Identification of functional markers for detecting vision loss in early and intermediate age-related macular degeneration

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List of abbreviations

AMD = Age-related macular degeneration

iAMD = Intermediate age-related macular degeneration

VA = Visual acuity

BCVA = Best corrected visual acuity

LLVA = Low luminance visual acuity

MAC = Moorfields Vanishing Optotypes Acuity Charts

IReST = International Reading Speed Texts

VRQoL = Vision-related quality of life

IVI = Impact of Vision Impairment questionnaire

CoR = Coefficient of repeatability

CNN = Convolutional neural network

ETDRS = Early Treatment Diabetic Retinopathy Study

RPE = Retinal pigment epithelium

BM = Bruch's membrane

OCT = Optical Coherence Tomography

ICH-GCP = International Conference on Harmonization of Good Clinical Practice

SLO = Scanning laser ophthalmoscope

PWS = Pointwise sensitivity

MS = Mean sensitivity

dB = Decibel

SD = Standard deviation

Publications

• **Welker SG**, Pfau M, Heinemann M, Schmitz-Valckenberg S, Holz FG, Finger RP. Retest Reliability of Mesopic and Dark-Adapted Microperimetry in Patients With Intermediate Age-Related Macular Degeneration and Age-Matched Controls. *Invest Ophthalmol Vis Sci 2018*; 59(4):AMD152-AMD159. doi: 10.1167/iovs.18-23878.

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• **Pondorfer SG**, Wintergerst MWM, Gorgi Zadeh S, Schultz T, Heinemann M, Holz FG, Finger RP. Association of Visual Function Measures with Drusen Volume in early stages of Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci 2020*

Henceforth referred to as *Drusen Volume Study*

1. Short Summary

Patients in the early and intermediate stages of age-related macular degeneration (AMD) often perform well in conventional visual function tests under high luminance and high contrast, whereas testing under dim light and low contrasts shows functional impairment. The functional deficit under reduced luminance and/or contrast has been well documented in patients with early and intermediate AMD (iAMD) using a number of different functional assessments such as low luminance visual acuity (LLVA), visual acuity (VA) measurements with the Moorfield Vanishing Optotypes Chart (MAC), contrast sensitivity tests and fundus-controlled perimetry. However, to date it is unclear which functional tests are most impacted in patients with early and iAMD and which tests are able to discriminate between different stages of AMD. As AMD affects vision-related quality of life (VRQoL) already in the earliest stages of the disease, it is important to investigate the relationship between visual function tests and VRQoL. Further, the relationship between visual function tests under low luminance and low contrast and measurements of retinal structure associated with AMD progression, such as drusen volume, has not been well characterized so far. Against this background we first determined the intrasession test-retest reliability of mesopic and dark-adapted fundus-controlled perimetry in patients with intermediate AMD and found good pointwise sensitivity test-retest among both testing types and in both groups (coefficient of repeatability of 4.4, 4.52, 3.96, and 4.56 dB, respectively). In order to assess which visual function measures are most strongly associated with VRQoL, we conducted a cross-sectional study and subjects were interviewed with the Impact of Vision Impairment (IVI) questionnaire. We found that patients with late AMD had significant lower IVI scores on all three subscales compared with iAMD and early AMD ($p < 0.011$). In the overall cohort, IVI subscales were associated with best corrected visual acuity (BCVA), LLVA, MAC-VA and contrast sensitivity (all $p < 0.001$), whereas a subgroup analysis, including only patients with early and iAMD, revealed that the IVI reading and mobility subscale was significantly associated with MAC-VA ($p < 0.013$). Moreover, we assessed which visual function measures are most strongly associated with overall retinal drusen volume in AMD. Drusen volume was automatically determined based on optical coherence tomography using a convolutional neural network (CNN) based approach. Mean drusen volume and MAC-VA significantly differed between

all AMD stages and controls ($p < 0.001$). After controlling for AMD stage, age and the presence of reticular pseudodrusen MAC-VA, mesopic and dark-adapted microperimetry were still significantly associated with drusen volume ($p = 0.008$, $p = 0.023$ and $p = 0.022$, respectively). Our results suggest that MAC-VA as well as mesopic and dark-adapted microperimetry might indicate structural changes related to drusen volume in early stages of AMD and are useful, patient-relevant measures of visual impairment in AMD.

2. Introduction

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the elderly in industrial countries (1). Late AMD can severely reduce visual acuity while patients in early and intermediate stages often perform well in conventional visual function tests under high-luminance and high-contrast conditions (2, 3). However, self-reported visual problems under low lighting and poor contrast have been documented in these stages (4, 5). Persons with early stages of AMD often complain about vision loss under low lighting, low contrast and changing light conditions, which also impact vision-related quality of life (VRQoL) (4, 6, 7). Several studies have demonstrated the functional deficit under reduced luminance and/or contrast in patients with early and intermediate AMD (iAMD) (8, 2) and the impairment of rod-mediated dark adaptation (9–12). Nevertheless best corrected visual acuity (BCVA) measured with a high-contrast high-luminance chart with single black optotypes on a white background, such as the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, is the most widely used functional outcome measure in ophthalmic research (13, 14). These conventional charts appear to be largely insensitive to the specific functional impairment in early and iAMD and underestimate the disease extent (15, 14, 13). Therefore, there is a lack of functional tests sensitive to disease severity and progression in the early stages of AMD (5, 16). Usually the first clinical sign of AMD are drusen located between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane (BM), in the sub-RPE-basal laminar space (17, 18) and drusen are among the most important biomarkers for staging AMD (19, 20). A recently developed convolutional neural network (CNN) based approach for a fully automated segmentation of drusen in OCT images (21) allows to compute the overall drusen volume. The relationship between visual function tests under low luminance and low contrast and measurements of retinal structure associated with AMD progression, such as drusen volume, has not been well described so far.

Recent studies have shown that functional deficits in early stages of AMD can be detected by measuring retinal sensitivity determined by fundus-controlled perimetry, also called “microperimetry” (14, 22–24). Impaired mesopic and scotopic sensitivity have been spatially correlated with the presence of both large soft drusen and focal abnormalities on fundus autofluorescence intensities (25–27). Studies have also reported that low

luminance visual acuity (LLVA) (5, 8, 28) and contrast sensitivity (29–32) are significantly reduced in early and iAMD. Visual acuity measurements with the Moorfields Vanishing Optotypes Acuity Charts (MAC-VA), which employs high-pass filtered letters, has also been demonstrated to be more sensitive in detecting early AMD compared to BCVA, hypothesizing that recognition of this high-pass letters is more vulnerable to photoreceptor dysfunctions (33). Thus, LLVA, MAC-VA, contrast sensitivity and microperimetry are believed to be more sensitive to earlier macular changes than BCVA and may be potential endpoints for clinical trials of early and iAMD patients (5, 34). However, to date no study has employed all functional tests previously identified as sensitive to the special functional impairment in early stages of AMD and compared their ability to discriminate between early and iAMD and healthy controls. This is required in order to inform selection of the best test or combination of tests in future observational or interventional studies assessing functional impairment in early and iAMD. Thus, we evaluated and compared an extensive battery of functional tests in patients with early, iAMD and healthy controls. We first determined the intrasession test-retest reliability of mesopic and dark-adapted fundus controlled perimetry in patients with iAMD (*Reliability Study*). To further characterize the relationship between function tests and their patient-relevance – which is an important pre-requisite for any functional test – we investigated which tests are most strongly associated with VRQoL in different stages of AMD (*VRQoL Study*). And we evaluated the relationship between drusen volume and a battery of visual function tests under low luminance and low contrast, as structural and functional measures may provide complementary information about disease status (*Drusen Volume Study*).

3. Materials and Methods

The studies took place at the Department of Ophthalmology, University of Bonn, Germany from December 2016 to January 2019. All projects were supported by the German Scholars Organization/Else Kröner Fresenius Stiftung (GSO/EKFS 16).

3.1 Ethics Approval

These investigations were approved by the Institutional Review Board of the University of Bonn, Germany (approval ID: 013/16). Written informed consent was obtained from all participants following an explanation of all tests involved. The protocol followed the tenets of the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice (ICH-GCP).

3.2 Participants

For all studies the participants were recruited from the AMD outpatient clinic at the Department of Ophthalmology, University of Bonn, the self-help organization Pro Retina and family members of patients. 23 patients with iAMD (67.3 ± 8.2 years; 78 % female) and 24 healthy controls (61.3 ± 5.2 years; 50 % female) were included in the *Reliability Study*. A total of 90 participants were included in the *VRQoL Study* including 10 patients with early AMD (69.9 ± 6.1 years; 70 % female), 42 patients with iAMD (69.7 ± 7.7 years; 73.8 % female) and 38 patients with late AMD (79.8 ± 5.9; 68.9 % female). In the *Drusen Volume Study* a total of 90 patients were recruited comprising 16 eyes with early AMD (70.0 ± 6.7 years; 68.8 % female), 62 with iAMD (69.7 ± 7.3 years; 67.7 % female) and 22 eyes from healthy controls (59.8 ± 6.3; 59.1 % female).

3.3 Functional testing

3.3.1 Reliability Study

a) Objective visual acuity

To compare visual function of every participant, they underwent automated refraction followed by visual acuity testing using an autorefractor (ARK-560A, Nidek; Gamagori, Japan), following the standard operating procedures for every patient in the eye hospital.

b) Mesopic and dark-adapted microperimetry

Microperimetric testing was performed using a modified version of the macular integrity assessment microperimeter for scotopic testing (S-MAIA; CenterVue, Padova, Italy). This device has two additional projection LEDs and the ability to reduce the line-scanning laser ophthalmoscope (SLO) laser power for scotopic testing. All patients underwent two mesopic and two dark-adapted microperimetric examinations using the modified S-MAIA device with small breaks (maximum 5 minutes) between the examinations. Prior to testing, pupillary dilation was performed using 1.0 % tropicamide. The S-MAIA performs fundus tracking using an SLO with a super-luminescent diode illumination with a central wave light of 850 nm for mesopic testing. An additional LED projecting red (627 nm) stimuli was used for dark-adapted testing. A customized stimulus grid was used that consisted of 33 points located at 0°, 1°, 3°, 5°, and 7° from fixation. First mesopic testing was performed. Patients were not dark-adapted, but the room light was switched off just before the examination. After mesopic testing, patients underwent 30 minutes of dark adaptation while waiting in the examination room (light was switched off, light level < 0.1 lux). Then dark-adapted testing was performed. For mesopic testing, achromatic stimuli (400–800 nm) were presented using a 4-2 staircase threshold strategy, while patients observed the fixation ring against a background of 1.27 cd/m^2. The dynamic range is 36 dB. For dark-adapted testing, red stimuli (627 nm) were presented, also using a 4-2 staircase strategy with a dynamic range for scotopic testing of 36 dB and no background illumination.

3.3.2 VRQoL Study

a) Visual function tests

All participants of the *VRQoL Study* underwent the following best-corrected visual function tests: BCVA, LLVA, MAC-VA and contrast sensitivity in measurement using Pelli-Robson Charts. BCVA was assessed according to the ETDRS method (35). LLVA was assessed in the way, but with a 2.0.-log unit density filter that reduces luminance by 110 fold placed in the trial frame (28). VA measurement with MAC charts followed the same procedure as BCVA testing. The MAC charts are based on the ETDRS charts and employ a high-contrast, high-pass letter design with a gray background of the same mean luminance as the letters to simulate lower contrast situations (33). Contrast sensitivity was measured using a Pelli-Robson chart presented at a distance of one meter (36–38). In patients with early and iAMD, we additionally assessed reading speed using the International Reading Speed Texts (IReST) (39) and macular sensitivity via mesopic and dark-adapted microperimetry. For the IReST, patients wore their best near correction and were asked to read one paragraph aloud while they were timed with a stopwatch. Mesopic and dark-adapted microperimetry were performed in the same manner than in the *Reliability study* (see 3.3.1. b)), with the exception that all patients underwent only one instead of two microperimetric examinations, i.e. one mesopic testing and then one dark-adapted testing.

b) Evaluation of VRQoL

VRQoL was evaluated using the German language version of the Impact of Vision Impairment (IVI) questionnaire (40). The IVI is a validated, reliable and commonly used VRQoL instrument and has been validated psychometrically for different ocular conditions and different levels of visual acuity (40, 41). It consists of 28 items with three to four responses options using Likert scales, ranging from “not at all” to “a lot”. The IVI has three specific subscales: “Reading and Accessing Information” (9 items; abbreviated as reading subscale), “Mobility and Independence” (11 items; abbreviated as mobility subscale) and “Emotional Well-being” (8 items; abbreviated as emotional subscale).

3.3.3 Drusen Volume Study

a) Visual function tests

For all patients the same visual function tests were performed as described in the VRQoL study in 3.3.2. a)

b) Automatic segmentation of drusen

For automated segmentation of drusen, the pipeline in (21) was used. In the first step, this drusen segmentation pipeline automatically segments RPE and BM bands, using a convolutional neural network (CNN), which transforms an input B-scan into RPE and BM probability maps. For the final hard segmentation of RPE and BM bands, probability maps are converted into cost maps so that pixels with higher probability have less cost. Dijkstra's algorithm is used to find a path with the minimum accumulated cost from the left to the right of each map (42). In the second step, a normal RPE is estimated through a rectification of RPE and BM bands. In the rectification step both RPE and BM are shifted vertically and column-wise until the BM band becomes a straight horizontal line. Then a low degree polynomial is fitted on the shifted RPE band and transformed back into the original image coordinates and is regarded as the drusen-free RPE. Finally any area that is between RPE and drusen-free RPE is classified as drusen. To eliminate falsely detected drusen, those with a height of 2 pixels or less are removed from the final segmentation.

3.4 Data analysis

Data of all studies were recorded electronically using Excel (Version 14.0, Microsoft, Washington, USA). For the *Reliability Study*, pointwise sensitivity (PWS) intrasession test-retest reliability for the mean sensitivity (MS) of all test points for mesopic and dark-adapted testing was assessed by the 95% coefficient of repeatability (CoR) as recommended by Bland and Altman (43). Statistical analyses were performed using the statistical software R (44). For the *VRQoL Study*, psychometric evaluation of the IVI was performed using Rasch analysis, a psychometric method that transforms ordinal scales into interval-level scales (expressed in logits) (45, 46). Rasch analysis was performed using commercial software (Winsteps software, ver. 3.92.1.2, Chicago, IL) (47). Groups

were compared using Wilcoxon rank-sum tests. Linear regression analysis was carried out to assess the relationship between IVI subscale scores (expressed in person measures in logits) and each of the visual function tests. We additionally performed a subgroup analysis, which included only patients with early and iAMD. Statistical analyses were performed using the statistical software STATA (48). For the *Drusen Volume Study* the Kruskal-Wallis test was used for group comparisons. Pairwise differences were calculated using the nonparametric Wilcoxon rank sum test. Univariate linear regression models against drusen volume were performed for each of the visual functional tests. If the relationship between a functional test and average person measures reached a p-value < 0.05 in univariate analysis, multiple regression was used to ensure that the findings were not confounded by different demographic characteristics across groups. Statistical analysis for the *Drusen Volume Study* was using the statistical software SPSS (49). For all three studies, *p*-values below 0.05 were considered statistically significant.

4. Results

Results of the three studies are described in the following paragraphs.

