Topographic Analysis of Photoreceptor Loss Correlated with Disease Morphology in Neovascular Age-Related Macular Degeneration

Abbreviated title: Topography of Photoreceptor Loss in nAMD

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Brief summary statement:

This study characterizes photoreceptor layer integrity during anti-VEGF therapy of neovascular AMD, based on SD-OCT data of a large prospective, multicentre trial and correlates photoreceptor status with visual function. Furthermore, we performed topographic analysis of photoreceptor integrity and fluid-related imaging biomarkers, enabling insight into spatiotemporal morphologic developments.

Abstract

Purpose: To quantify morphologic photoreceptor integrity during anti-vascular endothelial growth factor (anti-VEGF) therapy of neovascular age-related macular degeneration (nAMD) and correlate these findings with disease morphology and function.

Methods: This presents a post-hoc analysis on SD-OCT data of 185 patients, acquired at baseline, month 3 and month 12 in a multicenter, prospective trial. Loss of the ellipsoid zone (EZ) was manually quantified in all OCT volumes. Intraretinal cystoid fluid, subretinal fluid (SRF) and pigment epithelial detachments were automatically segmented in the full volumes using validated deep learning methods. Spatiotemporal correlation of fluid markers with EZ integrity as well as bivariate analysis between EZ integrity and best-corrected visual acuity (BCVA) were performed.

Results: At baseline, EZ integrity was predominantly impaired in the fovea, showing progressive recovery during anti-VEGF therapy. Topographic analysis at baseline revealed EZ integrity to be more likely intact in areas with SRF and vice versa. Moreover, we observed a correlation between EZ integrity and resolution of SRF. Foveal EZ integrity correlated with BCVA at all timepoints.

Conclusion: Improvement of EZ integrity during anti-VEGF therapy of nAMD occurred predominantly in the fovea. Photoreceptor integrity correlated with BCVA. EZ integrity was preserved in areas of SRF and showed deterioration upon SRF resolution.

Introduction

The benefit of intravitreal therapy with anti-vascular endothelial growth factor (anti-VEGF) agents in neovascular age-related macular degeneration (nAMD) is well established.[1] In pivotal clinical trials as well as in real world settings, patients on average experienced a gain in visual function following the initiation of anti-VEGF treatment.[2] Some patients, however, show limited gain, no gain or even a decline in visual function despite the intervention.[3] Permanent damage to the neurosensory retina caused by exudation, haemorrhage, neovascular ingrowth as well as scarring are the factors prohibiting a gain in visual function during the course of anti-VEGF therapy.[4]

Spectral domain–optical coherence tomography (SD-OCT) has become standard of care in the diagnosis and monitoring of patients affected by nAMD.[5] Furthermore, by enabling detailed visualization of distinct retinal structures, SD-OCT has opened the field of research on imaging biomarkers, enhancing our understanding of the individual course of disease and facilitating personalized treatment approaches.[6] Among various morphologic features, SD-OCT offers visualization of photoreceptor substructures via so-called outer retinal hyperreflective bands. These include among others the external limiting membrane, which also consists of Müller glia processes, and the EZ, also termed inner segment/outer segment junction. Several previous studies have shown a clear correlation between photoreceptor layer integrity and visual function.[7-9] These analyses, however, were relatively small case series based on qualitative OCT image assessment. Quantitative analyses of photoreceptor integrity in nAMD before and during anti-VEGF therapy are lacking. Our study aims to

characterize the condition of the photoreceptor layer during anti-VEGF therapy of nAMD in a multicentre trial using topographic as well as quantitative approaches. Moreover, we describe the restoration of EZ integrity, occurring predominantly in the fovea. We present three-dimensional analyses of the association between EZ integrity and major imaging biomarkers in nAMD, such as intraretinal cystoid fluid (IRC), subretinal fluid (SRF) and pigment epithelial detachment (PED), identified by methods of artificial intelligence.

