Microperimetry of Nascent Geographic Atrophy in Age-Related Macular Degeneration

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PURPOSE. To determine the microperimetric retinal sensitivity in areas with nascent geographic atrophy (nGA) compared with other pathological features in eyes with intermediate AMD.

METHODS. Participants with bilateral intermediate AMD underwent microperimetry examinations and high-resolution spectral-domain optical coherence tomography (SD-OCT) scans in a prospective study. Twenty-two participants (24 eyes) identified as having a microperimetric stimulus sampling an atrophic area (nGA or drusen-associated atrophy detected on SD-OCT) in an eye were analyzed, using three neighboring nonatrophic regions (with or without AMD-associated features) in the same eye as reference areas.

RESULTS. On average, the mean microperimetric retinal sensitivity was worse in areas with nGA than nonatrophic reference areas ($P \leq 0.008$), but better than areas with drusen-associated atrophy ($P = 0.008$). Considering all the microperimetry points in an eye, there were only 6 out of 16 eyes (37.5%) where the retinal sensitivity over nGA was the worst performing point in the eye, while all eight out of eight eyes (100.0%) with an area of drusen-associated atrophy detected on SD-OCT had the worst-performing point over that area.

CONCLUSIONS. Areas of nGA were characterized by worse microperimetric retinal sensitivity compared with nonatrophic areas in eyes with intermediate AMD, but better retinal sensitivity compared with areas of drusen-associated atrophy detected on SD-OCT. Areas of nGA were also not always the worst performing point in an eye. These findings further our understanding of the functional changes occurring in novel SD-OCT identified pathological changes in intermediate AMD.

Keywords: age-related macular degeneration, geographic atrophy, microperimetry, optical coherence tomography

The early stages of AMD are traditionally characterized by the presence of drusen and pigmentary changes visible on clinical examination or color fundus photography (CFP). However, recent advances in retinal imaging technology have allowed retinal features and microstructural changes in AMD to be visualized in greater detail.

High-resolution imaging modalities such as spectral-domain optical coherence tomography (SD-OCT) have been used in the early stages of AMD to identify features that confer an increased risk of developing areas of drusen-associated atrophy, including hyperreflective foci,1,2 drusen internal reflectivity, height of drusen, and the choroidal thickness beneath the druse.2 We have also recently used SD-OCT to describe unique characteristics that portend the development of drusen-associated atrophy, and we defined these features as nascent geographic atrophy (nGA); these features include the subsidence of the outer plexiform layer (OPL) and inner nuclear layer (INL), and development of a hyporeflective wedge-shaped band within the limits of the OPL.3 Areas of nGA preceded the development of drusen-associated atrophy by approximately 1 year on average, and were found to share similar topographical distribution and risk factors (including pigmentary abnormalities and the presence of nGA in the fellow eye) with areas of geographic atrophy (GA). However, areas of nGA were not detectable on typical clinical examination and CFP.3 We hypothesized that identifying the presence of nGA is important for determining the risk of future vision loss from the development of drusen-associated atrophy.

We have also observed previously that retinal sensitivity, as measured using flicker perimetry, is reduced and exhibits a more rapid decline in areas that subsequently developed GA many months later.4 Since nGA are areas that we have observed to precede the development of drusen-associated atrophy detected on SD-OCT, we hypothesized that areas of nGA would also exhibit reduced retinal sensitivity. An ideal tool to determine if this is the case is microperimetry, since the real-time compensation of eye movements through fundus tracking allows measurements of retinal sensitivity to be accurately made at individual retinal areas. Previous studies have reported reduced retinal sensitivity overlying pathological features in AMD, including drusen, pigmentary abnormalities, and reticular pseudodrusen (RPD).5-8

This study therefore sought to compare retinal sensitivity, as measured using microperimetry, in areas with nGA and drusen-associated atrophy detected on SD-OCT, with neighboring retinal areas without these changes.
METHODS

This study involved the analysis of microperimetric findings in participants who had been involved in a prospective study of structural and functional changes in intermediate AMD. For this study, we specifically identified participants with a microperimetric stimulus overlaying an atrophic area. The study was approved by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital and was conducted in adherence with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants following a thorough explanation of all the tests involved.