4.1 Reliability Study

Point-wise test-retest reliability was good among mesopic and dark-adapted testing in both iAMD patients and controls: the CoR was 4.4 dB for mesopic and 4.52 dB for dark-adapted testing in patients with iAMD and 3.96 dB for mesopic and 4.56 dB in control persons. For mesopic and dark-adapted testing, the CoRs were in the same range across all eccentricities in both groups. For all testing types and in both groups the average MS was higher in the first test. The mean difference of MS between test 1 and test 2 was 0.22 dB in mesopic and 0.40 dB in dark-adapted testing in iAMD patients. In the control group the difference between the two tests was greater with 0.41 dB in mesopic and 0.38 dB in dark-adapted testing. The differences were statistically significant (all p-values < 0.001). Pooled (test 1 + test 2) MS was significantly lower in iAMD patients compared to controls, while the difference was slightly higher for mesopic testing: in iAMD patients, pooled MS was 23.01 ± 3.3 dB and in the control group 25.63 ± 2.29 dB with a difference of 2.62 dB between the groups. For dark-adapted testing the difference was 2.49 dB: pooled MS in the iAMD group was 19.92 ± 4.06 dB vs. 22.41 ± 2.54 dB in the control group. To provide further information, the deviation in relation to the inter-eye variability in the control group (z-score) was calculated, which also takes in account the variability in the control group. On average the mean mesopic retinal sensitivity was 1.09 SD lower than the mean of control eyes, while mean dark-adapted retinal sensitivity was 1.07 SD lower. There was no difference of average MS between the different eccentricities in both groups for mesopic and dark-adapted testing. The pooled mean test duration was higher in controls than in iAMD patients with a greater difference for dark-adapted testing: For mesopic testing, the difference was 2.62 seconds (p = 0.04), while for dark-adapted testing the difference was greater with 8.55 seconds (p < 0.01).

4.2 VRQoL Study

All functional tests were significantly decreased in iAMD and late AMD compared to the early AMD group (all p-values < 0.001). There was no significant difference in BCVA between early and iAMD (p = 0.553), as well as in reading speed (p = 0.617) and mesopic

and dark-adapted microperimetry ($p = 0.274$ and $p = 0.141$). LLVA, MAC-VA and contrast sensitivity were significantly decreased in iAMD compared to the early AMD group ($p = 0.023$, $p = 0.041$ and $p < 0.001$ respectively). The data for the IVI were fitted to the Rasch model and key indicators of fit were explored. Overall, the psychometric testing supported the use of the three IVI subscales in this sample and demonstrated satisfactory PSI and PR for all subscales. No items had to be removed due to misfit or DIF. Mean person measures on all three subscales were significantly lower in subjects with early and iAMD compared to patients with late AMD. (Higher values of person measures indicate lower visual ability and indicate poorer VRQoL). There was no significant difference between early and intermediate AMD (all *p*-values > 0.662). Participants ≤ 75 years reported a better VRQoL in all subscales compared to the older age group > 75 years (all *p*-values < 0.011). In univariate regression analysis, age and AMD stage were significantly associated with all three scales of the IVI. In univariate linear regression, person measures of all three scales were negatively associated with BCVA, LLVA, MAC-VA and contrast sensitivity in the overall cohort. After controlling for age and AMD stage, multiple regression analysis showed that BCVA and MAC-VA remained significantly associated with all three IVI scores. LLVA was still significantly associated with the reading and mobility scales and contrast sensitivity only with the mobility scale. Contrast sensitivity and MAC-VA had the strongest associations with all scales. Analyzing only subjects with early and intermediate AMD, BCVA, LLVA and MAC-VA were associated with the reading scale and BCVA and MAC-VA with the mobility scale. In the adjusted analysis BCVA, LLVA and MAC-VA were still significantly associated with the reading scale and MAC-VA with the mobility scale. The IReST and macular sensitivity on mesopic and dark-adapted microperimetry showed no association with any of the scales of the IVI.

4.3 Drusen Volume Study

Mean drusen volume was found to be close to zero for controls (0.00024 mm^3 ± 0.0003). For early AMD, mean drusen volume was higher (0.00272 mm^3 ± 0.0015), and volume was again higher for iAMD (0.13582 mm^3 ± 0.1945). Age was not significantly associated with drusen volume ($p = 0.642$). Early and iAMD patients were found to have a significantly larger drusen volume when compared to controls (each p-value < 0.001) and iAMD patients also had significantly larger drusen volume compared to early AMD ($p < 0.001$).

All visual function tests were significantly decreased in iAMD compared to controls (all *p*-values < 0.05). In early AMD, BCVA and MAC-VA were also significantly decreased compared to controls ($p = 0.016$ and $p = 0.006$ respectively), but there was no difference in all other functional tests between the two groups (all *p*-values > 0.05). When comparing early AMD to iAMD, BCVA and reading speed did not differ significantly ($p = 0.31$ and $p = 0.07$), but there was a significant decrease in LLVA, MAC-VA, contrast sensitivity and mesopic and dark-adapted microperimetry in iAMD compared to the early AMD group (all p-values < 0.05). In univariate linear regression LLVA, MAC-VA, contrast sensitivity and mesopic and dark-adapted microperimetry were significantly negatively associated with the overall drusen volume (all $p < 0.006$). After controlling for AMD stage, age and the presence of reticular pseudodrusen, MAC-VA and global mesopic and dark-adapted microperimetry were still significantly associated with drusen volume ($p = 0.008$, $p = 0.023$ and $p = 0.022$ respectively). For mesopic and dark-adapted microperimetry, mean sensitivity at 0° – 1° and 3° degrees was significantly associated with drusen volume, while mean sensitivity at 5° and 7° was not associated with drusen volume after adjusting for AMD stage, age and the presence of reticular pseudodrusen.

5. Discussion

In the thesis projects I found that especially functional tests of central retinal function under low luminance and challenging contrast conditions were most impacted in early and iAMD. These tests were also mostly associated with VRQoL and drusen volume. The results of the *Reliability Study* show that the modified S-MAIA device allows for a reliable assessment of mesopic dark-adapted microperimetry in patients with iAMD. The results are comparable to previous findings from studies, which found mesopic microperimetry to be a good functional test for patients in the early and intermediate stages of AMD (36, 50, 8, 51, 5, 23). However, in the *VRQoL Study*, we did not find significant associations between any of the IVI scales with mesopic or dark-adapted microperimetric MS. Former studies revealed similar results: Wu et al. evaluated subjects with bilateral iAMD using a shorter 10-item Night Vision Questionnaire (NVQ-10) (3) and assessed the relationship of the NVQ scores with LLVA, low luminance deficit (LLD) and mesopic microperimetric mean sensitivity and central sensitivity. NVQ person measures were significantly associated with LLD, but not with LLVA or microperimetric measures. A study, which investigated whether scores of the Low Luminance Questionnaire were associated with objective measures of visual function in early and iAMD, did also not find any association with mesopic microperimetry (52). In regards to the association between VRQoL and visual function tests, BCVA, LLVA, MAC-VA and contrast sensitivity were significantly associated with all IVI subscales in patients with varying stages of AMD. However, MAC-VA and contrast sensitivity both showed a stronger relationship with the subscales of the IVI in the overall cohort than BCVA. A subgroup analysis, including only patients with early and iAMD, revealed a noticeable significant association between the reading and mobility subscales and MAC-VA. The MAC-VA is a relatively recent functional which simulates lower contrast situation. Shah and co-workers first demonstrated the MAC chart's ability to detect functional loss due to AMD when BCVA tested with EDTRS charts still was unaffected (33). They hypothesized that recognition of the high-pass letters is more vulnerable to photoreceptor dysfunction than conventional high luminance and high contrast letters. This is in accordance to our results as MAC-VA was not only significantly associated with VRQoL in the overall cohort, but also in the subgroup with only early and intermediate AMD patients. Moreover, results of the *Drusen Volume Study* revealed a

significant association between MAC-VA and drusen volume. We also found, that MAC-VA was the only functional test which differed significantly between all three groups, i.e. early AMD, iAMD and healthy controls. When comparing iAMD to controls, we found all visual function tests' performances significantly decreased in iAMD. These findings are in accordance with previous studies, which also reported a reduced visual function in these tests. Chandramohan and colleagues also found BCVA, LLVA and mesopic microperimetry significantly decreased in patients with iAMD compared to healthy controls (5). Similar results were reported by Wu et al. (8), who found these tests significantly reduced for all AMD groups except early AMD compared to controls, which is in line with our results. BCVA was on average four letters worse in the *Drusen Volume Study.* This is comparable to the results from Owsley et al. (53, 54) who reported a significant difference of two letters between patients with iAMD and controls. We also found LLVA and contrast sensitivity to be decreased in iAMD compared to control as well as compared to early AMD. However, we did not find a significant difference in these functional tests between early AMD and controls. Puell et al have shown that LLVA was impaired in early stages of AMD before changes in BCVA were observed (2). Feigl and colleagues reported decreased contrast sensitivity in early AMD compared to healthy controls (32). Results of the *Drusen Volume Study* revealed that drusen volume was found to be largest in the iAMD group and significantly lower in the early AMD group and in controls. Several other studies also have demonstrated that drusen volume increases with increasing AMD stage and is predictive to progression to late AMD (55–57). We calculated mean drusen volume of 0.0027 mm^3 for early AMD which is in lower than the values reported by Lei and coworkers, who found mean drusen volume of 0.03 (range 0.00 – 0.28) in eyes with early AMD (58). The difference could be explained by the small sample size in our early AMD group. The mean drusen volume measure we obtained for the iAMD group of 0.138 mm^3 is comparable to those reported by Yehoshua (59) who reported drusen volume measures of 0.095 – 0.375 mm^3 in the highest quintile for eyes with nonexudative AMD. We could show that drusen volume is associated with visual impairment detected by functional tests. This is in line with previous studies which found functional tests under low lighting to be correlated with retinal morphology in AMD (60, 61). Strengths of the *Reliability Study* was the use of a highly standardized testing protocol for mesopic and dark-adapted testing using a customized device performed by the same trained examiner. We could show that

the S-MAIA device yields highly reproducible microperimetric measurements in both testing types and therefore we used the same customized stimulus grid and testing procedure in the *VRQoL* and *Drusen Volume Study.* Strengths of the *VRQoL* and *Drusen Volume Study* include the wide range of functional tests including the relatively new MAC charts for which little data are available. In the *VRQoL Study* we assessed VRQoL using the German IVI, which is a validated instrument. We re-evaluated its psychometric performance and transformed responses into an interval-based scale for further statistical testing using Rasch Analysis. For the *Drusen Volume Study* we used a new CNN-based approach that allows for a fully automated segmentation of drusen in OCT images. Gorgi Zadeh et al. demonstrated that the CNN-based approach yields much better results than a previous state-of-the-art method by Chen et al. (62), and that it, therefore, allows for accurate automated assessment of drusen load in AMD (21). A limitation of all studies is the relatively small sample size. As common with exploratory studies, no adjustment for multiple testing was done in the *VRQoL* and *Drusen Volume Study*, which might lead to an over-estimation of statistical power.

In conclusion, we found that iAMD is associated with both reduced mesopic and dark-adapted retinal sensitivity, which can be assessed with the modified S-MAIA device that allows for reliable measurement. Performances of BCVA, LLVA, MAC-VA and contrast sensitivity are associated with all aspect of VRQoL in overall AMD, while in patients with earlier stages of AMD, BCVA, LLVA and MAC-VA are associated with VRQoL on the reading scale. In addition, MAC-VA is also correlated with VRQoL on the mobility scale, which suggests, that the MAC-VA might be a useful and patient-relevant measure of visual impairment in AMD, in particular in earlier stages. We also found that MAC-VA as well as mesopic and dark-adapted microperimetry are associated with drusen volume in early stages of AMD and might thus provide an indication of structural changes. Our results suggest that MAC-VA as well as mesopic and dark-adapted microperimetry might indicate structural changes related to drusen volume in early stages of AMD and are useful, patient-relevant measures of visual impairment in AMD.

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7. Attachments

Attachment 1:

- Paper 1: *Reliability Study*

Attachment 2:

- Paper 2: *VRQoL Study*

Attachment 3:

- Paper 3: *Drusen Volume Study*

Attachment 4:

- Impact of Vision Impairment (IVI) Questionnaire

Special Issue

Retest Reliability of Mesopic and Dark-Adapted Microperimetry in Patients With Intermediate Age-Related Macular Degeneration and Age-Matched Controls

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Purpose. To determine the intrasession test-retest reliability of mesopic and dark-adapted fundus-controlled perimetry in patients with intermediate age-related macular degeneration (iAMD).

Methods. We conducted a cross-sectional study with 23 iAMD patients (67.3 ± 8.2 years; range, 50–85; 78% female) and 24 healthy controls (61.3 ± 5.2 years; range, 50–71; 50% female) using a modified MAIA microperimeter. All patients underwent duplicate mesopic (achromatic stimuli, 400–800 nm) and dark-adapted (red stimuli, 627 nm) microperimetry, using a grid of 33 stimuli over 14° of the central retina. Main outcome measure was the intrasession test-retest reliability for pointwise sensitivity (PWS).

Results. PWS test-retest reliability was good among mesopic and dark-adapted testing in both patients and controls (coefficient of repeatability of 4.4, 4.52, 3.96, and 4.56 dB, respectively). Mean mesopic sensitivity in patients was 2.62 dB lower than in controls ($P < 0.01$); mean dark-adapted sensitivity was 2.49 dB lower than in controls ($P < 0.01$).

Conclusions. The modified MAIA device allows for reliable mesopic and dark-adapted microperimetry in iAMD patients. We found that iAMD is associated with both reduced mesopic and dark-adapted retinal sensitivity.

Keywords: microperimetry, age-related macular degeneration, rod function, test-retest, variability

Patients with intermediate age-related macular degeneration (iAMD) often perform well in visual function tests under high-luminance and high-contrast conditions, whereas testing under dim light and low contrasts shows functional impairment.[1,2] Furthermore, iAMD patients commonly require high ambient light for tasks such as reading and report difficulties, especially in performing daily activities, under low-luminance conditions.[3–5] Several studies have demonstrated that early AMD and iAMD patients have impairment of rod-mediated dark adaptation.[6–9] However, high-luminance high-contrast best corrected visual acuity (BCVA) is the most widely used functional outcome measure in clinical trials, although it underestimates the disease extent and is a poor measure for progression.[10,11] Therefore, there is a lack of functional tests sensitive to disease severity and progression in iAMD.

A good way to detect functional deficits in early stages of AMD is to measure retinal sensitivity determined by fundus-controlled perimetry (FCP), also called "microperimetry" or "gaze contingent perimetry."[11–16] Studies have shown that functional deficits detected by FCP are correlated to retinal pigment epithelium (RPE) elevation, thinning of the outer segment thickness, and disruption of the second hyperreflective band on spectral-domain optical coherence tomography.[17–19] Impaired mesopic and scotopic sensitivity have been spatially correlated with the presence of both large soft drusen and focal abnormalities on fundus autofluorescence intensities.[20–22]

While there are multiple reports on mesopic function in iAMD, less information about scotopic and dark-adapted FCP is available.[11,23–26] One study in AMD patients with reticular drusen (RDR) revealed that rod function is more severely affected than cone function in retinal areas with RDR.[22] This study was conducted with a modified version (MP-1S) of the MP-1 microperimeter (Nidek Technologies, Padua, Italy). A disadvantage of the MP-1S is the limited dynamic range of the stimulus-presenting liquid crystal display of 20 dB.[27] Different neutral density filters must be used for different patients based on their respective visual function.[27]

Recently, a modified version of the macular integrity assessment microperimeter for scotopic testing (S-MAIA; CenterVue, Padova, Italy) has been developed. This device has two additional projection LEDs and the ability to reduce the line-scanning laser ophthalmoscope (SLO) laser power for scotopic testing. A study with a prototype has yielded good test-retest reliability for the S-MAIA in mesopic and scotopic testing in normal subjects as well as in patients with various retinal diseases.[28–30] The latest version of the S-MAIA features an increased dynamic range for scotopic testing (36 instead of 20 dB). As it is important to establish the quality of measurement in order to be able to interpret the data generated, we assessed test-retest reliability of mesopic and dark-adapted microperimetry with the S-MAIA in iAMD patients and investigated the difference for mesopic and scotopic retinal sensitivity in iAMD patients compared to persons of normal retinal health in the same age range.

