMD and a well-demarcated area of RDR.

DESIGN, SETTING, AND PARTICIPANTS  Prospective case series in a referral center of 22 eyes from 18 patients (mean age, 74.7 years; range, 62-87 years). The study was conducted from June 1, 2014, to October 31, 2014.

INTERVENTIONS  With the use of combined confocal scanning laser ophthalmoscopy and spectral-domain optical coherence tomography imaging, retinal areas with RDR (category 1) and no visible pathologic alterations (category 2) were identified in each eye. Scotopic and photopic microperimetry (MP-1S; Nidek Technologies) was performed using a grid with 56 stimulus points.

MAIN OUTCOMES AND MEASURES  Comparison of mean threshold sensitivities for each category for scotopic and photopic microperimetry.

RESULTS  In all eyes, areas of category 1 showed a relative and sharply demarcated reduction of scotopic threshold values compared with areas of category 2, but only less-pronounced differences were seen for photopic testing. Statistical analysis in the 18 eyes in which the 1.0-log unit neutral density filter was applied revealed a difference of scotopic threshold values in areas of category 1 (mean, 13.5 dB [95% CI, 12.1-15.0]) vs category 2 (mean, 18.3 dB; [95% CI, 17.4-19.3] (P < .001). For photopic testing, the mean threshold values were 16.8 dB (95% CI, 15.5-18.2) in category 1 and 18.4 dB (95% CI, 17.1-19.6) in category 2 (P = .03).

CONCLUSIONS AND RELEVANCE  The results of this study suggest that rod function is more severely affected than cone function in retinal areas with RDR. This differential structural-functional correlation underscores the functional relevance of RDR in patients with AMD.
Microperimetry in Age-Related Macular Degeneration

Drsen represent a phenotypic hallmark in patients with age-related macular degeneration (AMD). Pseudodrusen visible en lumière bleu were initially described by Mimoun and coworkers and distinguished from soft, hard, calcified, and cuticular drusen. Subsequently, various nonuniform terms have been introduced, including reticular drusen (RDR), reticular pattern, reticular pseudodrusen, reticular macular disease, and subretinal drusenoid deposits. Previous studies using high-resolution imaging reported prevalence rates ranging from 54% to 62% in patients with late-stage AMD. Longitudinal analysis revealed an increase in the involved retinal area and a possible growth of single lesions. In addition, several lines of evidence indicate that RDR may represent a risk factor for AMD progression and the development of late AMD.

Alten and coworkers used multifocal electroretinography to evaluate retinal function over RDR areas and found that RDR did not show a definite interference of responses at baseline. Over time, a decline of function in eyes with progressive RDR was detected. Three studies have specifically evaluated retinal sensitivity of RDR areas using microperimetry. The study by Ooto et al (based on MP-1 microperimetry; Nidek Technologies) demonstrated reduced photopic retinal sensitivity of retinal areas covered with RDR compared with the sensitivity of healthy eyes. Furthermore, individual RDR lesions correlated with a larger decrease in photopic retinal sensitivity. Forte et al, using optical coherence tomography/scanning laser ophthalmoscopy (OCT/SLO) (Spectral OCT/SLO; OPKO-OTT), showed reduced photopic retinal sensitivity in RDR areas compared with control individuals with normal central visual acuity. Querques et al also using the Spectral OCT/SLO, reported a greater extent of reduced sensitivity in eyes with RDR compared with eyes with typical drusen. In this latter analysis, patients underwent dark adaptation for at least 15 to 30 minutes before testing, which allowed for mesopic retinal sensitivity assessment.

It is a general clinical experience that patients with AMD have particularly decreased vision in dim light conditions and require high ambient light in some settings (eg, when reading), which occurs even in early AMD stages. The observation that scotopic sensitivity loss exceeds photopic sensitivity loss in individuals with AMD is supported by histopathologic data that demonstrated a preferential vulnerability of rods.

Impaired photopic and scotopic sensitivity has been spatially correlated with the presence of large soft drusen and focal abnormalities of fundus autofluorescence intensities. Other authors have reported poor correspondence between the distribution of drusen and loss of sensitivity. Overall, the effect of conventional drusen and pigmentary changes on scotopic dysfunction in AMD remains controversial, particularly regarding the extent and distribution of morphologic changes in comparison with the extent of reported difficulties in scotopic light conditions by individuals with AMD and the extent of scotopic dysfunction as measured by functional tests.

The aim of the present study was to perform a structural-functional analysis with both scotopic and photopic fundus-controlled microperimetry in patients with AMD that showed well-demarcated areas of RDR. Furthermore, functional probing was performed outside areas with RDR with no visible pathologic alterations to allow direct comparison of sensitivity levels between unaffected and RDR-involved retinal areas in the same eye.