Methods

Study design and participants

This is a post-hoc analysis of a prospective, randomized, controlled clinical trial using 0.5mg Ranibizumab (Lucentis) in an as-needed regimen (OCTAVE; clinicaltrials.gov identifier: NCT01780935). Data acquired at baseline, month 3 and month 12 were included into our analysis. To be eligible for the OCTAVE trial, participants had to present with visual impairment due to active, newly diagnosed, untreated, center-involving macular neovascularization (MNV) due to AMD. Patients had to be aged at least 50 years. All patients provided written, informed consent and institutional review board approval was obtained at each participating centre. All study procedures were conducted in accordance with the Declaration of Helsinki. Patient data was fully anonymized. Approval for this analysis was obtained from the Ethics Committee at the Medical University of Vienna and the Study Sponsor (Novartis).

Imaging and functional assessments

Of the OCTAVE cohort (n=681), only patients imaged by Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) (n=473) were included. This selection was made because of better identification of the photoreceptor layer with Spectralis OCT due to automated real time averaging (ART).[10] The imaging protocol comprised OCT volume scans with 512 A-scans and 49 B-scans in a 20° by 20° field of view centered on the fovea and was performed by certified, masked examiners. ART was activated at 29 frames. Best-corrected visual acuity (BCVA) testing was performed by certified, masked examiners using Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

Image Analysis

SD-OCT resolves four hyperreflective bands in the outer retina, of which the second innermost has been termed EZ[11] or inner segment/outer segment junction. We specified the lack of EZ integrity as its complete focal absence in the z-axis, meaning full-thickness vertical disruptions/gaps over any distance. Annotation of focal photoreceptor disruptions was performed on each of the 49 OCT B-Scans of all patients and timepoints by a certified masked reader, supervised by a retina specialist. (Figure 1)

Automated segmentation of exudative components, comprising IRC, SRF and PED, was performed in the full volumes in order to assess spatial correspondence of these lesions with EZ integrity in a topographic manner. IRC and SRF were detected on a per-voxel basis using a fully automated, previously validated algorithm based on deep learning.[12] PED was segmented using the automated Iowa Reference Algorithm, developed to fit the outer boundary of retinal pigment epithelium (RPE) and Bruch's membrane.[13] We used a previously described size definition for PED including only

lesions of more than 400 μm in width and 75 μm in height, or more than 200 μm in height.[14]

In addition, the presence of subretinal hyperreflective material (SHRM), fibrosis and atrophy was evaluated in the central millimetre subfield for each timepoint by a certified, masked grader. SHRM was defined as hyperreflective material located external to the retina and internal to the RPE. The presence of compact, hyperreflective material with equal or higher reflectivity compared to the RPE was graded as fibrosis. Atrophy was defined by RPE attenuation or loss with concomitant subsidence of the outer retinal layers and signal transmission to the choroid. Fovea position was manually annotated as previously described[15] on the basis of minimization of the retinal nerve fibre layer thickness, presence of a foveal depression and focal elongation of the photoreceptor outer segment signal to enable ETDRS grid positioning as well as spatiotemporal correlation on an intra- and interpatient level.

Statistical Analysis

The data set was reported using descriptive statistics. To test for statistically significant differences of photoreceptor integrity (change) between ETDRS fields and timepoints appropriate tests were chosen depending on data distribution (paired comparisons were done using Wilcoxon signed ranks test or paired t-test, whereas for unpaired group comparisons Wilcoxon rank sum test and unpaired t-Test were used). EZ integrity was compared between groups defined by the presence or absence of imaging biomarkers using analysis of variance.

Topographic correlation or dissociation of EZ disruption with IRC, SRF and PED was assessed on a per A-scan level. For each en-face pixel chi square tests including these respective pairs of features were performed over all patients. Statistically significant

odds ratios (ORs), indicating the topographic correlation or dissociation between pairs, were calculated for each pixel across all patients and presented as color-coded en-face plots.[16]

To investigate correlations between EZ integrity change and change in IRC, SRF and PED area bivariate analysis was performed, reporting Pearson's correlation coefficient.