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Methods

We conducted a cross-sectional study at the Department of Ophthalmology, University of Bonn, Bonn, Germany, from December 2016 to July 2017. The study was approved by the Institutional Review Board of the University Bonn (approval ID: 013/16). Written informed consent was obtained from all participants following an explanation of all tests involved. The protocol followed the tenets of the Declaration of Helsinki.

Twenty-three patients with iAMD and 24 age-matched subjects with normal retinal health were recruited from the AMD outpatient clinic, the self-help organization Pro Retina, and family members of patients. Inclusion criteria for the iAMD group were drusen greater than 125 μm and/or any AMD pigmentary abnormalities according to the classification system introduced by Ferris et al.[31] For the control group, inclusion criteria was BCVA of 20/20 tested using an autorefractor (ARK-560A; Nidek, Gamagori, Japan). Exclusion criteria for both groups were age <50 years, the presence of choroidal neovascularization (CNV), geographic atrophy, significant cataract, any corneal pathology that could compromise vision, amblyopia, glaucoma, diabetes, neurologic or systemic disease affecting vision, refractive errors >6.00 diopters (D) of spherical equivalent and >2.00 D of astigmatism. One eye of each patient (the one with the better visual acuity) was included in the study. If both eyes fitted the inclusion criteria and had the same visual acuity, the right eye was chosen. In addition to BCVA and microperimetry, spectral-domain optical coherence tomography raster scanning was performed using a 25° × 25°-scan field (49 B-scans, automated real-time mode 20 frames, centered on the fovea), as well as fundus autofluorescence and infrared photography (Spectralis OCT2; Heidelberg Engineering, Heidelberg, Germany).

All patients underwent two mesopic and two dark-adapted microperimetric examinations using the modified S-MAIA device with small breaks (maximum 5 minutes) between the examinations. Prior to testing, pupillary dilation was performed using 1.0% tropicamide, and instructions were given to all patients regarding how to perform the examination. For mesopic testing patients who were not dark-adapted, the room light was switched off just before each examination.

The MAIA performs fundus tracking using an SLO with a super-luminescent diode illumination with a central wave light of 850 nm for mesopic testing. An additional LED projecting red (627 nm) stimuli was used for dark-adapted testing. A customized stimulus grid was used that consisted of 33 points located at 0°, 1°, 3°, 5°, and 7° from fixation (Fig. 1). The grid was designed in a manner to provide a relatively regular sampling density throughout the macular region with an increased density toward the fovea. This foveal-weighted design allows covering lesions of interest in AMD with adequate density while minimizing the number of stimuli to keep the examination time as short as possible.

A 645-nm red ring of 1° diameter was used as target of fixation. For mesopic testing, achromatic stimuli (400–800 nm) were presented using a 4-2 staircase threshold strategy, while patients observed the fixation ring against a background of 1.27 cd/m^2. The dynamic range is 36 dB. For dark-adapted testing, red stimuli (627 nm) were presented, also using a 4-2 staircase strategy with a dynamic range for scotopic testing of 36 dB and no background illumination.

Second tests were performed using the follow-up mode. All examinations were performed by a single experienced examiner in a darkened room. Room light was switched off during the mesopic testing and briefly switched on before the follow-up examination. After the two mesopic tests, all patients underwent 30 minutes of dark adaptation while waiting in the examination room (light was switched off, light level <0.1 lux). Two dark-adapted tests were then performed using the same device but presenting red stimuli. In all patients, only the study eye was tested, while the fellow eye was covered with an eye patch.

Statistical Analysis

Test reliability was assessed by the frequency of false-positive responses, measured by presentations of suprathreshold stimuli to the optic nerve head (i.e., blind spot, Heijl-Krakau method), which was manually located before the presentation of the first stimuli. Any participants with false-positive responses of more >33% were excluded from analysis.[11]

The primary outcome measure was the pointwise sensitivity (PWS) intrasession test-retest reliability for the mean sensitivity of all test points for mesopic and dark-adapted retinal sensitivity testing assessed by the 95% coefficient of repeatability (CoR) as recommended by Bland and Altman.[32] The CoR represents a value for which 95% of the test-retest differences for the same subject are expected to lie, which can be interpreted as the measurement error of the instrument combined with the subjective variability. A larger value of CoR hence represents a greater degree of test-retest variability.

The bivariate contour ellipse area (BCEA) was used for evaluation of fixation stability. It is the area of an ellipse (in degree) that covers either 63% or 95% of fixation points. After log transformation, the test-retest reliability was evaluated using the intraclass correlation coefficient (ICC) (after the Shapiro-Wilk test for normal distribution). Following this, retinal sensitivity in patients with iAMD and in persons with normal retinal health were compared using *t*-tests.

Statistical analyses were performed using the statistical software R.[33] Summary statistics (mean and standard deviation) were calculated for demographic and microperimetry performance data. Paired *t*-tests were used to compare the test duration and mean sensitivity between the first and second test. For comparison of mean sensitivity between the two persons group, an unpaired *t*-test was used. A P value < 0.05 was considered statistically significant.

Results

A total of 23 iAMD patients (67.3 ± 8.2 years; range, 50–85; 78% female) and 24 controls (61.3 ± 5.2 years; range, 50–71; 50% female) were included in the study. Twenty out of 23 iAMD patients had good BCVA of 20/25 or better, with the remaining three seeing at least 20/50. All controls had BCVA of at least 20/20. Nine patients were excluded from analysis because they did not match inclusion criteria (two patients with CNV, two patients with early AMD) or due to a false-positive response rate >33% (five individuals, three with iAMD and two healthy controls). Mean age of the five individuals with a high false-positive rate was 60.8 years, which did not significantly differ from the rest ($P = 0.39$). All patients and controls underwent the complete protocol, including duplicate mesopic and dark-adapted microperimetry. None of the persons had performed microperimetry previously.

Mean Sensitivity (MS)

For all testing types and in both groups the average MS was higher in the first test. In iAMD patients, the mean difference of MS between test 1 and test 2 was 0.22 dB in mesopic and 0.4 dB in dark-adapted testing. The difference between the two tests was greater in the control group with 0.41 and 0.38 dB, respectively. The difference was statistically significant ($P < 0.001$) (Table 1).

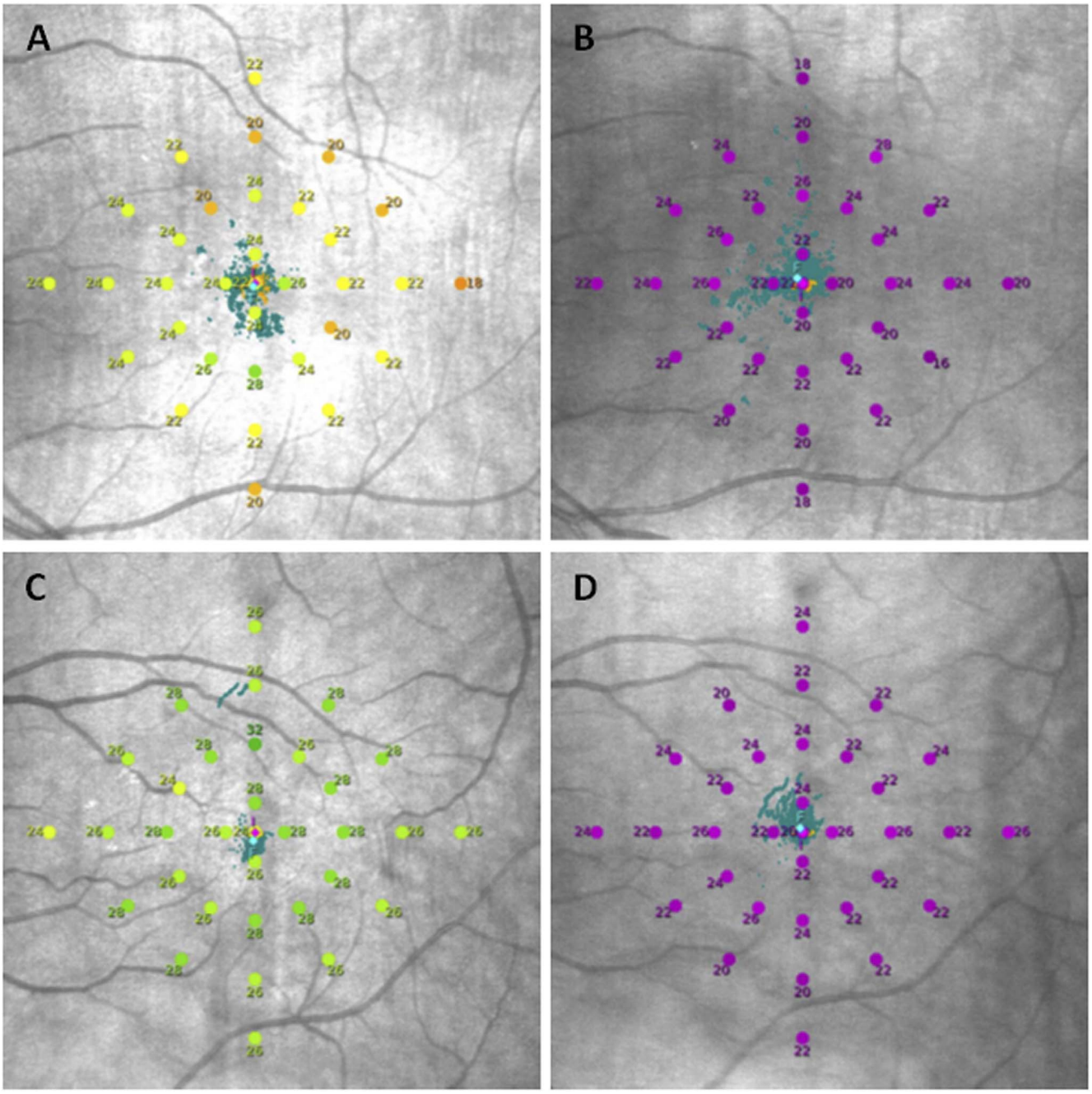

Figure 1. An exemplary report for mesopic and dark-adapted testing in iAMD patients and healthy controls. Each figure depicts the local retinal sensitivity of the patient superimposed on the SLO fundus photo. The numeric value represents the measured threshold in decibels. (**A**) Mesopic testing in iAMD. (**B**) Dark-adapted testing in in iAMD. (**C**) Mesopic testing in a control. (**D**) Dark-adapted testing in a control.

The pooled MS (first and second test) was significantly lower in iAMD patients compared to healthy controls as well as for mesopic and for dark-adapted testing ($P < 0.001$). The difference was slightly higher for mesopic testing: in iAMD patients, pooled MS was 23.01 dB (SD ± 3.3 dB) and in the control group 25.63 dB (SD ± 2.29 dB) (difference of 2.62 dB). For dark-adapted testing, the difference was 2.49 dB: the pooled MS in the iAMD group was 19.92 dB (SD ± 4.06 dB) versus 22.41 dB (SD ± 2.54 dB) in the control group. To provide detailed information we calculated the deviation in relation to the inter-eye variability in the control group (*z*-score), which also takes into account the variability in the control group. On average the mean mesopic retinal sensitivity was 1.09 SD lower than the mean of control eyes, while mean dark-adapted retinal sensitivity was 1.07 SD lower (Table 2). There was no difference of average MS between the different eccentricities in both groups for mesopic and dark-adapted testing. Table 1 shows the average MS for all eccentricities.

Test Duration

The pooled mean test duration (test 1 and test 2) was 4.25 minutes (SD ± 25.44 seconds) for iAMD patients and 4.2 minutes (SD ± 31.63 seconds) for controls in mesopic testing ($P = 0.04$) and 4.5 minutes (SD ± 28.43 seconds) and 4.41

Table 1. MS and SD Among Mesopic and Scotopic Red Testing in Cases and Controls in Both Testing Types

Type of Testing	Eccentricity	MS, dB (SD)		Test 1 − Test 2	Paired *t*-Test, 95% CI	
		First Test	Second Test			
Cases ($n = 23$)						
Mesopic	Global	23.13 (3.3)	22.9 (3.3)	0.22	$P = 0.01$	0.05–0.4
	0°–1°	22.69 (3.91)	22.45 (3.75)			
	3°	23.53 (3.34)	23.35 (3.16)			
	5°	23.09 (3.0)	22.96 (3.02)			
	7°	22.2 (3.13)	21.9 (3.4)			
Dark-adapted	Global	20.17 (4.02)	19.66 (4.07)	0.4	$P < 0.001$	0.2–0.6
	0°–1°	18.66 (4.46)	18.41 (4.58)			
	3°	20.76 (4.24)	20.12 (4.48)			
	5°	20.23 (3.65)	19.83 (3.58)			
	7°	19.76 (3.05)	19.11 (3.08)			
Controls ($n = 24$)						
Mesopic	Global	25.84 (2.27)	25.43 (2.29)	0.41	$P < 0.001$	0.25–0.56
	0°–1°	26.27 (2.36)	26.02 (2.28)			
	3°	26.53 (1.93)	26.14 (1.91)			
	5°	25.25 (2.13)	24.78 (2.25)			
	7°	24.47 (2.01)	24.2 (2.01)			
Dark-adapted	Global	22.61 (2.48)	22.22 (2.58)	0.38	$P < 0.001$	0.2–0.56
	0°–1°	22.87 (2.73)	22.24 (3.41)			
	3°	23.07 (2.29)	22.8 (2.38)			
	5°	22.18 (2.19)	21.7 (2.19)			
	7°	21.56 (2.54)	21.32 (2.24)			

CI, confidence interval.

minutes (SD ± 28.74 seconds) in dark-adapted testing, respectively ($P < 0.01$). On average, control persons performed both tests faster than did patients with iAMD, while the difference was greater for dark-adapted testing. For mesopic testing, the difference was 2.62 seconds ($P = 0.04$), while for dark-adapted testing the difference was greater with 8.55 seconds ($P < 0.01$) (Table 3). In all persons and tests, mean test duration was significantly shorter in the second test ($P < 0.01$). The difference was greater in iAMD patients than in controls (18.52 seconds for mesopic and 14.13 seconds for dark-adapted testing in iAMD and 10.08 and 9.29 seconds for controls, respectively) (Table 4).

CoR and Limits of Agreement

The CoR was 4.4 dB for mesopic and 4.52 dB for dark-adapted testing in iAMD patients and 3.96 dB for mesopic and 4.56 dB in control persons. For mesopic and dark-adapted testing, the CoRs were in the same range across all eccentricities in both groups. Table 5 shows the CoRs for all eccentricities and Figure 2 the CoRs in the four quadrants.