Participants

The inclusion criteria for all participants in this study were being aged 50 years or older and having best-corrected visual acuities of 20/40 or better in both eyes. For AMD participants, the inclusion criteria required participants to have at least drusen >125 μm (intermediate AMD), as determined on CFP in both eyes. The exclusion criteria for any eye included the presence of any GA (defined as a sharply delineated area of at least 175 μm in diameter of hypopigmentation where choroidal vessels are more visible than in surrounding areas, identified on CFP); choroidal neovascularization (CNV); any corneal pathology that would compromise vision; presence of significant cataract, glaucoma, or amblyopia. The exclusion criteria also included any participants with diabetes, any neurological or systemic disease affecting vision, taking medication known to affect retinal function, or any physical and/or mental impairment preventing them from participating in this study.

Microperimetry Examination

Assessment of retinal sensitivity to increment luminance was performed using the Macular Integrity Assessment (MAIA, CenterVue, Padova, Italy) microperimeter. All participants underwent microperimetric testing prior to performing any tests that could bleach the photoreceptors (such as FAF or CFP). Microperimetric testing was performed as outlined in detail previously.10 The MAIA microperimeter performs fundus-tracking using a line scanning laser ophthalmoscope that illuminates the fundus using a superluminescent diode with a central wavelength of 850 nm, using the entire fundus as a reference for fundus tracking at 25 frames per second. A red circle 1° in diameter was used as a fixation target, and Goldman III-sized stimuli (0.43°, or approximately 124 μm) were presented against a background of 1.27 cd/m² using a 4-2 staircase threshold strategy. The maximum and minimum luminance of the stimulus was 318 cd/m² and 1.37 cd/m², respectively, creating a dynamic range of 36 dB. A custom grid designed to assess the macular region was used and consisted of 37 points located at 0°, 1°, 2.33°, 4°, and 6° from fixation.

Test reliability was determined by the percentage of false-positive responses to suprathreshold stimuli at the optic nerve head, which was manually located prior to the start of the threshold measurements. Any test with false-positive responses of >25% was considered unreliable and the examination was repeated; this percentage was chosen since there were typically only four to five false-positive trials in each examination. Identical instructions were given to all participants before microperimetric examinations, and all AMD participants underwent two examinations in the right eye followed by one examination in the left eye during a single session and all control participants underwent two examinations of the study eye. The first examination of the right eye was discarded to minimize the influence of any intra-session learning effect.10

Imaging

Near-infrared reflectance (NIR), fundus autofluorescence (FAF), SD-OCT imaging (Spectrals HRA+OCT, Heidelberg Engineering, Heidelberg, Germany) and CFP (Canon CR-6-45NM; Canon, Saitama, Japan) were performed on all participants. Volume scans of SD-OCT were performed using a protocol consisting of 49 B-scans of the central 20 × 20° area, with 25 frames averaged for each B-scan.

Grading of Imaging and Analysis of Microperimetry

The grading of CFP was performed on all participants with commercial equipment (OptimizePro; Digital Healthcare Image Management System, Digital Healthcare Ltd.), by an experienced grader. Volume scans of SD-OCT were then examined to determine the presence of nGA and drusen-associated atrophy. Briefly, nGA was defined as the presence of features including the subsidence of the OPL and INL, and/or the development of a hyporeflective wedge-shaped band within the limits of the OPL.3 Drusen-associated atrophy detected on SD-OCT was defined as an area with a loss of the RPE and IS/OS bands, resulting in increased signal transmission below Bruch’s membrane (BM) that is accompanied by loss of the external limiting membrane (ELM) and outer nuclear layer (ONL) in this area (see example in Fig. 4C).3 Note that drusen-associated atrophy was defined using SD-OCT alone, independent of whether GA was present on CFP or not. However, all areas of drusen-associated atrophy detected on SD-OCT did not appear as GA on CFP since it was an exclusion criterion in this study.

The locations of these atrophic areas were then determined on the NIR image of the combined NIR and SD-OCT volume scans. The near-infrared reflectance image obtained from the microperimeter was then used to determine whether these areas were sampled by one of the microperimetric test stimuli; the entire test stimuli had to fall within the nGA area (outlined by the loss of the ELM band on SD-OCT), otherwise the area was not included in the analyses. For eyes where the atrophic area was sampled on microperimetry, three reference areas were then chosen to allow a comparison of the retinal sensitivity with other areas within the same eye. These areas were chosen using the three opposite and perpendicular points within the same ring on microperimetry (Fig. 2), and the AMD pathology present in these areas were then determined on CFP, NIR, and SD-OCT. Pigmentary changes associated with AMD were defined as the presence of hyperpigmented material on CFP that appeared as hyperreflective foci on SD-OCT.