Due to the exploratory nature of the analysis, p-values below <0.05 indicate exploratory significance and adjustment for multiple testing was not performed.

Results

Data disposition and patient characteristics

Data acquired at baseline, month 3 and month 12 was available for 223 patients.38 eyes (17%) were excluded due to insufficient image quality and segmentation errors. Therefore, a total of 185 eyes of 185 patients were included into this post-hoc analysis, yielding 555 OCT volume scans and 27,195 B-scans for annotation. Mean BCVA (letter score) at baseline, month 3 and month 12 was 61.4 (±12.83), 69.3 (± 13.45) and 69.7 (±15.77), respectively. Descriptive statistics of OCT morphology in our cohort are presented in Table 1.

Photoreceptor integrity at baseline and during therapy

Figure 2A shows median EZ integrity in the nine ETDRS fields at baseline, month 3 and month 12. At baseline, EZ integrity impairment was most affected pronounced in the fovea with a median integrity as low as 31% [interquartile range 0; 73]. With treatment, the EZ signal (mean ±SD) showed better recovery in the foveal area

compared to the extrafoveal retina from baseline to month 3 (+6% ±33 vs. -3% ±10, p<0.001), from month 3 to month 12 (+12% ±26 vs. +4% ±7, p<0.001), as well as from baseline to month 12 (+18% ±37 vs. +1% ±12, p<0.001). To reflect the response of photoreceptor integrity in a spatiotemporal manner, cumulative distribution plots of EZ, which topographically illustrate photoreceptor recovery, were designed (Figure 2B). Exemplary cases are provided in Figure 3A-C.

Development of OCT morphology during therapy

All fluid-related markers showed a decrease in affected area following initiation of therapy (Table 1). While IRC and SRF maintained low levels of fluid area following treatment (median 0.02-0.04mm^2), PED area remained higher at a median 2.88mm^2 and 3.32mm^2 at month 3 and 12, respectively. Prevalence of SHRM in the central mm decreased by 56% from baseline to month 3, while fibrosis was present in a quarter of patients at month 3 and 12 in the central mm compared to 4% at baseline. Similarly, the prevalence of atrophy tripled from baseline to 36% at month 12.

Correlation between photoreceptor integrity and vision

Foveal EZ integrity showed a moderate positive correlation with BCVA, with a correlation coefficient of r=-0.47 at baseline, r =-0.64 at month 3 and r=-0.63 at month 12, respectively (Supplementary Figure 1). BCVA change and change in EZ integrity, however, did not show any meaningful correlation, neither from baseline to month 3, nor from month 3 to month 12 (r =-0.18 and r =-0.26, respectively).

Topographic correlation of fluid markers with photoreceptor integrity

Topographic per-pixel correlation between morphological photoreceptor integrity and fluid markers at baseline showed that EZ integrity was more likely to be preserved in areas with SRF and vice versa (Figure 4). Co-localization of PED and impaired EZ integrity appeared throughout the entire macular area. IRC showed a sparse positive topographic correlation with the distribution of impaired EZ integrity, i.e. areas with PED and IRC were more likely to exhibit concomitant photoreceptor impairment (Figure 4).

Fluid-related morphologic changes were assessed from baseline to month 3, as the majority of fluid resolution has been shown to occur after the initial injection[17]. No meaningful correlations between the change in EZ integrity from baseline to month 3 and the change in IRC or PED area could be observed. However, an inverse correlation with the change in SRF area from baseline to month 3 was revealed, i.e. resolution of SRF correlated with an decrease in EZ integrity ($r=-.351$, $p<0.001$, Supplementary Figure 2). This change in EZ integrity following resolution of SRF is also evident in spatiotemporal en-face maps (Figure 3). En-face maps occasionally also showed re-appearance of EZ integrity at month 12 in regions affected by SRF at baseline.