The 95% limits of agreements (LoA) according to Bland-Altman statistics ranged in the iAMD group from −4.51dB (95% confidence interval [CI], −4.82 to −4.2 dB) to 4.97 dB (95% CI, 4.66–5.27 dB) for mesopic testing and −5.0 dB (95%, −5.35 to −4.65 dB) to 5.81 dB (95% CI, 5.46–6.15 dB) for dark-adapted testing. For the control group, the LoA was between −3.76 dB (95% CI, −4.03 to 3.5 dB) and 4.59 dB (95% CI, 4.32–4.86 dB) for mesopic testing and −4.44 dB (95% CI, −4.72 to −4.13 dB) and 5.21 dB (95% CI, 4.9–5.52 dB) for dark-adapted testing. Bland-Altman plots did not show correlations between the average and difference of MS of both groups in mesopic and dark-adapted testing (Fig. 3).

Stability of Fixation

After log transformation to reduce skew, the analysis of the stability of fixation revealed in the iAMD group low agreement between the first and the second test for mesopic and high agreement for dark-adapted testing (ICC values 0.274 and 0.863, respectively). In the control group with healthy persons, the analysis showed moderate agreement for mesopic and high agreement for dark-adapted testing with ICC values of 0.657 and 0.898, respectively.

Discussion

The modified S-MAIA device allows for a reliable assessment of mesopic and dark-adapted microperimetry in patients with iAMD and persons with normal retinal health in the same age range. Both mesopic and dark-adapted retinal sensitivity were reduced in iAMD patients compared to controls.

Our results are comparable to findings from previous studies, which found mesopic microperimetry to be a good functional test for patients in the early and intermediate stages of AMD.[26,34–38] Wu and colleagues[11] showed a pointwise CoR

Table 2. Pooled MS (First and Second Test) in Decibels and SD in Mesopic and Scotopic Red Testing

	MS, dB (SD)				
	Cases	Controls	Difference	Unpaired *t*-Test	*z*-Score
Mesopic	23.01 (3.3)	25.63 (2.29)	−2.62	$P < 0.01$	−1.09
Dark-adapted	19.92 (4.06)	22.41 (2.54)	−2.49	$P < 0.01$	−1.07

Table 3. Pooled (First and Second Test) Mean Test Duration in Cases and Controls for Both Testing Types

	Mean Test Duration, s (SD)			
	Cases	Controls	Difference	Unpaired *t*-Test
Mesopic	255.3 (25.44)	253.62 (31.63)	−2.62	$P = 0.04$
Dark-adapted	273.23 (28.43)	264.68 (28.74)	−8.55	$P < 0.01$

for iAMD patients and controls of 4.12 and 3.74 dB, respectively, using mesopic microperimetry with the unmodified MAIA device with 37 test stimuli. These results were similar compared to the CoRs found in this study performed under mesopic conditions using 33 test stimuli (for iAMD 4.4 dB and for controls 3.96 dB).

A study by Pfau et al.[30] assessing test-retest reliability of scotopic microperimetry using the same modified S-MAIA device found CoRs of 4.75 dB for mesopic and 4.06 dB for dark-adapted testing in persons with normal retinal health. The CoRs were slightly higher than our findings for control persons. The perimetry grid with 49 testing points, resulting in longer test durations and a higher proportion of test points at eccentricities of 5° and 7°, may explain the difference in the CoR.

The assessment of dark-adapted microperimetry is more complex and time-consuming since it requires 30-minute dark adaptation in a room completely darkened. In total, the procedure is more prone to interference than is mesopic testing and requires good participant compliance. Due to the testing grid with 33 stimuli, we could keep test duration below 5 minutes for both mesopic and dark-adapted testing. This examination time was well accepted by patients and controls. In total, the whole examination procedure, including repeat testing and dark adaption time, took about 1 hour. The examination time for dark-adapted testing was about 20% longer than for mesopic testing in both groups. As the dark-adapted testing with red stimuli reflects rod and cone photoreceptor function, the longer reaction time might result from the lower stimulus intensities and the increasing recruitment of rod photoreceptors in this test.[30,39]

Nebbioso and colleagues[24] conducted a cross-sectional study to investigate the correlation between the presence of hard drusen only and a reduction in mesopic and scotopic retinal sensitivity measured with the Nidek MP-1S. They found that in patients with hard drusen, scotopic sensitivity was statistically significantly reduced, while mesopic sensitivity was not different. In our study both tests revealed reduced retinal sensitivity. The results showed no greater difference in dark-adapted testing between patients and controls than in mesopic testing (2.62 vs. 2.49 dB). However, in our study we included patients with iAMD, that is, a more advanced stage of AMD compared to Nebbioso and coworkers.[24]

Table 5. 95% CoR and 95% CI of 95% CoR

Type of Testing	Eccentricity	95% CoR, dB	95% CI of 95% CoR
Cases			
Mesopic	Global	4.4	0.15–8.66
	0°	4.6	−1.37–10.57
	1°	4.34	0.31–8.36
	3°	4.73	1.29–8.17
	5°	4.06	0.22–7.91
	7°	4.27	0.52–8.03
Dark-adapted	Global	4.52	0.19–8.8
	0°	3.15	−2.37–8.67
	1°	5.26	0.87–9.66
	3°	5.16	1.68–8.63
	5°	4.37	1.98–6.77
	7°	4.64	0.41–8.88
Controls			
Mesopic	Global	3.96	−0.73–8.65
	0°	4.41	−3.85–12.67
	1°	3.78	−0.39–7.95
	3°	3.9	1.44–6.67
	5°	3.72	1.42–6.02
	7°	3.9	0.23–7.72
Dark-adapted	Global	4.56	−0.52–9.66
	0°	4.65	−3.88–13.19
	1°	5.07	−0.44–10.58
	3°	4.65	1.36–7.94
	5°	4.29	2.27–6.32
	7°	4.16	0.39–7.93

In our study we found a slightly better MS for the first test and for mesopic compared to dark-adapted testing in cases and in controls. This finding differs from results in previous studies, which investigated test-retest variability, where a slight improvement in MS between the first and the second test was observed.[30,40] Wu and colleagues[11] demonstrated in their study a significant learning effect between the first and second examination of mesopic microperimetry with the MAIA. In our study, we could not confirm such a large learning effect. One explanation for the slightly better MS in the first test in our study could be the examination procedure: before scotopic testing, 30 minutes of dark adaptation in a completely darkened room is required, and thus fatigue can easily occur. Although we tried to keep the examination time as short as possible with a 33-stimuli grid, nevertheless it is a demanding testing procedure requiring patient concentration. In further examinations, longer breaks and higher motivation should be tested to assess both the impact of a learning effect as well as fatigue.

For dark-adapted testing, we found high ICCs for the BCEA, indicating high test-retest repeatability for the assessment of fixation stability. For mesopic testing, the ICC was low for iAMD patients and moderate for controls. However, absolute BCEA

Table 4. Mean Test Duration in the First and Second Test for Both Testing Types in Cases and Controls

	Mean Test Duration, s (SD)			Paired *t*-Test	
	First Test	Second Test	Difference	95% CI	*P* value
Cases					
Mesopic	264.56 (27.82)	246.04 (18.7)	18.52	16.87–20.16	$P < 0.001$
Dark-adapted	280 (25.77)	266.17 (29.21)	14.13	12.74–15.51	$P < 0.001$
Controls					
Mesopic	258.66 (34.8)	248.58 (27.2)	10.08	9.07–11.09	$P < 0.001$
Dark-adapted	269.33 (28.69)	260.04 (28.04)	9.29	7.86–10.71	$P < 0.001$

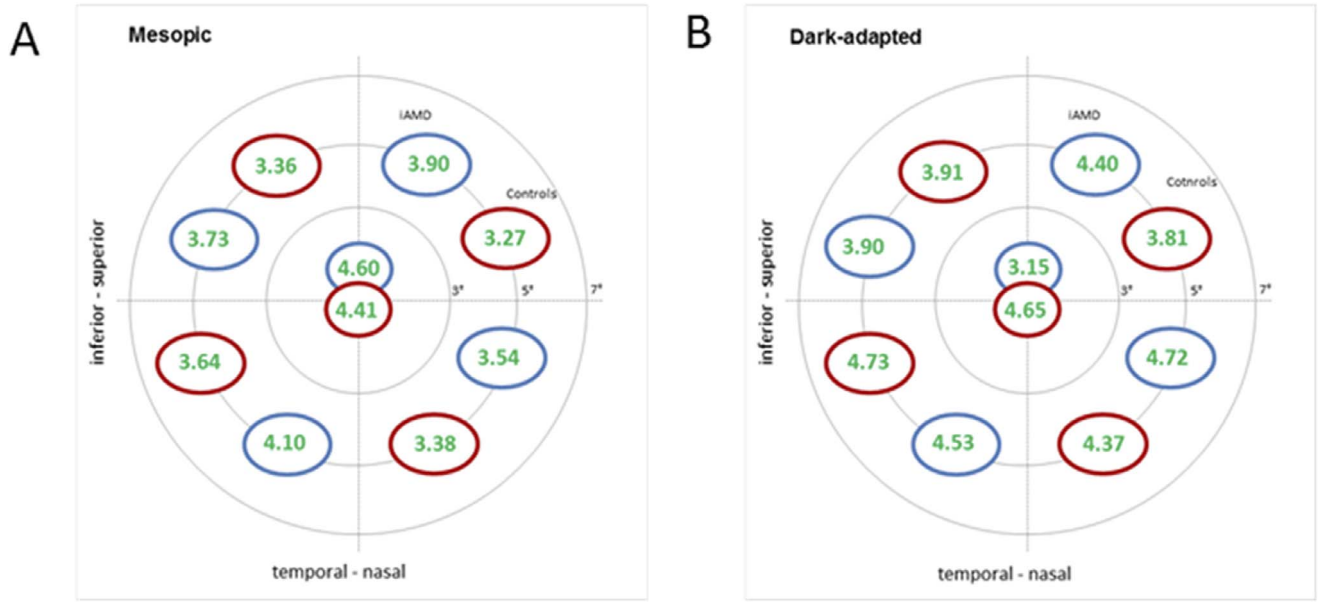

Figure 2. For (**A**) mesopic and (**B**) dark-adapted testing, 95% CoR in each quadrant and central in iAMD patients (*blue circles*) and controls (*red circles*)

was lower in all eyes for mesopic testing. Several previous publications have reported difficulties ensuring a stable fixation under scotopic conditions compared to mesopic conditions.[22,30,41] It would be conceivable that the different ICCs in our study result from a learning effect in fixation. Mesopic testing was always performed before dark-adapted testing.

Strengths of our study include the use of a highly standardized testing protocol for mesopic and scotopic testing

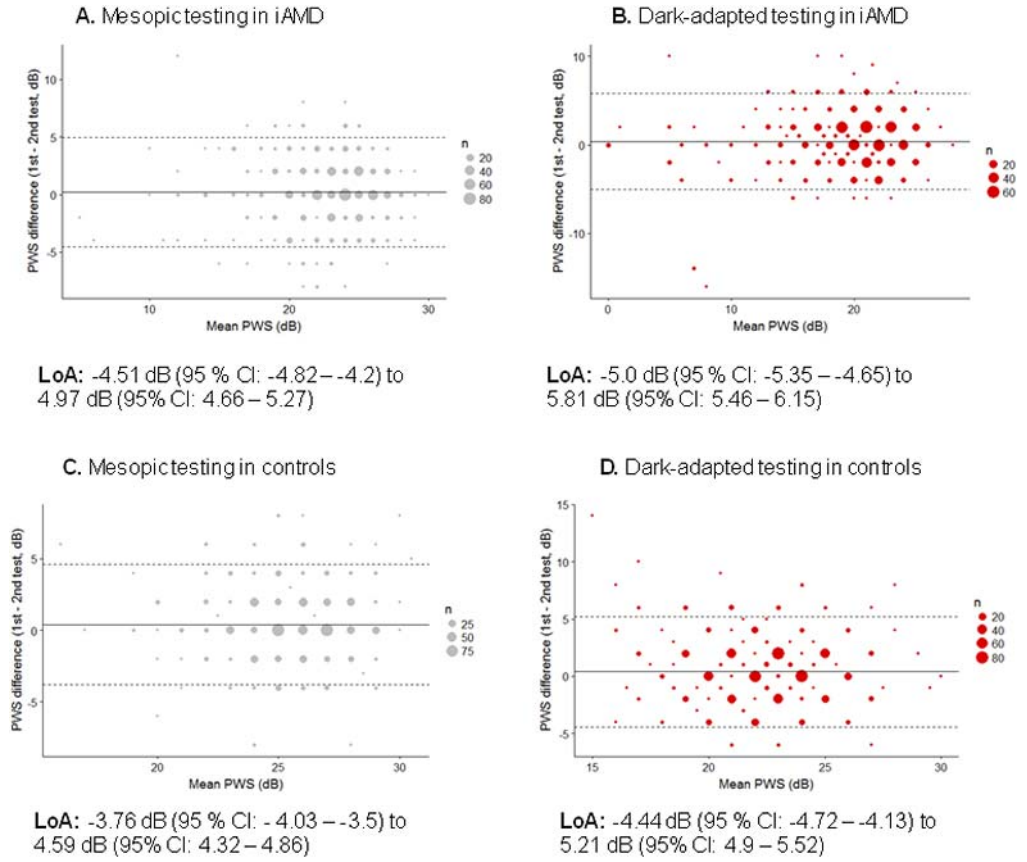

Figure 3. Bland-Altman plots for mesopic and scotopic red testing in cases (**A**, **B**) and in controls (**C**, **D**). The *x*-axis shows the mean PWS for each pair of repeated tests, the *y*-axis the PWS difference between the two tests (first test – second test). The overall mean is represented by the *central line*, and the 95% LoA are marked by the *upper* and *lower dashed lines*.

using a customized device performed by the same trained examiner as well as the comprehensive phenotyping of our participants. A possible limiting factor of our study might be the small study population. However, the sample was sufficient to demonstrate good reliability as well as differences in MS between the groups.

Another limitation is the fact that it is not possible to test exactly the same retinal locations with mesopic and dark-adapted testing. This is a limitation of the device, which allows for an exact overlap of the testing points in the follow-up mode within each testing mode, that is, mesopic or scotopic testing. The difference in the location of the testing points is very small ($<1^{\circ}$), thus it is unlikely to have an influence on the results. An additional limitation is the lack of assessment of intersession reliability days or weeks apart.

In conclusion, we found the modified S-MAIA device to yield highly reproducible mesopic and scotopic MS measurements in both iAMD patients and persons in normal retinal health. In addition, iAMD patients had lower MS on both mesopic and scotopic testing.

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Association of Vision-related Quality of Life with Visual Function in Age-Related Macular Degeneration

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The purpose of this study was to assess which visual function measures are most strongly associated with vision-related quality of life (VRQoL) in age-related macular degeneration (AMD). A cross-sectional study of subjects with early AMD (n = 10), intermediate AMD (n = 42) and late AMD (n = 38) was conducted. Subjects were interviewed with the Impact of Vision Impairment (IVI) questionnaire. Functional tests performed included best-corrected visual acuity (BCVA), low luminance visual acuity (LLVA), visual acuity measured with the Moorfields Acuity Charts (MAC), contrast sensitivity, reading speed, mesopic and dark-adapted microperimetry. The relationship between VRQoL and visual function was assessed with multiple regressions controlling for confounders. Rasch analysis demonstrated the validity of the IVI to assess VRQoL through three subscales: reading and accessing information, mobility and independence, and emotional well-being. Subjects with late AMD had significant lower IVI scores on all subscales compared with intermediate and early AMD ($p < 0.011$). In the overall cohort, IVI subscales were associated with BCVA, LLVA, MAC-VA and contrast sensitivity (all $p < 0.001$). Among the subgroup of early and intermediate AMD subjects, reading and mobility subscales were significantly associated with MAC-VA ($p < 0.013$). These results suggest that MAC-VA is a useful, patient-relevant measure of visual impairment in AMD.