The pathological changes in the reference areas were then separated into three groups of different disease severity with an adequate sample size in each group to provide meaningful analyses for comparison with areas with nGA or drusen-associated atrophy detected on SD-OCT. These groups included: group 1, areas without any AMD-associated pathology or with drusen ≤125 μm; group 2, areas with drusen >125 μm and/or RPD only; and group 3, areas with at least AMD-associated pigmentary changes. Areas of nGA and drusen-associated atrophy detected on SD-OCT were considered groups 4 and 5, respectively.

Statistical Analysis

A linear mixed-effects model was used to compare the mean retinal sensitivity between different groups of different pathological changes, while accounting for the correlation between eyes and between points in an eye. The group of pathological change and eccentricity of the point were
considered fixed effects, while the points nested within an eye nested within a patient was considered a random effect; a post-hoc Bonferroni correction was used to account for multiple comparisons. All statistical analyses were performed using commercially available statistical software (SPSS, software version 21; IBM/SPSS, Inc., Chicago, IL, USA).

RESULTS

From the participants involved in our prospective study of structural and functional changes in intermediate AMD, a total of 24 eyes from 22 AMD participants were identified as having a microperimetric stimulus location sample an atrophic area (nGA or drusen-associated atrophy detected on SD-OCT). These participants were on average aged 70.3 ± 6.4 years (ranged between 57 to 83 years).

In the AMD participants, 17 areas of nGA in 16 eyes and 11 areas of drusen-associated atrophy detected on SD-OCT in eight eyes fell within areas sampled by microperimetry. From these 28 areas of nGA and drusen-associated atrophy detected on SD-OCT, 84 reference areas within the same eccentricity were included. These reference areas were grouped according to the pathological features present: group 1 consisted of 26 areas without any AMD-associated pathology or having drusen ≤125 μm; group 2 consisted of 39 areas with drusen >125 μm and/or RPD only; and group 3 consisted of 19 areas with at least AMD-associated pigmentary changes. Areas of nGA and drusen-associated atrophy detected on SD-OCT were considered groups 4 and 5, respectively.

The mean microperimetric retinal sensitivity of each group of different AMD pathology severity was then determined, with 27.6 ± 0.6 dB, 27.1 ± 0.5 dB, 23.8 ± 0.7 dB, 20.4 ± 0.8 dB and 16.4 ± 0.9 dB for groups 1 through 5, respectively. Retinal sensitivity was significantly worse with increasing severity of AMD pathology in all pairwise comparisons \((P < 0.008)\), except between group 1 (areas without any AMD-associated pathology or drusen ≤125 μm) and group 2 (areas with drusen >125 μm and/or RPD only; \(P = 1.000\)). Specifically, areas with nGA (group 4) had worse retinal sensitivity than nonatrophic areas (groups 1 through 3; \(P < 0.008\)), but better retinal sensitivity than areas with drusen-associated atrophy (group 5; \(P = 0.008\)). These findings are illustrated in Figure 3.

However, we also observed that the retinal sensitivity overlying nGA was not always the worst performing point (the test point in an eye with the lowest sensitivity amongst the 37 points tested) in an individual eye. There were only 6 out of 16 eyes (37.5%) where the retinal sensitivity overlying an area of nGA (the area of nGA with the poorest retinal sensitivity was used if more than one area was present) was the worst performing point in that eye (examples in Fig. 4).

However, in eight out of eight eyes (100.0%), the worst-performing point was overlying an area of atrophy detected on SD-OCT (again, using the area with the worst retinal sensitivity

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**Figure 1.** Features defined as nGA as visualized on SD-OCT. **Top row:** The first characteristic feature is the subsidence of the INL and OPL. Other features are typically present at this stage, including a break in the ELM, disruption of the ISe and RPE bands, and traces of increased signal transmission below the RPE. The solid black line outlines the border between the INL and OPL from this scan, and the dashed black line outlines its border at a previous visit when these features were not present. **Bottom row:** The second characteristic feature was the presence of a hyporeflective wedge-shaped band (outlined by black solid lines) within the limits of the OPL that subsequently develops as the characteristic feature of this stage. There is also typically drusen regression (white dashed line) that is accompanied by a vortex-like subsidence of the INL and OPL at this stage.