Correlation of the presence of SHRM, fibrosis, atrophy and fluid markers with photoreceptor integrity

Table 2 details the results of analysis of variance between photoreceptor integrity and the presence of the most characteristic nAMD features. Patients presenting with SHRM at baseline showed significantly lower EZ integrity in the central mm at baseline (70.6% [±31] vs. 33.5% [±36.7], $p=0.002$). We observed trends of lower baseline and month 12 EZ integrity in patients with baseline IRC in the central mm

and higherbaseline and month 12 EZ integrity in patients with baseline SRF in the central mm. Both did not reach exploratory significance. Furthermore, impairment of foveal EZ integrity at month 12 was greater in patients showing atrophy at month 12 (66.5% [±32.4] vs. 27.5% [±31.2], p<0.001). Less than 20 patients presented with fibrosis or no PED at baseline, which is why these parameters were excluded from the model. Analysis of patients presenting with SRF in the central mm at baseline, revealed greater impairment of mean foveal EZ integrity (57.3% ±37.4) at month 12 in patients presenting SRF resolution (n=39) compared to 27.9% (±33.4) in patients showing persistent/recurring SRF in central mm at month 12 (n=84) (p<0.001).

Discussion

Research in retinal morphology during anti-VEGF therapy of nAMD has intensively focused on the presence and resolution of IRC and SRF, on changes in PED and on the development of atrophic and fibrotic stages of disease. OCT, however, also allows more detailed and difficult assessment of neurosensory retinal morphology relevant for visual function, such as that of the photoreceptor layers. Previous approaches have categorized photoreceptor integrity on OCT into presence, disruption and absence. In small studies, the recovery of photoreceptor integrity following the initiation of therapy, has been shown.[7,9,18] This post-hoc analysis assesses and characterizes the course of photoreceptor integrity during anti-VEGF therapy in a much larger cohort, undergoing a standardized treatment protocol. Images were not captured specifically for this analysis, but for a previously performed, prospective, multicentre, randomized trial. Furthermore, we apply a fully quantitative approach, which highlights the spatiotemporal characteristics of the course of EZ integrity and recovery.

In our cohort, foveal photoreceptors were profoundly impaired at baseline. This may be due to the mostly subfoveal location of the neovascular membrane, as requested by the inclusion criteria. MNV (type I, II or III) may be causative for disrupting the photoreceptor layers morphologically. Furthermore, intraretinal exudation has been shown to be mostly restricted to the macular center.[16] The presence of IRC is known to have a detrimental effect on the neurosensory architecture, leading to disruptions in the outer retinal layers.[19]

Following initiation of therapy, photoreceptor integrity increased throughout the entire macular area, predominantly in the fovea (Figure 2). Resolution of SHRM, due to regression of the MNV and consecutive realignment of photoreceptors is one possible explanation for this observation. In numerous cases the en-face topographies of EZ integrity following treatment initiation showed explicit "foveal sparing" (Figure 2B), i.e. impairment of photoreceptors was less in the fovea than in surrounding areas. This topographic distribution phenomenon of retinal lesions is seen in several acquired as well as hereditary retinal diseases, such as Bull's eye macular dystrophies and geographic atrophy and suggests protective properties of foveal microstructure.[20] Regeneration of the cone-enriched fovea compared to the rod-dominated parafovea might underline the increased vulnerability of rods over cones, which has been shown in normal ageing as well as in all stages of AMD.[21,22]

Since in this analysis assessment of photoreceptor integrity was solely based on the evaluation of OCT images, one has to keep in mind the implications for interpretation of layer disruption and supposed "regeneration". Loss of EZ signal may imply loss of

photoreceptor cells. Further reasons, however, might include lateral displacement or misalignment of photoreceptor cells. Axial directionality of the incoming light has been shown to affect reflectivity in OCT, a property linked to the Stiles Crawford effect.[23] Consequently, axial orientation of photoreceptors might influence structural reflectivity in a similar way.