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the elderly in industrial countries and an important public health problem[1,2]. Approximately 30–50 million people are affected by AMD worldwide[3]. Late stages can severely reduce visual acuity while patients with early and intermediate AMD often perform well in conventional visual function tests under high luminance and high contrast. Nevertheless, persons with early stages of AMD often complain about vision loss under low lighting, low contrast and changing light conditions, which also impacts vision-related quality of life (VRQoL)[4–6]. Standardized visual function tests under low luminance and low contrast have been met with increasing interest in particular in early stages of AMD as these tests might be more sensitive to the specific functional impairment in early and intermediate AMD[7] than the currently most widely used outcome measure in ophthalmic research, namely high-contrast, high-luminance best-corrected visual acuity (BCVA)[8].

However, to date we do not know which visual tests or combination of tests with or without structural data might best allow for this. From a regulatory perspective, an important pre-requisite of any functional test is its patient-relevance which can be approximated by VRQoL[9]. A validated and commonly used VRQoL instrument is the Impact of Vision Impairment (IVI) questionnaire which is reliable[10] and has been validated psychometrically for different ocular conditions and different levels of visual acuity[9,11,12]. Therefore, in this study, we used the IVI to investigate the relationship between VRQoL and several visual functional tests under low luminance and low contrast in patients with different stages of AMD. The aim of our study was to identify which functional tests are able to discriminate between different stages of AMD and to investigate whether these tests are correlated with VRQoL in order to assess patient-relevance of the tests.

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Methods

We conducted a cross-sectional study at the Department of Ophthalmology, University of Bonn, Germany, from January 2017 until January 2019. The study was approved by the Institutional Review Board of the University Bonn (approval ID: 013/16), where patients were recruited from outpatients clinics. Written informed consent was obtained from all participants following an explanation of all tests involved. The protocol followed the tenets of the Declaration of Helsinki.

Participants were categorized into "early AMD", "intermediate AMD" and "late AMD" based on the classification system introduced by Ferris *et al.*[13]. One eye of each patient (the more advanced eye) was included. If both eyes fitted the inclusion criteria and had the same visual acuity, the right eye was chosen. Inclusion criterion for all groups was the ability to converse, read and write German. Exclusion criteria were age <50 years, any corneal pathology that could compromise vision, amblyopia, diabetes, glaucoma, neurological or systemic disease affecting vision, refractive errors >6.00 dioptres (D) of spherical equivalent and >2.00 dioptres (D) of astigmatism. In addition to the functional tests spectral domain optical coherence tomography raster scanning was performed using a 25° × 25° scan field (49 B-scans, automated real-time mode 20 frames, centred on the fovea) as well as fundus autofluorescence and infrared reflectance imaging (Spectralis OCT2, Heidelberg Engineering, Heidelberg, Germany). All patients also underwent a clinical examination including dilated funduscopy.

The IVI questionnaire. The IVI is an instrument to assess different dimensions of VRQoL. It consists of 28 items with three to four responses options using Likert scales, ranging from "not at all" to "a lot". The IVI has three specific subscales: "Reading and Accessing Information" (9 items; abbreviated as reading subscale), "Mobility and Independence" (11 items; abbreviated as mobility subscale) and "Emotional Well-being" (8 items; abbreviated as emotional subscale). We used the validated German language version of the IVI[9].

Functional testing. All participants underwent the following visual function tests: Best-corrected visual acuity (BCVA) in Early Treatment Diabetic Retinopathy Study (ETDRS) letters, low luminance VA (LLVA), BCVA in Moorfields Acuity Chart (MAC) letters and contrast sensitivity measurement using Pelli-Robson Charts.

Visual acuity and functional tests were performed before fundus imaging. BCVA (in letters) was assessed according to the ETDRS method[14] at a testing distance of 4 m. If the patient was unable to read the first four rows of the chart, the distance was reduced to one meter[14]. LLVA was assessed in the same manner, but with a 2.0-log unit neutral density filter that reduces luminance by 100 fold[15] placed in the trial frame. VA measurement with a MAC chart followed the same procedure as BCVA. The MAC charts are based on the ETDRS charts and employ a high-contrast, high-pass letter design with a gray background of the same mean luminance as the letters to simulate lower contrast situations[16]. The letters are also called "vanishing optotypes", because, for normal vision, the detection and recognition thresholds are very similar and the letters seem to disappear soon after the recognition limit has been reached[16]. Contrast sensitivity was measured using a Pelli-Robson Chart presented at 1 m distance[17–19]. To avoid fatigue patients were allowed to take small breaks (maximum five minutes) between the tests if required.

In patients with early and intermediate AMD, we additionally assessed reading speed using the International Reading Speed Texts (IReST)[20] and macular sensitivity via mesopic and dark-adapted microperimetry. For the IReST, patients wore their best near correction and were asked to read one paragraph aloud while they were timed with a stopwatch. Mesopic and dark-adapted microperimetry were performed after pupillary dilation with 1.0% tropicamide. Macular sensitivity was measured using the modified S-MAIA device (S-MAIA, CenterVue, Padova, Italy), which performs fundus tracking using a line-scanning laser ophthalmoscope (SLO) with a super-luminescent diode illumination with a central wave light of 850 nm for mesopic testing and with an additional LED projecting red (627 nm) stimuli for dark-adapted testing. As previously described, a customized stimulus grid was used that consisted of 33 points located at 0°, 1°, 3°, 5° and 7° from fixation[21]. First, mesopic testing was performed, followed by dark-adapted testing after 30 minutes of dark adaptation while waiting in the examination room (light was switched off, light level <0.1 lx). The microperimetric outcome measure was the mean sensitivity (MS) in dB. Due to feasibility issues (fixation stability, grid centration and age/patient fatigue) patients with late AMD did not undergo microperimetry examination. We did also not test reading speed in late AMD patients as most of them did not reach the minimum required visual acuity of 55 letters[22]. All tests were performed in one eye with the non-study eye covered with an eye-patch.

Psychometric evaluation of the IVI. Rasch analysis was used to evaluate the instrument in our cohort. It is a psychometric method that transforms ordinal scales into interval-level scales (expressed in logits)[23,24]. Item difficulty (item measure) in relation to person ability (person measure) is calculated by placing both in the same linear continuum. The ability of the scale to discriminate different strata of person ability was assessed using person separation index (PSI) and person reliability coefficient (PR)[25]. Values of >2.0 and >0.8, respectively, were considered adequate and represented the capacity of the scale to distinguish three levels of person ability[26,27].

Unidimensionality – the ability that a scale measures a single underlying latent trait and that the items "fit" the underlying trait – was assessed in two ways. First, we determined item fit through an "infit" mean square standardized residuals statistic[28]. Values between 0.7 and 1.3 are considered acceptable, while lower or higher values may indicate redundancy or an unacceptable level of "noise" in the responses[28]. Second, the principle component analysis (PCA) of the residuals was examined to test for local independence. The PCA of residuals for the first factor should explain at least 50% of the variance and the first contrast of residuals should be <2.0 eigenvalue[29].

The targeting of the instrument, i.e. how well item difficulty targets person ability, was assessed by visual inspection of the person-item map and the difference between person and item mean logits. A difference of >1.0 logits indicates notable mistargeting[30].

Characteristics	AMD group		
	Early	Intermediate	Late
Mean Age (SD)	69.8 (±6.1)	69.7 (±7.7)	79.8 (±5.9)
Patients, n (eyes)	10	42	38
Women	7 (70.0%)	31 (73.8%)	62 (68.9%)

Table 1. Characteristics of Participants in each AMD Category. *SD = standard deviation.

Furthermore, each item was assessed for differential item functioning (DIF), which is a statistical method for detecting whether sample subgroups (sex and age groups) systematically respond differently to certain items, despite having similar underlying ability. A DIF contrast of >1.0 logits is notable and suggests that the item may be biased for some participants subgroups.

Person measures (in logits) were recalibrated to a 0 to 100 scale. Higher values indicate lower visual ability and indicate poorer VRQoL. Rasch analysis was performed using commercial software (Winsteps software, ver. 3.92.1.2, Chicago, IL)[31]. The Andrich rating scale model was used for analysis[29].

Statistical analysis. Descriptive statistics were performed to assess baseline demographic variables for the AMD groups. The nonparametric Wilcoxon rank sum test was used to compare person measures on the three subscales of the IVI in early, intermediate AMD and late AMD subjects. Univariate linear regression was carried out to assess the relationship between the person measures and demographic variables. In the overall cohort, separate univariate linear regression models against the IVI subscale person measures were performed with each of the visual function test. If the relationship between a function test and average person measures reached a p-value < 0.05 in univariate analysis, multiple regression was used to ensure that the findings were not confounded by AMD stage and age. We additionally performed a subgroup analysis, which included only patients with early and intermediate AMD. Statistical analyses were performed using the statistical software STATA[32].

Results

Sociodemographic and clinical characteristics of the participants. A total of 90 participants were recruited comprising 10 patients with early AMD (11%), 42 (47%) with intermediate AMD and 38 (42%) patients with late AMD. Participants' mean age was 73.9 ± 8.4 years and there were more female (69%) than male participants. Patients in the late AMD group were significantly older compared to early and intermediate AMD patients ($p < 0.05$), while patients with early and intermediate AMD were in the same age range ($p = 0.96$) (Table 1). All functional tests were significantly decreased in intermediate and late AMD compared to the early AMD group (all p-values < 0.001). There was no significant difference in BCVA between early and intermediate AMD ($p = 0.553$), as well as in reading speed ($p = 0.617$) and mesopic and dark-adapted microperimetry ($p = 0.274$ and $p = 0.141$). However, LLVA, MAC-VA and contrast sensitivity were significantly decreased in intermediate AMD compared to the early AMD group ($p = 0.023$, $p = 0.041$ and $p < 0.001$ respectively). Detailed results can be found in Table 6 in Supplement 1.

Psychometric validation of the IVI questionnaire. The data for the IVI were fitted to the Rasch model and key indicators of fit were explored. Overall, the psychometric testing supported the use of the three IVI subscales in this sample and demonstrated satisfactory PSI and PR for all subscales. No items had to be removed due to misfit or DIF. Detailed results of the Rasch analysis can be found in Supplement 2.

Relationship between visual function tests and VRQoL. Mean person measures (in logits) for the three subscale scores are shown in Fig. 1. Subjects with early and intermediate AMD had significantly lower person measures on all three subscales compared to subject with late AMD. There was no significant difference between early and intermediate AMD. Similarly, participants ≤75 years reported a better VRQoL compared to the older age group >75 years (Table 2). Age and AMD stage were also significantly associated with all three scales of the IVI in univariate linear regression analysis (Table 3).

In univariate linear regression, person measures of all three scales were negatively associated with BCVA, LLVA, MAC-VA and contrast sensitivity in the overall cohort. After controlling for age and AMD stage, multiple regression analysis showed that BCVA and MAC-VA remained significantly associated with all three IVI scores. LLVA was still significantly associated with the reading and mobility scales and contrast sensitivity only with the mobility scale (Table 4). Contrast sensitivity and MAC-VA had the strongest associations with all scales.

Analyzing only subjects with early and intermediate AMD, BCVA, LLVA and MAC-VA were associated with the reading scale and BCVA and MAC-VA with the mobility scale. In the adjusted analysis BCVA, LLVA and MAC-VA were still significantly associated with the reading scale and MAC-VA with the mobility scale (Table 5).

The IReST and macular sensitivity on mesopic and dark-adapted microperimetry showed no association with any of the scales of the IVI.

Discussion

In our study we found that especially functional tests of central retinal function under low luminance and challenging contrast conditions were associated with VRQoL. LLVA, MAC-VA and contrast sensitivity were significantly reduced in patients with intermediate and late AMD compared to early AMD, while no significant difference was found for BCVA between intermediate and early AMD.

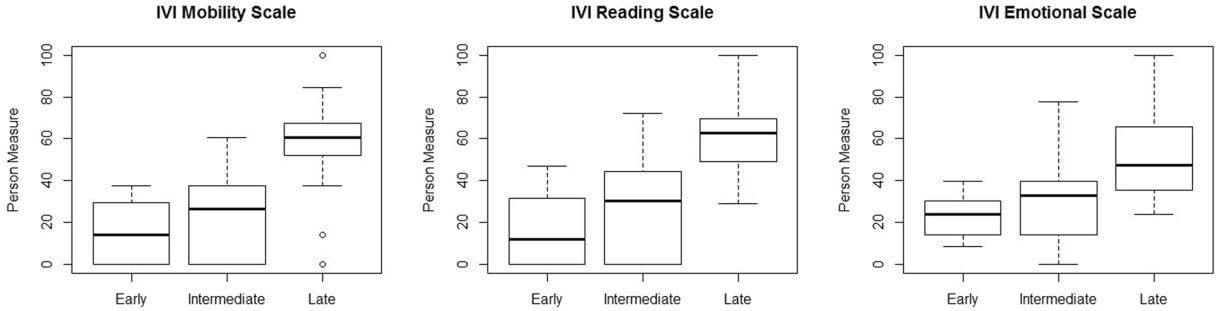

Figure 1. Boxplots showing IVI Reading Scale Scores, Mobility Scale Scores and Emotional Scale Scores for early, intermediate and late AMD. Each boxplot includes the maximum (upper whisker), upper quartile (top of the box), median (horizontal line in box), lower quartile (bottom of the box) and minimum (lower whisker) values.

Variable	*n* (%)	Reading IVI		Mobility IVI		Emotional IVI	
		Mean ± SD	*P* Value	Mean ± SD	*P* Value	Mean ± SD	*P* Value
Total sample, $n=90$		40.00 ± 25.54		36.79 ± 26.01		38.69 ± 21.62	
Age, y							
≤75	44 (48.8)	28.92 ± 22.39	<**0.001**	25.01 ± 21.82	<**0.001**	32.82 ± 18.19	**0.011**
>75	46 (51.1)	50.67 ± 23.96		48.06 ± 25.01		44.29 ± 23.28	
Sex							
Female	62 (68.9)	38.93 ± 27.02	0.662	37.46 ± 26.95	0.786	38.69 ± 22.66	0.937
Male	28 (31.1)	42.49 ± 22.18		35.29 ± 24.49		38.66 ± 19.50	
AMD stage							
Early	10 (11.1)	17.61 ± 19.39	<**0.001***	15.27 ± 15.10	<**0.001***	23.78 ± 9.71	<**0.001***
Intermediate	42 (46.7)	26.97 ± 20.81		22.98 ± 19.95		30.65 ± 20.27	
Late	38 (42.2)	60.38 ± 15.58		57.71 ± 18.70		51.49 ± 18.55	

Table 2. Baseline IVI subscales scores and comparisons between age groups, sex and AMD stage. *Significant differences found between early and late AMD, and between intermediate and late AMD, but not between early and intermediate AMD; p-values based on the Wilcoxon rank sum test.