Methods

Participants were recruited from the AMD outpatient clinic at the Department of Ophthalmology, University of Bonn, Bonn, Germany. The study, conducted from June 1 to October 31, 2014, was approved by the local ethics committee (Ethik-Kommission, Medizinische Fakultät, Rheinische Friedrich-Wilhelms-Universität; Lfd. No. 125/14), and written informed consent was obtained from each patient. The participants did not receive financial compensation.

For inclusion, individuals had to have received a diagnosis of AMD in both eyes (according to the classification system proposed by Ferris et al). Only persons with stable fixation and clear media in at least 1 eye to allow central visual function testing and retinal imaging were enrolled in the study. A clearly distinguishable area with RDR encompassing the size of at least 2 disc areas had to be present in the study eye as detected by combined confocal SLO infrared reflectance and spectral-domain (SD)-OCT imaging. Furthermore, this RDR area had to be at least partially surrounded by retinal areas that did not show any pathologic alterations (ie, healthy-appearing retina); the microperimetry test pattern had to cover at least 2 disc areas with no visible pathologic changes to compare retinal sensitivity results between RDR-affected and normal retinal areas in the same eye. Any other AMD-related pathologic characteristics, such as drusen, pigmented changes, and small areas of atrophy or regressed choroidal neovascularization, could be adjacent to the RDR area and present within the central 20° × 20° field. Exclusion criteria were any signs of active choroidal neovascularization. In addition, patients with vascular or inflammatory retinal abnormalities, glaucoma, or history of retinal or refractive surgery or laser treatment were not included.

All participants underwent a complete ophthalmologic examination, including visual acuity assessment, slitlamp examination, and fundus biomicroscopy. Retinal imaging was conducted using color fundus photography and combined confocal SLO and SD-OCT imaging (eMethods in the Supplement [detailed imaging protocol]). Functional testing was performed using both scotopic (following 30 minutes of dark adaptation before testing) and photopic microperimetry (Nidek MP-1; Nidek Technologies) (eMethods [detailed microperimetry assessment] and eFigure 1 in the Supplement).

Statistical Analysis

The microperimetry test grid was superimposed on the enface SLO near-infrared reflectance image for each study eye. The location of each of the 56 threshold values was then assessed to determine whether they were located over RDR areas (category 1) or over areas with no visible pathologic alterations (category 2).

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The mean values of all 56 threshold values were calculated separately for each eye and then separately for the 2 structural categories. Furthermore, the number of test points with a loss of more than 10 dB compared with the best possible sensitivity (20 dB) was assessed for each study eye. Because the range and behavior of the 1.0- and 2.0-log unit neutral density filters are different, the participants were split in 2 groups. The calculations of threshold values were conducted for the 1.0-log unit neutral density filter. The Wilcoxon signed rank test on differences was used to compare the classifications under the 2 different categories. Statistical analysis was conducted using SPSS, version 22.0.0.0 (SPSS Inc).

Results

Demographics

Twenty-two eyes of 18 patients (6 men and 12 women) underwent scotopic and photopic microperimetry examinations and were included in the final analysis. The median age was 74.7 years (interquartile range, 71-80; range, 62-87 years). The median visual acuity was 20/25 Snellen (range, 20/20 to 20/40). According to the AMD classification system recently proposed by Ferris et al,30 8 eyes were classified as early-stage AMD, 11 eyes as intermediate-stage AMD, and 3 eyes as late-stage AMD (1 had geographic atrophy and 2 had choroidal neovascularization). (eFigure 2 in the Supplement gives an example of a patient with RDR and early AMD.) There were 9 pseudophakic and 13 phakic eyes, respectively.

Microperimetry

Details for the periods of microperimetry assessments, data on fixation stability, and results of the filter selection examination are given in the Table and in the eResults in the Supplement. The overall mean scotopic sensitivity was 14.5 dB (95% CI, 12.7-16.3; range, 4.5-18.9) for the 18 eyes with the 1.0-log unit filter and 15.6 dB (95% CI, 14.8-16.4; range, 14.4-16.1) for the 4 eyes with the 2.0-log unit filter. The overall mean photopic sensitivity was 17.2 dB (95% CI, 15.4-19.0; range, 6.1-20.0) for the group with 18 eyes and 18.1 dB (95% CI, 17.0-19.2; range, 17.1-19.5) for the group with 4 eyes. The mapping of the stimuli location to the structural changes as seen by en-face confocal SLO and SD-OCT imaging disclosed a mean number of 29.4 (95% CI, 24.0-34.7; range 7-49) test points per eye that were spatially confined to areas with RDR (category 1); a mean number of 21.6 (95% CI, 17.2-26.1; range, 6-37) test points were spatially confined to areas with no visible pathologic alterations (category 2). In all eyes (both with the 1.0- and the 2.0-log unit neutral density filter), there was a relative reduction of scotopic threshold values over areas with RDR compared with areas with no visible pathologic alterations. In addition, this difference in scotopic threshold values was sharply demarcated and spatially confined to the clearly distinguishable borders of the RDR area as shown by retinal imaging. In most eyes, photopic function appeared to be similar or only relatively slightly reduced at areas of category 1 compared with areas of category 2 in the same eyes. However, neither an obvious and consistent difference in photopic retinal sensitivity values comparing the 2 different categories nor a sharply demarcated photopic threshold change from one category to another was clearly visible. Two representative examples are illustrated in Figure 1 and Figure 2 (for further details, see the eResults in the Supplement).