While comprehensive histologic analyses of the EZ integrity status with the actual cellular photoreceptor condition are lacking, studies investigating photoreceptors via multimodal imaging, including adaptive optics (AO) imaging, revealed decreased cone mosaic density on AO to correlate with disruption of the EZ band in OCT.[24] Photoreceptor morphology, whether it be rod vulnerability in AMD or cone mosaic disruption in retinal dystrophies, visualized by AO, is reported to correlate with function.[24,25]

In our analysis, a correlation between photoreceptor integrity and retinal function (BCVA) was observed at baseline, month 3 and month 12. Change in photoreceptor integrity, however, did not correlate with vision change. Photopic contrast sensitivity and retinotopic ocular sensitivity measurements, derived from microperimetry testing have been shown to present more appropriate parameters when attempting to correlate visual function with morphologic changes.[25,26] For example, microperimetric analyses have shown areas with SRF to show higher functional improvements than areas with intraretinal fluid or fibrovascular PED during anti-VEGF therapy of nAMD.[27] In general, there is a correlation between structure and function, which, however, is not tight enough to detect when morphologic changes occur over time. This has to be

considered when interpreting OCT morphology in its impact on function, as structural changes do not necessarily relate to functional loss.

In depth per-pixel analysis of the topographic correspondence between EZ integrity and IRC, SRF and PED at baseline revealed that areas with SRF are more likely to exhibit intact photoreceptors. While the EZ band often presented delineable overlying SRF, we did, however, observe qualitative alterations in thickness and reflectivity. Photoreceptor- and function-preserving properties have already previously been attributed to SRF, for instance in a post-hoc analysis of the Comparison of Age-related Macular Degeneration Treatment Trials.[28,29] Furthermore, a large, prospective, multicentre, randomized treat-and-extend trial showed patients, in which some SRF was tolerated to have non-inferior functional outcomes to patients, in which treatment aimed to resolve SRF completely.[30] In this post-hoc analysis, the structure-preserving nature of SRF shows in a trend toward greater photoreceptor integrity at month 12 in patients presenting SRF at baseline. Most interestingly, a decrease in SRF area at month 12 correlated inversely with EZ integrity. This may be due to a real decay of photoreceptors as a results of e.g. the lack of a spatial buffer between photoreceptors and toxic metabolites or the mere misalignment of photoreceptors resulting in a decreased/missing EZ signal. By month 12, EZ recurrence was found in some parts of the region previously occupied by SRF (Figure 3). Nevertheless resolution of SRF by month 12 was associated with decreased morphologic photoreceptor integrity at the same timepoint. We may speculate recurrence of photoreceptor signal to be linked to the realignment of photoreceptors after previous misalignment caused by the “drying” effect of anti-VEGF. Functional studies, applying microperimetry testing in cases of

SRF before and after resolution, might provide more detailed insight into structure-function correlation of the photoreceptor layers, visualized by OCT.

SHRM in the central millimetre was associated with decreased photoreceptor integrity at baseline, however did not entail significantly decreased photoreceptor integrity at month 12. This may be due to the predominant foveal recovery, frequently observed following resolution of SHRM (Figure 3). A recently published retrospective analysis on the correlation between SHRM and visual acuity goes in line with our findings.[31] In this analysis, disappearance of SHRM was more closely associated with intact EZ, whereas compact SHRM correlated with disrupted EZ. Furthermore, visual acuity was there shown to improve upon SHRM disappearance. We observed a trend toward less photoreceptor integrity at month 12 in patients with IRC at baseline, which is in line with the previously reported vision-deteriorating effect of intraretinal fluid.[32,33]As to be expected, the development of atrophy at month 12 was associated with decreased photoreceptor integrity, as was the development of fibrosis.

This analysis presents another excellent example of an application of deep learning, as this innovative, in-depth topographic approach determining localization, area and volume of features was made possible only by the automated segmentation of fluid compartments. Efforts are being undertaken to facilitate automated segmentation of the photoreceptor layers on OCT to further enhance our understanding of the condition of this layer during disease and therapy (Automated Quantification of Photoreceptor alteration in macular disease using Optical Coherence Tomography and Deep Learning, Orlando J et al, submitted for publication).