	Reading IVI		Mobility IVI		Emotional IVI	
	β Coefficient	*P* Value	*β* Coefficient	*P* Value	*β* Coefficient	*P* Value
Age	1.414	<**0.001**	1.608	<**0.001**	0.644	**0.017**
Age-Groups						
≤75 (Reference)						
>75	21.75	<**0.001**	23.054	<**0.001**	11.475	**0.011**
Sex						
Male (Reference)						
Female	−3.550	0.545	2.177	0.716	0.029	0.995
AMD Stage						
Early (Reference)						
Intermediate	9.361	0.156	7.709	0.251	6.872	0.299
Late	42.77	<**0.001**	42.438	<**0.001**	27.712	<**0.001**

Table 3. Univariate Linear Regression of Baseline Demographics against IVI subscales.

This is in accordance with previous studies, which investigated these tests in early and intermediate AMD, with any visual impairment in early stages of AMD being present on LLVA but not BCVA[33]. Similarly, Feigl and associates reported decreased contrast sensitivity in early AMD compared to healthy controls[34]. In line with previous other studies we found late AMD and older age to be associated with decreased VRQoL on all three subscales, namely the reading, mobility and emotional scales[35,36]. MAC-VA and contrast sensitivity both showed a stronger relationship with the subscales of the IVI in the overall cohort than BCVA. This is of special relevance because poor contrast sensitivity has been shown to be sensitive in discriminating early stages of AMD[34,37]. Furthermore, in previous studies contrast sensitivity was a factor impacting VRQoL of AMD patients: Roh and colleagues[38] demonstrated that contrast sensitivity was an important factor affecting VRQoL in patients with

	Reading IVI		Mobility IVI		Emotional IVI	
	β Coefficient	*P* Value	*β* Coefficient	*P* Value	*β* Coefficient	*P* Value
BCVA	−1.030	<**0.001***	−1.053	<**0.001***	−0.641	<**0.001***
LLVA	−0.936	<**0.001***	−0.916	<**0.001***	−0.558	<0.001
MAC	−1.175	<**0.001***	−1.217	<**0.001***	−0.781	<**0.001***
CS	−1.915	<0.001	−2.094	<**0.001***	−1.345	<0.001
IReST[#]	−0.88	0.393	−0.102	0.303	0.017	0.851
Sensitivity (Mesopic)[#]	1.094	0.213	0.700	0.412	−0.201	0.807
Sensitivity (Dark-adapted)[#]	−0.594	0.522	−0.539	0.549	−0.277	0.748

Table 4. Linear Regression of Visual Function Measures against IVI subscales for the complete cohort. *After controlling for AMD status and age in multiple linear regression, the point estimate remained statistically significant. BCVA = best-corrected visual acuity, LLVA = low luminance visual acuity, CS = contrast sensitivity, IReST = International Reading Speed Texts. [#]Data incomplete.

	Reading IVI		Mobility IVI		Emotional IVI	
	β Coefficient	*P* Value	*β* Coefficient	*P* Value	*β* Coefficient	*P* Value
BCVA	−1.627	**0.010***	−1.463	0.033	−0.134	0.806
LLVA	−0.949	**0.033***	−0.878	0.069	0.139	0.712
MAC	−1.574	**0.011***	−1.651	**0.013***	−0.669	0.207
CS	−0.452	0.699	−0.619	0.622	−0.680	0.485
IReST	−0.122	0.399	−0.156	0.301	0.029	0.812
Sensitivity (Mesopic)	1.555	0.210	1.116	0.418	−0.322	0.762
Sensitivity (Dark-adapted)	−0.818	0.532	−0.871	0.548	−0.464	0.677

Table 5. Subgroup Analysis: Linear Regression of Visual Function Measures against IVI subscales for early and intermediate AMD. *After controlling for age in multiple linear regression, the point estimate remained statistically significant. BCVA = best-corrected visual acuity, LLVA = low luminance visual acuity, CS = contrast sensitivity, IReST = International Reading Speed Texts.

vision impairment due to bilateral advanced AMD. Bansback *et al.*[39] found a relationship between contrast sensitivity and health-related quality of life, suggesting that benefits of ocular treatments may be underestimated if contrast sensitivity is not taken into account.

A subgroup analysis, only including early and intermediate AMD subjects, revealed a noticeable association between MAC-VA and VRQoL for both the IVI reading and mobility subscales. The MAC-VA is a relatively recent functional test which simulates lower contrast situations. Shah *et al.*[16] demonstrated the MAC-VA to be more sensitive in detecting early AMD compared to conventional BCVA and hypothesized that recognition of the high-pass letters is more vulnerable to photoreceptor dysfunction than conventional high luminance and high contrast letters. This is in accordance with our findings as MAC-VA was not only significantly associated with VRQoL in the overall cohort, but also in the subgroup of early and intermediate AMD. As the MAC-VA is a relatively new test, no previous studies have investigated whether it is associated with VRQoL. We could show that MAC-VA as well as LLVA and contrast sensitivity had a stronger effect on VRQoL compared to BCVA. A review by Mones and colleagues revealed that the use of contrast sensitivity as an outcome measure in clinical trials may be a better predictor of activities of daily living, mobility and orientation than BCVA[40]. Also in the Blue Mountains Eye Study, contrast sensitivity was strongly associated with self-reported measures of visual disability[41].

We did not find significant associations between any of the IVI scales with mesopic or dark-adapted microperimetry mean sensitivity. This is in accordance with the findings of Wu and co-authors[42]. They evaluated subjects with bilateral intermediate AMD using a shorter 10-item Night Vision Questionnaire (NVQ-10)[42] and assessed the relationship of the NVQ scores with LLVA (also using a standard 2.0.-log neutral density filter), low luminance deficit (LLD), mesopic microperimetric mean sensitivity and central sensitivity. NVQ-10 person measures were significantly associated with LLD, but not with BCVA, LLVA, microperimetric mean sensitivity or central sensitivity. Thompson *et al.*, who determined in their study whether Low Luminance Questionnaire scores were associated with objective measures of visual function in early and intermediate AMD, did also not find any association with mesopic microperimetry measures[36]. Interestingly, we did not find reading speed to be correlated with VRQoL.

Strengths of our study include the wide range of functional tests including the relatively new MAC charts for which little data are available to date as well as reading performance and dark-adapted microperimetry. VRQoL was assessed using a validated instrument available in German – the German IVI. We re-evaluated its psychometric performance and transformed responses into an interval-based scale for further statistical testing using modern psychometric methods. Participants were phenotyped based on current gold-standard retinal imaging in combination with a clinical examination. One of the major limitations of our study is the relatively small

sample size, especially for the early AMD group. For microperimetry and reading speed measures the sample size was even smaller, as we only performed these test in subjects with early and intermediate AMD. As common with exploratory studies, no adjustment for multiple testing was done which might lead to an over-estimation of statistical power. The IVI assesses overall VRQoL with no particular focus on activities under low luminance and low contrast which might have decreased our ability to detect any associations with functional tests under those conditions. However, general VRQoL is most closely related to daily visual functioning and thus very suitable to assess patient-relevance of visual function tests.

In conclusion, our study showed that performance on BCVA, LLVA, MAC-VA and contrast sensitivity are associated with all aspects of VRQoL in overall AMD. In patients with earlier stages of AMD, BCVA, LLVA and the MAC-VA are associated with VRQoL on the reading scale. In addition, MAC-VA is also correlated with VRQoL on the mobility scale in these patients, which suggests that the MAC-VA might be a useful and patient-relevant measure of visual impairment in AMD, in particular in earlier stages.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

S.G.P., J.H.T. and R.P.F. wrote the manuscript. S.G.P. and J.H.T. analyzed the data. S.G.P., J.H.T., M.H., M.W.M.W., F.G.H. and R.P.F. reviewed the manuscript. R.P.F. supervised the study.

Competing interests

CenterVue SpA, Padua, Italy has provided research material (S-MAIA) for the conduct of this study. Center-Vue had no role in the design or conduct of the experiments. S. G. Pondorfer: Heidelberg Engineering (F), Optos (F), Carl Zeiss MedicTec (F), CenterVue (F); J. H. Terheyden: Heidelberg Engineering (F), Optos (F), Carl Zeiss MedicTec (F), CenterVue (F); M. Heinemann: Heidelberg Engineering (F), Optos (F), Carl Zeiss MedicTec (F), CenterVue (F); M. W. M. Wintergerst: Heidelberg Engineering (F), Optos (F), Carl Zeiss MedicTec (F), CenterVue (F), Heine Optotechnik (C, F), DigiSight Technologies (F), D-Eye (F) F.G. Holz: Heidelberg Engineering (F, C, R), Optos (F), Carl Zeiss MedicTec (F, C), CenterVue (F), Allergan (F, R), Alcon/Novartis (F, R), Genentech/Roche (F, R), Bayer (F, R), Acucela (F, R), Boehringer Ingelheim (F, R); R.P. Finger: Heidelberg Engineering (F), Optos (F), Carl Zeiss MedicTec (F),, CenterVue (F), Bayer (C), Novartis (C), Santen (C), Opthea (C), Novelion (C), Retina Implant (C), Oxford Innovation (C), Novartis (F).

Additional information

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SUPPLEMENTARY INFORMATION

Association of Vision-related Quality of Life with Visual Function in Age-Related Macular Degeneration

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SUPPLEMENT 1

Table 6: Functional tests measures: Descriptive analysis and group comparisons

Functional Test (Mean [SD])	Early AMD	iAMD	Late AMD	P-value* Early vs. iAMD	P-value* Early vs. late AMD	P-value* iAMD vs. late
BCVA	84.40 (3.81)	83.02 (6.78)	55.84 (14.85)	0.553	<0.001	<0.001
LLVA	72.00 (4.59)	65.78 (9.53)	38.82 (15.73)	0.023	<0.001	<0.001
MAC	65.70 (3.74)	60.76 (6.63)	42.47 (13.65)	0.041	<0.001	<0.001
Contrast Sensitivity	37.90 (2.81)	33.33 (3.11)	24.53 (8.05)	<0.001	<0.001	<0.001
IReST	159.40 (21.37)	153.84 (29.19)		0.617		
Mesopic Microperimetry	23.98 (3.04)	22.95 (3.24)		0.274		
Dark-adapted Microperimetry	21.28 (4.63)	20.51 (2.50)		0.141		

*P-values based on the Wilcoxon rank sum test, iAMD = intermediate AMD, SD = standard deviation, BCVA = best corrected visual acuity, LLVA = low luminance visual acuity, MAC = Moorfields Vanishing Optotype Charts, IReST = International Reading Speed Text

SUPPLEMENTARY INFORMATION

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SUPPLEMEMT 2

During Rasch analysis, the total IVI scale suggested evidence of multidimensionality, with an eigenvalue of 3.38 (although PCA for the first factor explained > 50% of the variance) and three misfitting items, and it was subsequently split into its three component scales: “Reading and Accessing Information” (9 items), “Mobility and Independence” (10 items) and “Emotional well-being” (8 items). For the reading subscale, PSI and PR were 2.28 and 0.84, respectively, indicating that three levels of person strata can be detected. There was minimal evidence of multidimensionality with the PCA for the first factor explaining >60% of the variance and the eigenvalue for the first contrast of 1.86. No DIF was found was for sex and age. The mobility subscale had a PSI of 2.06 and a PR of 0.81. Item 4 displayed misfit, but was retained as its removal did not improve fit statistics. The PCA of the residuals was 58.9%, and the first contrast of the residuals was 1.97 eigenvalue, which is acceptable for the requirements of unidimensionality. No DIF was found for sex but item 19 displayed DIF for age (the younger age group responded differently to this item compared to the older age group >75 years). As we adjusted for age in the regression models we did not remove the item. For the emotional subscale, PSI and PR were 2.02 and 0.80, also indicating that three levels of person strata can be detected. There was no evidence of multidimensionality with the PCA of the residuals of 67.5% and the eigenvalue for the first contrast of 1.85. DIF was found for age, where item 21 had a DIF contrast of 1.47 logits. The item was retained, as it captures important information pertaining to emotional well-being and its removal did not alter the fit statistics relevantly. Targeting was suboptimal for the total IVI scale as well as for the three domains (difference between person and item mean >1.0 for all) indicating that participants had higher ability levels than the mean difficulty of the items.

41 **Table 7**: Fit parameters of the Complete IVI, Reading IVI, Mobility IVI and Emotional IVI compared with Rasch
42 Model Requirements

Parameters	**Rasch Model**	**IVI_C**	**IVI_R**	**IVI_M**	**IVI_E**
Misfitting items, n	0	**3** (23, 21, 25)	1 (6)	1 (4)	1 (21)
PSI	>2.0	2.92	2.28	2.06	2.02
PR	>0.8	0.90	0.84	0.81	0.80
Difference in person and item mean	<1	**-1.80**	**-1.58**	**-2.32**	**- 2.45**
Variance by the first factor	>50%	55.6 %	61.0 %	58.9%	67.5 %
PCA (eigenvalue for 1st contrast)	<2.0	**3.38**	1.86	1.97	1.85
Differential item functioning (Item number [DIF contrast])	<1.0				
Gender		6 (-1.14)	None	None	None
Age group (≤75; ≥76)		19 (1.12) 21 (-1.27) 25 (-1.36)		19 (1.06)	21 (-1.47)

43 IVI_C, complete IVI; IVI_R, Reading and accessing information subscale of the IVI; IVI_M, Mobility and
44 independence subscale of the IVI; IVI_E, Emotional well-being subscale of the IVI. Bold values represent misfit
45 to the Rasch model.

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Clinical and Epidemiologic Research

Association of Visual Function Measures with Drusen Volume in Early Stages of Age-Related Macular Degeneration

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Purpose. To assess which visual function measures are most strongly associated with overall retinal drusen volume in age-related macular degeneration (AMD).

Methods. A total of 100 eyes (16 eyes with early AMD, 62 eyes with intermediate AMD, and 22 eyes from healthy controls) were recruited in this cross-sectional study. All subjects underwent several functional assessments: best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA), visual acuity (VA) measured with the Moorfields Acuity Chart (MAC-VA), contrast sensitivity with the Pelli–Robson test, reading speed using the International Reading Speed texts, and mesopic and dark-adapted microperimetry. Drusen volume was automatically determined based on optical coherence tomography using an approach based on convolutional neural networks. The relationship between drusen volume and visual function was assessed with linear regressions controlling for confounders.

Results. Mean drusen volume and MAC-VA differed significantly among all AMD stages and controls ($P < 0.001$). In univariate linear regression, LLVA, MAC-VA, contrast sensitivity, and mesopic and dark-adapted microperimetry were significantly negatively associated with the overall drusen volume (all $P < 0.006$). After controlling for AMD stage, age, and the presence of subretinal drusenoid deposits, MAC-VA and mesopic and dark-adapted microperimetry were still significantly associated with drusen volume ($P = 0.008$, $P = 0.023$, and $P = 0.022$, respectively).

Conclusions. Our results suggest that MAC-VA, as well as mesopic and dark-adapted microperimetry, might indicate structural changes related to drusen volume in early stages of AMD.