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**Subsidence of the OPL and INL**

(i) Subsidence of the OPL and INL

Features frequently present:

(ii) Disruption of the ISe and RPE band

(iii) Break in ELM

(iv) Traces of increased signal transmission below BM

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**Hyporeflective Wedge-Shaped Band**

(i) Hyporeflective wedge-shaped band

Features frequently present:

(ii) Vortex-like subsidence of OPL and INL

(iii) Drusen regression

(iv) Traces of increased signal transmission below RPE

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if more than one area was present). Areas of atrophy detected by SD-OCT (and not detected on CFP as GA) were not associated with absolute scotomas, unlike areas of established GA (examples in Fig. 5).

**DISCUSSION**

In this study, we used microperimetry to explore retinal sensitivity overlying areas of nGA, which we have recently described as features on SD-OCT that have been found to portend the development of drusen-associated atrophy in AMD. On SD-OCT, the features of nGA include the subsidence of the OPL and INL, and development of a hyporeflective wedge-shaped band within the limits of the OPL. In this study, reference areas within the same eye were examined to allow a comparison of the retinal sensitivity in areas of nGA with other pathological features of intermediate AMD.

It has been previously found that retinal sensitivity was reduced in areas with large drusen, RPD and pigmentary abnormalities when compared with areas or eyes without such features. In this study, we found that retinal sensitivity was on average further significantly reduced in areas of nGA compared with areas with drusen, pigmentary abnormalities, or RPD. These areas of nGA were usually, but not always, the worst-performing point in an eye (among the 37 points tested), whereas when areas of drusen-associated atrophy were detected on SD-OCT, we found that they were consistently the worst-performing point in the eye.

Changes in function over areas of drusen may relate to changes in associated structures such as the integrity of the ISe band, or the RPE elevation as previously reported. For example, areas with large confluent drusen that are characterized by marked elevation of the RPE and disruption of the overlying ISe band can have dramatically reduced retinal sensitivity.

**FIGURE 2.** Microperimetry findings (retinal sensitivity overlaid on the near-infrared reflectance image captured by the microperimeter) in an eye with drusen-associated atrophy as detected on SD-OCT are shown to illustrate how reference areas were determined. The microperimetric point overlying an atrophic area (either nascent geographic atrophy or drusen-associated atrophy detected on SD-OCT) was first identified (as indicated by the solid box in this example). Reference areas (without any atrophic changes) were then determined using the three points within the same ring that was opposite and perpendicular to the atrophic area (as indicated by the dashed boxes in this example).

**FIGURE 3.** Mean microperimetric retinal sensitivity for areas grouped by AMD pathological features, showing worsening of retinal sensitivity with more severe features; the retinal sensitivity was significantly different between pairwise comparisons of all groups ($P < 0.008$; Bonferroni adjusted), except between groups 1 and 2 ($P = 1.000$). The microperimetric retinal sensitivity was worse in areas of nGA (group 4) compared with nonatrophic areas (groups 1 through 3; $P < 0.008$), but better when compared with areas of drusen-associated atrophy (group 5; $P = 0.008$). The *error bars* represent 95% confidence intervals of the mean.

**Group Number**

1. Drusen ≤125 μm / No Pathology
2. Drusen >125 μm or RPD
3. AMD Pigmentary Changes
4. Nascent Geographic Atrophy
5. Drusen-Associated Atrophy detected on SD-OCT

**Abbreviations**

SD = Standard Deviations  
AMD = Age-related Macular Degeneration  
RPD = Reticular Pseudodrusen  
SD-OCT = Spectral-Domain Optical Coherence Tomography
FIGURE 4. Examples of microperimetry over nGA (indicated by black arrows) shown on a SD-OCT B-scan and the CFP. Note that areas of nGA and its adjacent reference area are highlighted by vertical-colored boxes. (A) Example showing reduced retinal sensitivity (red box) overlying an area of nGA (black arrow) compared with an adjacent reference area (green box); this was the worst-performing point of this eye among the 37 points tested. (B) Example showing reduced retinal sensitivity (yellow box) overlying an area of nGA (black arrow) similar to its adjacent reference area (orange box), but the retinal sensitivity overlying the large, elevated druse (indicated by the white arrow) was the worst-performing point in this eye.