Limitations of this analysis include its retrospective character and its restriction to the EZ band, as only one of the morphologic structures attributable to photoreceptors on OCT images. Furthermore, assessment of the EZ, as a very fine structure, is naturally dependent on local image quality. Visualization by OCT entails inherent artefacts, such as shadowing of the photoreceptor layers by overlying structures. Advanced imaging and functional testing modalities, such as AO and microperimetry present promising approaches, which will provide further insight into these findings.

During progressive exudative disease and the course of anti-VEGF therapy it is the dysfunction, degeneration and irreversible alteration of photoreceptors that causes permanent vision loss. This pathological progression can be characterized by the course of photoreceptor integrity, visualized by SD-OCT, and correlates with concomitant features pathognomonic for nAMD. We show profound impairment of foveal photoreceptors at baseline as well as a solid recovery of photoreceptor (signal) within the foveal area. Moreover, photoreceptor integrity correlated well with BCVA. We demonstrated a photoreceptor-protective effect of SRF. In contrast, SHRM, fibrosis, PED and IRC have a negative impact on photoreceptor integrity. The availability of automated photoreceptor segmentation methods based on artificial intelligence will offer novel horizons to quantify photoreceptor status in clinical practice in the future.

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References

1. Maguire MG, Martin DF, Ying GS, et al. Five-Year Outcomes with Anti-Vascular Endothelial Growth Factor Treatment of Neovascular Age-Related Macular Degeneration: The Comparison of Age-Related Macular Degeneration Treatments Trials. Ophthalmology 2016.
2. Brown DM, Michels M, Kaiser PK, et al. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology 2009;116:57-65 e5.
3. Nguyen CL, Gillies MC, Nguyen V, et al. Characterisation of poor visual outcomes of neovascular age-related macular degeneration treated with anti-vascular endothelial growth factor agents. Ophthalmology 2018.
4. Daniel E, Toth CA, Grunwald JE, et al. Risk of scar in the comparison of age-related macular degeneration treatments trials. Ophthalmology 2014;121:656-66.
5. Schmidt-Erfurth U, Klimscha S, Waldstein SM, Bogunovic H. A view of the current and future role of optical coherence tomography in the management of age-related macular degeneration. Eye (Lond) 2017;31:26-44.
6. Schmidt-Erfurth U, Waldstein SM. A paradigm shift in imaging biomarkers in neovascular age-related macular degeneration. Prog Retin Eye Res 2016;50:1-24.
7. Kim YM, Kim JH, Koh HJ. Improvement of photoreceptor integrity and associated visual outcome in neovascular age-related macular degeneration. Am J Ophthalmol 2012;154:164-73 e1.
8. Shin HJ, Chung H, Kim HC. Association between foveal microstructure and visual outcome in age-related macular degeneration. Retina 2011;31:1627-36.
9. Coscas F, Coscas G, Lupidi M, et al. Restoration of Outer Retinal Layers After Aflibercept Therapy in Exudative AMD: Prognostic Value. Invest Ophthalmol Vis Sci 2015;56:4129-34.
10. Sander B, Larsen M, Thrane L, Hougaard JL, Jorgensen TM. Enhanced optical coherence tomography imaging by multiple scan averaging. Br J Ophthalmol 2005;89:207-12.
11. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF, International Nomenclature for Optical Coherence Tomography P. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN*OCT consensus. Ophthalmology 2014;121:1572-8.
12. Schlegl T, Waldstein SM, Bogunovic H, et al. Fully Automated Detection and Quantification of Macular Fluid in OCT Using Deep Learning. Ophthalmology 2018;125:549-58.