For further statistical analysis between photopic and scotopic test results, only the 18 eyes with the 1.0 log unit were analyzed. The group with 4 eyes with the 2.0-log unit filter was not included because of the inability to directly compare the threshold values with the ones from the 1.0-log unit eyes (range and behavior different; no mathematical conversion factor validated) and because the number of eyes in this group was considered too small for a proper separate statistical evaluation.

Comparing the mean threshold values in the 2 categories, there was an obvious overall difference for scotopic microperimetry (category 1: mean, 13.5 dB [95% CI, 12.1-15.0]; category 2: mean, 18.3 dB [95% CI, 17.4-19.3]; P = .001). There was a less-pronounced difference for photopic testing (category 1: mean, 16.8 dB [95% CI, 15.5-18.2]; category 2: mean, 18.4 dB [95% CI, 17.1-19.6]; P = .03) (Figure 3). In a similar manner, when counting the number of test points with a loss of more than 10 dB, there was an overall clear difference between the 2 categories for scotopic (P = .001) and, again to a lesser extent, for photopic (P = .04) testing (Figure 3).

Discussion

This study demonstrates localized and severe scotopic functional impairment over areas with RDR in the presence of preserved central visual acuity. These findings are in accordance with previous studies19-21,23,24 that have reported difficulties particularly with vision under dark-adapted conditions in individuals with the early stages of AMD.

We found reductions in retinal sensitivity for both photopic and scotopic threshold values over areas with RDR, although the depth of the relative scotopic scotoma was greater than the depth of photopic scotomata. The greater number of test points with a localized severe reduction in retinal sensitivity for scotopic testing would also be conceivable with greater scotopic compared
with photopic sensitivity loss. Furthermore, there was an obvious and clear spatial correlation between the size of the scotopic scotoma and the visible extent of the RDR areas noted on retinal imaging. These observations suggest that rod function is more severely affected than cone function over RDR areas. This result is in accordance with histologic findings of a preferential vulnerability of the rod system and relative preservation of cone photoreceptors in the early stages of AMD. \textsuperscript{25,32,33} Furthermore, Curcio and coworkers\textsuperscript{31} have recently shown a spatial correlation of damage to rods and the presence of subretinal deposits, ie, RDR, suggesting that rods may be an important pathophysiologic stimulus for the formation of RDR.

The exact histopathologic correlate of RDR remains unclear and has been a matter of debate. Some studies favor an
origin within the choroid; however, other findings that are mainly based on simultaneous SD-OCT and confocal SLO imaging suggest alterations at the level of the photoreceptors with focal accumulations of hyperreflective material above the retinal pigment epithelium (RPE) and with disintegration of the external limiting membrane.\textsuperscript{34-37} Both hypotheses would be in accordance with a relative reduced retinal sensitivity over areas with RDR. Although an impaired choriocapillaris blood flow due to atrophy or fibrosis could subsequently affect the RPE and photoreceptors, the accumulation of material above the RPE might interfere with normal metabolisms of the RPE/photoreceptor complex. Recently, Spaide\textsuperscript{38} reported the de-
Development of outer retinal atrophy after regression of RDR and also speculated that outer retinal atrophy might particularly interfere with rod function, based on the clinical experience in patients with AMD and excessive reticular drusen having good central visual acuity but severe difficulties in dim light conditions. This finding has been confirmed by the results of the present study showing a high spatial correlation of scotopic dysfunction with the extent of the RDR area.

Rod function in patients with AMD has been assessed by using scotopic electroretinography, dark adaptation, or dark-adapted perimetry, but not specifically correlated with the area affected by RDR. In this study, we used the recently introduced Nidek MP-1S device, which allows for both photopic and scotopic fundus-controlled perimetry. Crossland et al reported that the 2.0-log unit neutral density filter would be the most appropriate filter in healthy eyes. In this study, we performed a filter selection examination and identified the 1.0-log unit neutral density filter as the most appropriate filter in most participants. This observation might be in accordance with a general reduction of scotopic threshold values in patients with AMD compared with controls, although we cannot exclude other confounders.