FIGURE 5. Examples of microperimetry over (A) an area of drusen-associated atrophy detected on SD-OCT and (B) over GA. The microperimetry findings in these areas are overlaid on an SD-OCT B-scan (bottom row) that was taken through an area on the corresponding CFP (top row). (A) The first example shows residual retinal sensitivity (vertical red box) present in an area with drusen-associated atrophy detected on SD-OCT (black arrow) and the adjacent reference area (vertical green box); the area with drusen-associated atrophy was the worst-performing point in that eye among the 37 points tested. (B) The second example (which was not a participant included in this study) shows areas of absolute scotoma (indicated by a “#,” and two examples along the SD-OCT B-scan that are highlighted by vertical black boxes) in an eye with GA that is visible on CFP.
sensitivity. In areas of nGA, there is disruption of the ISe band, but often not large elevations of the RPE since drusen regression has typically occurred in these areas. Reduction in retinal sensitivity may therefore be quite substantial in areas with confluent drusen, and at times be the worst-performing point in an eye as we observed in this study, even when nGA is present. Note that these findings may also account for the absence of a significant difference between areas with drusen < 125 μm or no AMD-associated pathology (group 1) and areas with drusen > 125 μm or RPD (group 2) observed in this study, since the integrity of the ISe band or extent of RPE elevation are not captured in the grading of these pathological features.

This study also noted that areas with nGA were not characterized by absolute scotomas, and the retinal sensitivity in areas of nGA was on average better than areas with drusen-associated atrophy detected on SD-OCT. These findings suggest the possibility that areas with nGA may not yet be characterized by a complete loss of photoreceptors. Although we have found previously that the ISe band was often disrupted or absent in areas with nGA, it has been shown that the integrity of the ISe band can improve following drusen regression, suggesting that the absence of the ISe band does not necessarily indicate that photoreceptors are irretrievably lost. However, it is not possible to determine from the findings of this study the true underlying pathological changes contributing to the decreased retinal function.

All areas identified as having drusen-associated atrophy on SD-OCT (but not on CFP) also had residual retinal function, unlike areas of well-established GA that are associated with absolute scotomas. These findings are interesting given our previous findings that flicker perimetry sensitivities were reduced and show an increased rate of reduction in areas that subsequently went on to develop GA. We speculate that either nGA or drusen-associated atrophy were likely to be present, but undetected (since SD-OCT was not available when that study was conducted), prior to the detection of GA on CFP and contributed to the reduction in retinal sensitivity. It is possible that alignment error, shifts in the area of retina sampled between frames tracked by the imaging system of microperimetry and light scatter contribute to the finding of residual function, especially when measuring these smaller areas of atrophic changes. However, it is also possible that residual function is present because some photoreceptors may still be present in these areas of drusen-associated atrophy, but may be missed between the B-scans of the SD-OCT or not visualized on the B-scans when disrupted due to their altered reflective properties.

The findings of this study have important implications for our understanding of the structural and functional changes in eyes with intermediate AMD, especially in areas that are on the pathway to developing atrophic changes. It is important that we recognize and appreciate these changes that help us determine the disease severity, and the potential reversibility of these changes, as we enter an era where targeted interventions for the early stages of AMD are being developed. For interventions targeted at preventing the progression of drusen-associated atrophy, it may be better to intervene at an earlier stage when nGA is present, since retinal function is not yet completely lost. Novel interventions could potentially examine whether the progression from nGA to drusen-associated atrophy can be slowed, halted, or even potentially reversed. For the evaluation of other interventions targeted at the earlier stages of AMD, the development of nGA may be a more useful endpoint than the current endpoint of GA detected on CFP. It is also important to appreciate through the findings of this study that significantly reduced retinal sensitivity is not specific to the underlying pathological changes, and may confer different long-term implications. However, given the limitations of the cross-sectional nature of this study, future longitudinal studies are required to examine these important implications.

In summary, retinal sensitivity was on average reduced in areas with nGA compared with other pathological features of intermediate AMD, although areas of nGA were not always the worst-performing point in an eye. Areas with drusen-associated atrophy detected on SD-OCT (but not on CFP) were always the worst-performing point, but were not associated with an absolute scotoma, unlike GA. These findings provide further insight into the structural and functional changes in intermediate AMD and provide important new information to consider when planning clinical interventional studies for the early stages of AMD.

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References