13. Garvin MK, Abramoff MD, Wu X, Russell SR, Burns TL, Sonka M. Automated 3-D intraretinal layer segmentation of macular spectral-domain optical coherence tomography images. IEEE Trans Med Imaging 2009;28:1436-47.
14. Simader C, Ritter M, Bolz M, et al. Morphologic parameters relevant for visual outcome during anti-angiogenic therapy of neovascular age-related macular degeneration. Ophthalmology 2014;121:1237-45.
15. Wu J, Waldstein SM, Montuoro A, Gerendas BS, Langs G, Schmidt-Erfurth U. Automated Fovea Detection in Spectral Domain Optical Coherence Tomography Scans of Exudative Macular Disease. Int J Biomed Imaging 2016;2016:7468953.
16. Klimscha S, Waldstein SM, Schlegl T, et al. Spatial Correspondence Between Intraretinal Fluid, Subretinal Fluid, and Pigment Epithelial Detachment in Neovascular Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci 2017;58:4039-48.
17. Waldstein SM, Simader C, Staurenghi G, et al. Morphology and Visual Acuity in Aflibercept and Ranibizumab Therapy for Neovascular Age-Related Macular Degeneration in the VIEW Trials. Ophthalmology 2016;123:1521-9.
18. Sayanagi K, Sharma S, Kaiser PK. Photoreceptor status after antivascular endothelial growth factor therapy in exudative age-related macular degeneration. Br J Ophthalmol 2009;93:622-6.
19. Pelosini L, Hull CC, Boyce JF, McHugh D, Stanford MR, Marshall J. Optical coherence tomography may be used to predict visual acuity in patients with macular edema. Invest Ophthalmol Vis Sci 2011;52:2741-8.
20. Bird AC, Bok D. Why the macula? Eye (Lond) 2018;32:858-62.
21. Curcio CA. Photoreceptor topography in ageing and age-related maculopathy. Eye (Lond) 2001;15:376-83.
22. Curcio CA, Millican CL, Allen KA, Kalina RE. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. Invest Ophthalmol Vis Sci 1993;34:3278-96.
23. Gao W, Cense B, Zhang Y, Jonnal RS, Miller DT. Measuring retinal contributions to the optical Stiles-Crawford effect with optical coherence tomography. Opt Express 2008;16:6486-501.
24. Chen Y, Ratnam K, Sundquist SM, et al. Cone photoreceptor abnormalities correlate with vision loss in patients with Stargardt disease. Invest Ophthalmol Vis Sci 2011;52:3281-92.
25. Mones J, Rubin GS. Contrast sensitivity as an outcome measure in patients with subfoveal choroidal neovascularisation due to age-related macular degeneration. Eye (Lond) 2005;19:1142-50.
26. Parravano M, Oddone F, Tedeschi M, et al. Retinal functional changes measured by microperimetry in neovascular age-related macular degeneration treated with ranibizumab: 24-month results. Retina 2010;30:1017-24.
27. Sulzbacher F, Roberts P, Munk MR, et al. Relationship of retinal morphology and retinal sensitivity in the treatment of neovascular age-related macular degeneration using aflibercept. Invest Ophthalmol Vis Sci 2015;56:1158-67.
28. Sharma S, Toth CA, Daniel E, et al. Macular Morphology and Visual Acuity in the Second Year of the Comparison of Age-Related Macular Degeneration Treatments Trials. Ophthalmology 2016;123:865-75.