To minimize the effects of test-retest variability, at least 2 scotopic microperimetry assessments were performed that had to show a difference of the mean retinal sensitivity threshold values of 3.0 dB or less. This requirement was met in 19 eyes, suggesting that reproducible results can be obtained in individuals with retinal diseases. It also has been forming an exact structural-functional correlation. The dynamic range of test stimuli intensities is limited with the current MP-1S device. Therefore, neutral density filters are used. Crossland et al reported that the 2.0-log unit neutral density filter would be the most appropriate filter in healthy eyes. In this study, we performed a filter selection examination and identified the 1.0-log unit neutral density filter as the most appropriate filter in most participants. This observation might be in accordance with a general reduction of scotopic threshold values in patients with AMD compared with controls, although we cannot exclude other confounders.

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Figure 3. Statistical Analysis

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean Sensitivity, dB</th>
<th>Number of Test Points With &gt;10-dB Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category 2</td>
<td></td>
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</tbody>
</table>

Statistical analysis for the mean retinal sensitivity threshold values (upper row) and the number of test points with greater than 10-dB loss (lower row) between both categories. The circles and asterisks represent, respectively, mild and extreme outliers. The boxes indicate the interquartile range (IQR); horizontal line, median; and whiskers are the 1.5 IQR. The classification of the retinal areas into 2 categories (category 1 indicates retinal areas with RDR; category 2, retinal areas with no visible pathological alterations) is based on the evaluation of retinal imaging data.
reported by Crossland et al\(^2\) that, in addition to control and correction of fixation, the testing time with the MP-1S was faster than scotopic perimetry that was not fundus controlled. Based on the present study, we can confirm that scotopic retinal sensitivity testing using microperimetry is also practical in individuals with AMD. The observations suggest that the time for testing is similar to that with photopic microperimetry, although the 30-minute period for dark adaptation prior to testing needs to be considered. Particularly along with the development of new innovative therapeutic strategies in retinal diseases, including AMD, we would support considering scotopic microperimetry as an outcome measure for interventional trials.

Changes in photopic and mesopic retinal sensitivity in patients with AMD using fundus-controlled perimetry have been reported in several studies.\(^{26-29,45,46}\) Despite preserved visual acuity, significant reduction of sensitivity in patients with “regular” soft drusen and RPE changes have been demonstrated.\(^{45}\) Furthermore, relative reduction of retinal sensitivity over areas with increased fundus autofluorescence intensities has been shown.\(^{26,27}\) Three studies\(^{16-18}\) have specifically assessed photopic or mesopic retinal sensitivity using fundus-controlled perimetry over areas with RDR and have consistently reported on a spatial correlation to impaired retinal function. The results of the present study with a loss of photopic sensitivity over RDR areas compared with unaffected retinal areas would be in accordance with the earlier reports in the literature. Although significant results have been obtained in systematic analyses for photopic sensitivity loss over RDR areas, we believe that this finding is difficult to see at first glance when looking at the illustrations in previous publications and most of the individual topographic test results of this study. By contrast, there were obvious and sharply demarcated relative scotomatous for scotopic thresholds visible in all eyes in this study that could be clearly distinguished from unaffected retinal areas. This observation would be well conceivable with a more severe affection of the rod compared with the cone systems in RDR areas. Curcio and coworkers\(^3\) demonstrated that RDR preferably localize to the perifovea. Using in vivo imaging, the occurrence of foveal sparing and a subsequent slower spread of the RDR areas toward the fovea by in vivo imaging has recently been reported.\(^4\) This observation might be explained by the different photoreceptor distribution and the predominance of cones within the fovea. Furthermore, this observation would be in accordance with a preferred vulnerability of rods compared with cones to the RDR development.

Various limitations of the study have to be considered. First, the number of participants and eyes examined is relatively small, and we have included only eyes with a clearly distinguishable RDR area that had to be surrounded by unaffected retina within the total area of retina assessed. However, we assume that the strict inclusion and exclusion criteria applied together with the repeated scotopic microperimetry testing allowed the recording of meaningful results. The exclusion of 4 patients from the systematic statistical analysis because of the use of the 2.0–instead of the 1.0–log unit neutral density filter must also be regarded as a limitation. We believe that by using the filter selection examination and identifying the most appropriate individual neutral density filter, we could largely reduce the likelihood of floor and ceiling effects for microperimetry. Although we compared cone function under photopic conditions with rod function under scotopic conditions, we did not look at cone function under scotopic conditions (following dark adaptation).

Overall, microperimetry represents a time-consuming assessment, particularly in elderly individuals with poor fixation. The topographic resolution of test stimuli is inferior compared with the resolution of retinal imaging technology. These limitations must be considered for large-scale natural history as well as interventional studies.

**Conclusions**

The results of this study suggest that rod function is more severely affected than cone function in retinal areas with RDR. This differential structural-functional correlation underscores the functional relevance of RDR in patients with AMD.

**REFERENCES**