Keywords: Automatic segmentation of drusen, drusen volume, age-related macular degeneration, contrast sensitivity

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the elderly in developed countries, with a worldwide prevalence of 8.1% for early AMD and 8.69% for any AMD in people over 45 years of age.[1,2] Due to current demographic trends, the burden of AMD is estimated to grow to 288 million by 2040.[1] Late stages can lead to a severe loss of visual acuity, whereas early stages of the disease are often not associated with obvious visual symptoms. In conventional visual function tests under high luminance and high contrast, patients with early and intermediate stages of the disease usually achieve good scores; however, they often report difficulties and vision loss under low lighting, low contrast, and changing light conditions.[3–5] Therefore, standardized visual function tests under low luminance and low contrast have attracted increasing interest, particularly with regard to early stages of AMD, as they might allow better monitoring of the disease and aid in predicting disease progression.[6]

Usually the first clinical sign of AMD are drusen located between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane (BM), in the sub-RPE-basal laminar space.[7,8] Retinal cells overlying drusen exhibit structural and molecular abnormalities indicative of photoreceptor degeneration and Müller glial activation, suggesting that photoreceptor cell function is compromised as a consequence of drusen formation.[9] Drusen are among the most important biomarkers for staging AMD.[10–13] A recently developed convolutional neural network (CNN)-based approach for a fully automated segmentation of drusen in optical coherence tomography (OCT) images[14] allows us to compute the overall drusen volume. Compared to manual segmentation of drusen,

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automated segmentation has been shown to be highly reproducible and accurate.[15,16] The relationship between visual function tests under low luminance and low contrast and measurements of retinal structure associated with AMD progression, such as drusen volume, has not been well described so far. It may be that structural and functional measures provide complementary information about disease status. Thus, we evaluated the relationship between drusen volume and a battery of visual function tests under low luminance and low contrast.

Methods

We conducted a cross-sectional study at the Department of Ophthalmology, University of Bonn, Germany, from January 2017 until January 2019. The study was approved by the Institutional Review Board of the University Bonn (approval ID 013/16). Written informed consent was obtained from all participants following an explanation of all non-invasive tests involved. The protocol followed the tenets of the Declaration of Helsinki.

Sixteen eyes from patients with early AMD, 62 eyes from patients with intermediate AMD (iAMD), and 22 healthy eyes were recruited from patients from the AMD outpatient clinic, the self-help organization Pro Retina, and family members of patients. Participants were categorized as early AMD, iAMD, or healthy controls (no apparent aging changes and normal aging changing), based on the classification system introduced by Ferris et al.[11] Exclusion criteria were age < 50 years; any media opacity that could compromise vision; amblyopia, diabetes, glaucoma, or neurological or systemic disease affecting vision; refractive errors > 6.00 diopters (D) of spherical equivalent; and >2.00 D of astigmatism. Spectral-domain OCT (SD-OCT) raster scanning was performed using a 20° × 25° scan field (121 B-scans, automated real-time mode 20 frames, centered on the fovea); fundus autofluorescence (FAF) and infrared reflectance (IR) imaging (Spectralis OCT2, Heidelberg Engineering, Heidelberg, Germany); and color fundus photography (CFP) of the macula (Canon CR-2 AF; Tokyo, Japan). For a diagnosis of subretinal drusenoid deposits (SDDs) and pigment changes, characteristic changes had to be present on at least two imaging methods including CFP, SD-OCT, FAF, and IR. All patients also underwent a clinical examination including dilated funduscopy. Pupillary dilatation was achieved using 1% tropicamide.

Functional Testing

All participants underwent the following visual function tests: best-corrected visual acuity (BCVA), Early Treatment Diabetic Retinopathy Study (ETDRS) letters, low-luminance visual acuity (LLVA), Moorfields Acuity Chart (MAC-VA), contrast sensitivity measurement using Pelli–Robson charts, reading speed using the International Reading Speed Texts (IReST), and mesopic and dark-adapted microperimetry using modified Macular Integrity Assessment (S-MAIA) microperimetry (CenterVue, Padova, Italy).

BCVA for letters was assessed according to the ETDRS method[17] at a testing distance of 4 meters. LLVA was assessed in the same manner, but with a 2.0-log unit neutral density filter that reduces luminance by 100-fold[18] placed in the trial frame. MAC-VA measurement followed the same procedure as for BCVA. The MAC charts are based on the ETDRS charts and employ a high-contrast, high-pass letter design with a gray background of the same mean luminance as the letters to simulate lower contrast situations.[19] The letters are also referred to as vanishing optotypes, because, for normal vision, the detection and recognition thresholds are very similar, and the letters seem to disappear soon after the recognition limit has been reached.[19] Contrast sensitivity was measured using a Pelli–Robson chart presented at a distance of 1 meter.[20–22] For the IReST, patients wore their best near correction and were asked to read one paragraph aloud while they were timed with a stopwatch.[23] BCVA and reading speed were performed under photopic conditions, whereas LLVA, MAC-VA, and contrast sensitivity were performed under mesopic conditions. Based on the test characteristics and the lighting conditions in which these tests were administered, we presumed a more cone-mediated function in the BCVA and reading test and a partially rod-mediated function in the LLVA, MAC-VA, contrast sensitivity, and, in particular, mesopic microperimetry.

Prior to microperimetry testing with the S-MAIA device, pupillary dilatation was performed. The S-MAIA performs fundus tracking using a line-scanning laser ophthalmoscope with a super-luminescent diode illumination with a central wave light of 850 nm for mesopic testing and an additional light-emitting diode projecting red (627 nm) stimuli for dark-adapted testing. The dark-adapted testing with red (627 nm) stimuli is more influenced by cone-mediated function, reflecting a mixture of both rod- and cone-mediated responses.[24] As previously described, a customized stimulus grid was used that consisted of 33 points located at 0°, 1°, 3°, 5°, and 7° from fixation.[25] First, mesopic testing was performed. Patients were not dark-adapted, but the room light was switched off just before the examination. After mesopic testing, patients underwent 30 minutes of dark adaptation while waiting in the examination room (light was switched off, light level was <0.1 lux), and then dark-adapted testing was performed. The microperimetric outcome measure was the global mean sensitivity (in dB) and mean sensitivity at eccentricities of 0°–1°, 3°, 5°, and 7°. All tests were performed in one eye, with the non-study eye covered with an eye patch.

Automatic Segmentation of Drusen

For automated segmentation of drusen, the pipeline reported by Gorgi Zadeh et al.[14] was used. In the first step, this drusen segmentation pipeline automatically segments RPE and BM bands, using a CNN, which transforms an input B-scan into RPE and BM probability maps. For the final hard segmentation of RPE and BM bands, probability maps are converted into cost maps so that pixels with higher probability have lower costs. Dijkstra's algorithm is used to find a path with the minimum accumulated costs from the left to the right of each map.[26] The extracted paths are considered as final RPE and BM band segmentation.

In the second step, an ideal (normal) RPE is estimated through a rectification of RPE and BM bands.[14] In the rectification step, both RPE and BM are shifted vertically and column-wise until the BM band becomes a straight horizontal line, then a low-degree polynomial is fitted on the shifted RPE band and transformed back into the original image coordinates and is regarded as the drusen-free RPE. Finally, any area between the RPE and drusen-free RPE is classified as drusen. To eliminate falsely detected drusen, those with a height of 2 pixels or less are removed from the final

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Table 1. Characteristics of Participants in Each Group

Characteristic	AMD Group		
	Control	Early	Intermediate
Age (y), mean ± SD	59.8 ± 6.3	70.0 ± 6.7	69.7 ± 7.3
Eyes, n	22	16	62
Women, n (%)	13 (59.1)	11 (68.80)	42 (67.7)
Subretinal drusenoid deposits, n (%)	0	5 (31.3 %)	12 (19.4)
Pigment changes, n (%)	0	0	31 (50)

segmentation. More details on the automated drusen segmentation pipeline can found in the Supplementary Material.

Statistical Analysis

Descriptive statistics were performed to assess baseline demographic variables for the AMD groups and controls. Due to the sample size, most results were not normally distributed (by the Shapiro–Wilk test), so non-parametric tests were used for analysis. The Kruskal–Wallis test was used for group comparisons. Pairwise differences were calculated using the nonparametric Wilcoxon rank-sum test. The relationship between drusen volume and demographic variables was assessed with univariate linear regression. In the overall cohort, separate univariate linear regression models against drusen volume were performed for each of the visual functional tests. If the relationship between a functional test and average person measures reached a $P < 0.05$ in univariate analysis, multiple regression was used to ensure that the findings were not confounded by different demographic characteristics across groups. Statistical analyses were performed using the statistical software SPSS Statistics (IBM, Armonk, NY, USA).[27] $P < 0.05$ was considered statistically significant.

Results

Sociodemographic and Clinical Characteristics of the Participants

The 100 eyes studied included 16 eyes with early AMD (16%), 62 eyes with iAMD (62%), and 22 eyes from healthy controls (22%). The mean age of participants was 67.5 ± 8.1 years, and there were more female participants (66%) than male. The controls were significantly younger than the early AMD and iAMD patients ($P < 0.001$), but there was no significant difference in age between the two AMD groups ($P = 0.88$) (Table 1). Mean image quality of our sample was 30.158 dB (SD 4.395). Mean drusen volume was found to be close to zero for controls (0.00024 ± 0.0003 mm^3). For early AMD, mean drusen volume was higher (0.00272 ± 0.0015 mm^3), and volume was again higher for iAMD (0.13582 ± 0.1945 mm^3). Age was not significantly associated with drusen volume ($P = 0.642$). Early AMD and iAMD patients were found to have a significantly larger drusen volume when compared to controls (each $P < 0.001$), and iAMD patients also had significantly larger drusen volume compared to early AMD ($P < 0.001$) (Fig.). SDDs were present in five eyes with early AMD (31.3 %) and in 12 eyes with iAMD (19.4%) but not in control eyes. Pigment changes were present in 31 eyes with iAMD (50%). All functional vision tests were significantly decreased in iAMD compared

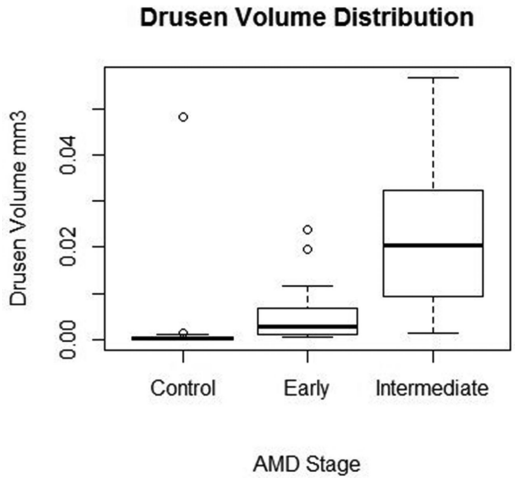

Figure. Boxplot showing drusen volume (in mm^3) for controls and early and intermediate AMD (excluding outliers). Each boxplot includes the maximum (upper whisker), upper quartile (top of the box), median (horizontal line in box), lower quartile (bottom of the box), and minimum (lower whisker) values.

to controls (all $P < 0.05$). BCVA and MAC-VA were also significantly decreased in early AMD compared to controls ($P = 0.016$ and $P = 0.006$, respectively), but there was no significant difference in all other functional tests between the two groups (all $P > 0.05$). When comparing early AMD to iAMD, BCVA and reading speed did not differ significantly ($P = 0.31$ and $P = 0.07$, respectively), but there was a significant decrease in LLVA, MAC-VA, contrast sensitivity, and mesopic and dark-adapted microperimetry in the iAMD group compared with the early AMD group (all $P < 0.05$) (Table 2). Univariate linear regression revealed no significant association between drusen volume and age (β coefficient = 0.001; $P = 0.642$).

Relationship Between Drusen Volume and Visual Function Tests

In univariate linear regression, LLVA, MAC-VA, contrast sensitivity, and mesopic and dark-adapted microperimetry were significantly negatively associated with the overall drusen volume (all $P < 0.006$) (Table 3). After controlling for AMD stage, age, and the presence of SDD, MAC-VA and global mesopic and dark-adapted microperimetry were still significantly associated with drusen volume ($P = 0.008$, $P = 0.023$, and $P = 0.022$, respectively). For mesopic and dark-adapted microperimetry, mean sensitivity at 0°–1° and 3° degrees was significantly associated with drusen volume, whereas mean sensitivity at 5° and 7° was not associated with drusen volume after adjusting for AMD stage, age, and the presence

Table 2. Descriptive Analysis and Group Comparisons

	Mean (SD)			*P*[*]		
	Control	Early AMD	iAMD	Early AMD vs. iAMD	Early AMD vs. Control	iAMD vs. Control
Drusen load, mm^3	0.000236 (0.0003033)	0.002718 (0.0015339)	0.135821 (0.1945323)	**<0.001**	**<0.001**	**<0.001**
BCVA	87.6 (4.1)	83.5 (4,1)	81.6 (6.8)	0.306	**0.016**	**<0.001**
LLVA	74.0 (4.6)	70.0 (6.4)	64.0 (9.3)	**0.008**	0.06	**<0.001**
MAC-VA	68.4 (3.9)	64.1 (4.2)	60.1 (6.7)	**0.026**	**0.006**	**<0.001**
Contrast sensitivity	38.2 (2.9)	37.0 (3.6)	33.6 (3.1)	**<0.001**	0.137	**<0.001**
IReST	166.8 (21.9)	162.7 (23.9)	149.6 (28.1)	0.077	0.693	**0.012**
Mesopic microperimetry	26.2 (1.7)	24.9 (3.2)	23.0 (2.8)	**0.016**	0.056	**<0.001**
Dark-adapted microperimetry	22.7 (1.4)	23.1 (5.7)	20.5 (2.4)	**0.004**	0.715	**<0.001**

[*] *P* values are based on the Wilcoxon rank-sum test.
Bold numbers indicate statistical significance.

Table 3. Linear Regression of Visual Function Measures Against Drusen Volume (in mm^3)

	Univariate Regression		Adjusted for AMD Stage and Age		Adjusted for AMD Stage, Age, and Presence of SDD	
	β Coefficient	*P*	β Coefficient	*P*	β Coefficient	*P*
BCVA	–0.001	0.571	0.001	0.581	0.001	0.588
LLVA	**–0.005**	**0.007**	–0.003	0.091	–0.003	0.093
MAC-VA	**–0.009**	**<0.001**	**–0.007**	**0.008**	**–0.007**	**0.008**
Contrast sensitivity	**–0.014**	**0.001**	–0.007	0.148	–0.008	0.130
IReST	–0.001	0.125	0.000	0.368	–0.000	0.349
Mesopic microperimetry						
Global	**–0.019**	**0.001**	**–0.012**	**0.040**	**–0.014**	**0.023**
0°–1°	**–0.022**	**<0.001**	**–0.018**	**<0.001**	**–0.018**	**<0.001**
3°	**–0.018**	**<0.001**	**–0.012**	**0.015**	**–0.015**	**0.006**
5°	**–0.012**	**0.033**	–0.005	0.306	–0.007	0.195
7°	–0.007	0.130	–0.001	0.784	–0.001	0.609
Dark-adapted microperimetry						
Global	**–0.017**	**0.001**	**–0.011**	**0.022**	**–0.012**	**0.022**
0°–1°	**–0.016**	**<0.001**	**–0.010**	**0.031**	**–0.010**	**0.037**
3°	**–0.021**	**<0.001**	**–0.016**	**0.001**	**–0.017**	**0.001**
5°	**–0.010**	**0.029**	–0.006	0.185	–0.006	0.166
7°	–0.005	0.270	–0.001	0.983	–0.000	0.951
Pigment changes	**0.089**	**0.013**	0.038	0.319	0.034	0.372

Bold numbers indicate statistical significance.

of SDD (Table 3). In univariate regression, the presence of pigment changes was significantly associated with drusen volume ($P = 0.013$), but adjusting for pigment changes in multivariate regression showed no effect on overall results.