29. Philip AM, Podkowinski D, Pablik E, et al. Presence of subretinal fluid at baseline preserves from photoreceptor alterations in age-related macular degeneration, diabetic macular edema and cystoid macular edema due to central retinal vein occlusion. ARVO Poster 2016.
30. Guymer RH, Markey CM, McAllister IL, et al. Tolerating Subretinal Fluid in Neovascular Age-Related Macular Degeneration Treated with Ranibizumab Using a Treat-and-Extend Regimen: FLUID Study 24-Month Results. Ophthalmology 2018.
31. Kumar JB, Stinnett S, Han JIL, Jaffe GJ. Correlation of Subretinal Hyperreflective Material Morphology and Visual Acuity in Neovascular Age-Related Macular Degeneration. Retina 2019.
32. Waldstein SM, Philip AM, Leitner R, et al. Correlation of 3-Dimensionally Quantified Intraretinal and Subretinal Fluid With Visual Acuity in Neovascular Age-Related Macular Degeneration. JAMA Ophthalmol 2016;134:182-90.
33. Schmidt-Erfurth U, Waldstein SM, Deak GG, Kundi M, Simader C. Pigment epithelial detachment followed by retinal cystoid degeneration leads to vision loss in treatment of neovascular age-related macular degeneration. Ophthalmology 2015;122:822-32.

Figure and Table Legends:

Figure 1: Annotation of EZ disruption. (A) From left to right: Example-Bscan showing the border region of EZ integrity within a white rectangle, border region displayed in an enlarged view, A-scan based annotation of disrupted EZ (area marked yellow). External limiting membrane (ELM) is marked for descriptive purpose only. (B) Example case at baseline, month 3 and month 12: Central B-scans without (a) and with (b) annotation of EZ disruption. (c) En-face view of the area of EZ disruption per OCT volume with central B-scan marked by red line. ETDRS grid is superimposed on the en-face view.

Figure 2: A, Median EZ integrity in ETDRS subfields at baseline, month 3 and month 12. Photoreceptor integrity was lowest in the foveal center at baseline and improved with therapy. B, Spatiotemporal plots of EZ integrity. The frequency of photoreceptor disruptions over all patients is indicated in greyscale on a per-pixel level. Graphs are centered on the fovea and right eyes are mirrored to conform to left eyes. EZ integrity

was most impairmed at baseline in the foveal center. While in parafoveal areas loss of photoreceptor integrity was maintained at month 12, the foveal center demonstrated a clear recovery of the EZ signal.

Figure 3 A-C: Exemplary developments from individual patients. From top to bottom row: Central B-scan, en-face EZ integrity loss in yellow, en-face maps of IRC, SRF and PED presence at baseline (first column), month 3 (second column) and month 12 (third column). Case A shows an eye with focal impairment of EZ integrity in the fovea at baseline. Progressive resolution of SHRM and concomitant improvement in foveal EZ integrity are seen following treatment. Increasing loss of EZ integrity in the temporal para- and perifovea topographically corresponds to the area of SRF resolution. A similar pattern concerning topographic correlation of SRF resolution with loss of EZ integrity is evident in Case B and C, whereby distinct foveal sparing can be noted in the former. Case B, exhibiting fluid only in the subretinal compartment, presents largely intact EZ integrity at baseline. In contrast, case C, showing extensive IRC, SRF and PED presents with impaired EZ integrity on a larger scale, in particular in areas affected by IRC and PED. Note the patchy re-appearance of EZ at month 12 in the area affected by SRF at baseline.

Table 1: OCT morphology at baseline, month 3 and month 12

Figure 4: En-face per pixel odds ratio maps showing areas with significant topographic correspondence between features at baseline. Positive correspondence (log odds ratio > 0) are colored in light to dark green and dissociation (log odds ratio < 0) in yellow to red. Graphs are centered on the fovea and right eyes are mirrored to

conform to left eyes. A dissociation between the presence of subretinal fluid and photoreceptor impairment was observed.

Table 2: Mean EZ integrity impairment in the central millimetre at baseline and month 12 depending on morphologic characteristics

Supplementary Figure 1: Correlation between best-corrected visual acuity and foveal EZ impairment at baseline, month 3 and month 12. The correlation between morphology and function strengthens as the retina is progressively dried out in later timepoints of the study.

Supplementary Figure 2: Scatter dot plots between change in EZ integrity and change in IRC (upper left), SRF (upper right) and PED (bottom) area, reveal a correlation for SRF and EZ integrity area change from baseline to month 3, i.e. resolution of SRF correlated with a decrease in EZ integrity.