Discussion

In our study, drusen volume was associated with visual impairment detected in functional tests under low luminance and challenging contrast conditions in early stages of AMD. Specifically, MAC-VA and both mesopic and dark-adapted microperimetry were significantly associated with drusen volume. These results indicate a structure–function relationship in early stages of AMD that may not be detectable using conventional high-luminance, high-contrast functional tests.

The association between drusen volume and microperimetric sensitivity found in this study is consistent with previous studies in early stages of AMD[28–31]; however, in those studies only mesopic microperimetry was assessed. We also found global dark-adapted microperimetric sensitivity, as well as sensitivities at 0°–1° and 3° eccentricities but not at 5° and 7° eccentricities, to be associated with drusen volume. Our results revealed a significant association between MAC-VA and drusen volume. We also found that MAC-VA was the only functional test that differed significantly among all three groups. When comparing the iAMD group to controls, we found that the performance of all visual function tests decreased significantly in the iAMD group. These findings are comparable with previous studies that have also reported a reduced visual function in these tests. Chandramohan and colleagues[32] also found BCVA, LLVA, and mesopic microperimetry significantly decreased in patients with iAMD compared to healthy controls. Similar results were found by Wu et al.,[33] who found that results of these tests were significantly reduced for all AMD groups except early AMD compared to controls, which is in line with our results. BCVA was on average four letters worse in eyes with early AMD, and this difference was statistically significant. This is comparable to the findings from Owsely et al.[34] and Klein et al.,[35] who reported a significant difference of two letters between these two groups. We did not find a significant relationship between the presence

of SDDs and drusen volume, and adjusting for SDDs in multivariate regression analysis demonstrated no changes in the results. Interestingly, we found a higher prevalence of SDDs in eyes with early AMD (31.3%) than in eyes with iAMD (19.4%), which is in contrast to many other studies and likely a spurious finding due to our small sample size.[36]

We found that LLVA and contrast sensitivity were decreased in the iAMD group compared to controls and early AMD; however, we did not find a significant difference in these functional tests between early AMD and controls. Puell et al.[37] showed that LLVA was impaired in early stages of AMD before changes in BCVA were observed. Decreased contrast sensitivity in early AMD compared to healthy controls has been reported by Feigl and coworkers.[38]

In our study, drusen volume was found to be largest in the iAMD group and significantly lower in the early AMD group and controls. This is in accordance with several other studies demonstrating that drusen volume increases with increasing AMD stage and is predictive of progression to late AMD.[39–41] For early AMD, we calculated a mean drusen volume of 0.0027 mm^3, which is lower than the values reported by Lei and coworkers,[42] who found a mean drusen volume of 0.03 (range, 0.00–0.28) in eyes with early AMD. This could also be explained by the small sample size in our early AMD group. The mean drusen volume measure we obtained for the iAMD group of 0.138 mm^3 is comparable to the results of a study by Yehoshua et al.,[43] who reported drusen volume measures of 0.095 to 0.375 mm^3 in the highest quintile for eyes with nonexudative AMD.

We showed that drusen volume is associated with visual impairment detected by functional tests. This is in line with previous studies that have found that functional tests under low lighting are correlated with retinal morphology in AMD.[44,45]

Strengths of our study include the comprehensive panel of functional tests, including the relatively new MAC charts, for which few data are available, as well as the use of reading performance and dark-adapted microperimetry. All participants were phenotyped according to the current reference-standard retinal imaging in combination with a clinical examination. For drusen volume calculation, we used a new CNN-based approach that allows for the fully automated segmentation of drusen in OCT images. Gorgi Zadeh et al.[14] demonstrated that the CNN-based approach yields much better results than a previous state-of-the-art method reported by Chen et al.[46] and therefore allows for accurate automated assessment of drusen load in AMD. A limitation of our study is the relatively small sample size of the early AMD group and the controls, leading to less statistical power. As common with exploratory studies, no adjustment for multiple testing was done which might lead to an overestimation of statistical power. Another limitation of our study is the fact that the control group was younger than both AMD groups, although univariate linear regression revealed no significant association between age and drusen volume ($P = 0.642$), and we adjusted for age in the multivariate regression analyses.

In conclusion, our study showed that MAC-VA and mesopic and dark-adapted microperimetry are associated with drusen volume in early stages of AMD and thus might provide an indication of structural changes. Our findings suggest that these visual function tests might be useful measurements in monitoring and diagnosing early AMD and iAMD and could be used as functional endpoints in clinical studies. However, more research is warranted, and a longitudinal follow-up will be needed to evaluate the performance of these functional tests as intended, for example, by the MACUSTAR consortium.[47]

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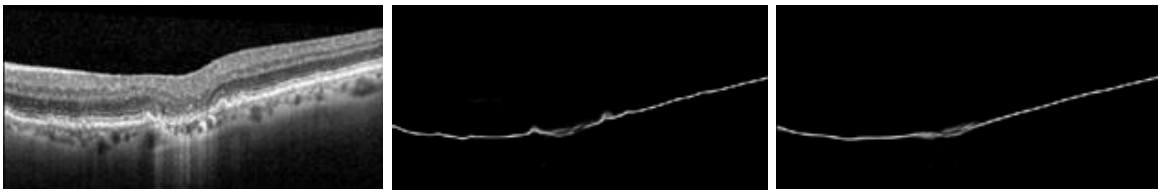
Supplementary Material

In the work by Gorgi Zadeh et al. [1] for automated segmentation of drusen, first the retinal pigment epithelium (RPE) and bruch's membrane (BM) layers are automatically segmented. To do this, they use a convolutional neural network (CNN), which was trained using an independent set of OCT volumes, from patients that were not part of this study. Throughout the training phase, the CNN learns to produce four probability maps, each having the same size as the input image. Two of these maps can be used to assess the location of RPE and BM layers in the input: As it is shown in **Supplementary Figure 1**, the RPE probability map (center) indicates locations that are likely to be part of the RPE layer; analogously, the BM probability map (right) indicates locations that are likely to contain part of the BM.

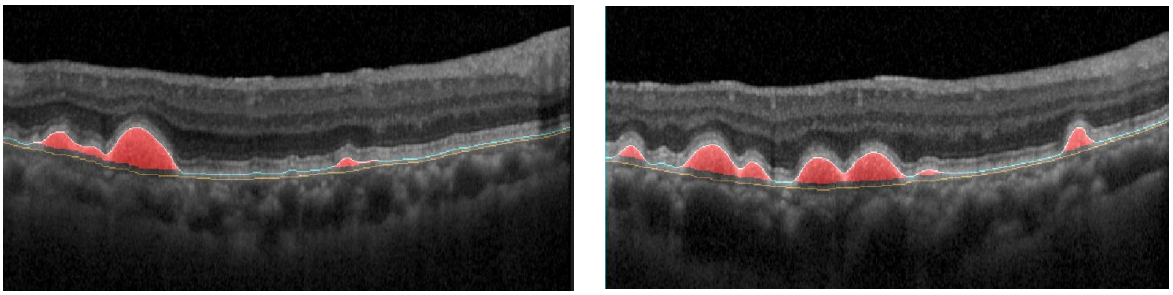
Gorgi Zadeh et al. then use Dijkstra's algorithm to turn these probability maps into the final segmentation of RPE and BM layers, examples of which are shown in **Supplementary Figure 2** [2]. This algorithm is used to ensure that each layer is represented as a curve that connects the left and right boundaries of the image. It achieves this by finding a continuous path through the image, from the leftmost column to the rightmost one, which has a minimum accumulated cost. Gorgi Zadeh et al. define this traversal cost so that the path is drawn towards the pixels in which RPE or BM, respectively, have been detected with high probability.



Supplementary Figure 1: On the left is a sample input B-scan to CNN. In the middle is the probability map produced by the CNN for RPE layer. On the right is the probability map for BM layer.

From the resulting RPE layer segmentation, an ideal (normal) RPE is estimated through a rectification of RPE and BM layers [1]. In the rectification step both RPE and BM are shifted vertically and column-wise so that the BM layer becomes a straight horizontal line. This rectification ensures that the natural curvature of the retina does not lead to false positive

drusen detections. The ideal (drusen-free) RPE is then fitted as a low degree polynomial, and transformed back so that the BM layer is restored to its original shape. Finally, any area that is between RPE and drusen-free RPE is classified as drusen (as shown in red in **Supplementary Figure 2**). To eliminate false positive detections, those with a height of 2 pixels or less are removed. One pixel corresponds to 0.0039 mm in axial direction. Therefore the 2 pixel criterion removes drusen that have a height bellow 0.0078 mm. Details of the CNN have been published previously and can be found in [1].



Supplementary Figure 2: Automatically segmented drusen (red), RPE (cyan) and BM (yellow) layers overlayed on two sample B-scans.

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cera

Impact of Vision Impairment Profile

Anleitung

Bitte lesen Sie jede Frage aufmerksam und umkreisen Sie diejenige Antwort, die am ehesten auf Sie zutrifft.

Machen Sie in jeder Zeile einen Kreis.

Falls Sie eine Brille, Kontaktlinsen oder Lupen für einige Ihrer Tätigkeiten tragen bzw. benutzen, beantworten Sie bitte die Fragen je nachdem wie Sie mit diesen sehen können.

Hier zwei Beispiele: Wie oft waren Sie im vergangenen Monat über Ihr Sehvermögen beunruhigt oder besorgt beim…

	überhaupt nicht	**ab und zu**	**Häufig**	**sehr häufig**	**Ich mache das aus anderen Gründen nicht.**
Überqueren von Straßen?	0	1	2	(3)	8
Zubereiten einer Mahlzeit für sich selbst?	0	1	(2)	3	8

Bitte fangen Sie hier an und vergessen Sie nicht:

Machen Sie in jeder Zeile einen Kreis. Bitte lassen Sie keine Zeile aus.

Bitte geben Sie Auskunft über Ihr Sehvermögen mit einer Brille, Kontaktlinsen oder Lupen, falls Sie diese benutzen.

Wie sehr hat Ihr Sehvermögen Sie im vergangenen Monat bei folgenden Aktivitäten behindert:

	überhaupt nicht	**ab und zu**	**Häufig**	**sehr häufig**	**Ich mache das aus anderen Gründen nicht.**
1. Ihre Fähigkeit fern zu sehen und es auch zu genießen?	0	1	2	3	8
2. Teilnahme an Freizeitaktivitäten wie z.B. Kegeln, Spazieren gehen, Schwimmen?	0	1	2	3	8
3. Einkaufen? (Das Gewünschte zu finden und es zu bezahlen)	0	1	2	3	8
4. Familie oder Freunde zu besuchen?	0	1	2	3	8
5. Leute erkennen oder treffen?	0	1	2	3	8
6. Die Pflege Ihres äußeren Erscheinungsbildes im Allgemeinen? (Gesicht, Haare, Kleidung etc.)	0	1	2	3	8
7. Öffnen von Verpackungen? (z.B. von Lebensmittel, Medikamenten)	0	1	2	3	8
8. Lesen von Beschriftungen oder Anleitungen auf Medikamenten?	0	1	2	3	8
9. Bedienen von Haushaltsgeräten und Telefon?	0	1	2	3	8

Wie sehr hat Ihr Sehvermögen Sie im vergangenen Monat bei folgenden Aktivitäten behindert:

	überhaupt nicht	**ab und zu**	**Häufig**	**sehr häufig**	**Ich mache das aus anderen Gründen nicht.**
10. Wie sehr hat Ihr Sehvermögen Sie dabei behindert, sich draußen zurecht zu finden (auf dem Bürgersteig oder beim Überqueren der Straße)?	0	1	2	3	8
11. Wie oft mussten Sie im vergangenen Monat aufgrund Ihres Sehvermögens vorsichtig gehen, um einen Fall oder ein Stolpern zu vermeiden?	0	1	2	3	8
12. Im Allgemeinen, wie sehr hat Ihre Sehfähigkeit Sie beim Reisen oder Benutzen von Transportmitteln wie z.B. Bus oder Zug beeinträchtigt?	0	1	2	3	8
13. Hinabsteigen von Stufen, Treppen oder Bordsteinkanten?	0	1	2	3	8

Wie sehr hat Sie Ihre Sehfähigkeit im vergangenen Monat bei folgenden Aktivitäten beeinträchtigt?

63

	überhaupt nicht	**ab und zu**	**Häufig**	**Ich mache das aus anderen Gründen nicht.**
14. Lesen von normal groß Gedrucktem? (z.B. Zeitung)	0	1	2	8
15. Erlangen von Informationen, die Sie brauchen? (z.B. Telefonnr. und Adressen)	0	1	2	8

Bitte geben Sie Auskunft über Ihr Sehvermögen mit einer Brille, Kontaktlinsen oder Lupen, falls Sie diese benutzen.

Wie oft waren Sie aufgrund Ihres Sehvermögens im vergangenen Monat beunruhigt oder besorgt über Folgendes:

	überhaupt nicht	**ab und zu**	**Häufig**	**sehr häufig**
16. Ihre allgemeine Sicherheit zu Hause?	0	1	2	3
17. Verschütten oder Kaputtmachen von Dingen?	0	1	2	3
18. Ihre allgemeine Sicherheit, wenn Sie außer Haus sind?	0	1	2	3
19. Wie oft im letzten Monat hat Ihr Sehvermögen Sie davon abgehalten, Dinge zu tun, die Sie tun wollten?	0	1	2	3
20. Wie oft haben Sie aufgrund Ihres Sehvermögens im vergangenen Monat Hilfe von anderen Personen benötigt?	0	1	2	3

Bitte denken Sie darüber nach, wie Sie sich aufgrund Ihres Sehvermögens im vergangenen Monat gefühlt haben.

	überhaupt nicht	**ab und zu**	**Häufig**	**sehr häufig**
21. Haben Sie sich wegen Ihres Sehvermögens geniert?	0	1	2	3
22. Waren Sie wegen Ihres Sehvermögens frustriert oder verärgert?	0	1	2	3
23. Haben Sie sich wegen Ihres Sehvermögens einsam oder isoliert gefühlt?	0	1	2	3
24. Haben Sie sich wegen Ihres Sehvermögens traurig oder niedergeschlagen gefühlt?	0	1	2	3
25. Wie oft waren Sie im vergangenen Monat besorgt, dass Ihr Sehvermögen sich verschlechtern könnte?	0	1	2	3
26. Wie oft haben Sie sich wegen Ihres Sehvermögens im vergangenen Monat Sorgen gemacht, wie Sie Ihren Alltag bewältigen?	0	1	2	3
27. Haben Sie sich wegen Ihres Sehvermögens wie eine Belästigung oder Belastung gefühlt?	0	1	2	3
28. Wie sehr hat Sie Ihr Sehvermögen im vergangenen Monat in Ihrem Leben im Allgemeinen beeinträchtigt?	0	1	2	3

Bitte überprüfen Sie, ob Sie alle Fragen beantwortet haben. Vielen Dank!

Statement – Bescheinigung über Anteil der an der Dissertation geleisteten Arbeit

Hiermit wird erklärt, dass die Promovendin Susanne G. Pondorfer den Großteil der Arbeit der für die Dissertation eingereichten Publikationen geleistet hat.

Hiermit versichere ich außerdem schriftlich, dass ich keine anderen als die in den Dissertationen angegebenen Quellen und Hilfsmittel benutzt habe.

Bonn, 10.02.2020

Ort, Datum

Unterschrift