Effects on Choroidal Neovascularization after Anti-VEGF Upload Using Intravitreal Ranibizumab, as Determined by Spectral Domain-Optical Coherence Tomography

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PURPOSE. It is unclear whether anti-VEGF monotherapy in age-related macular degeneration (AMD) achieves morphologic CNV regression or only stops further CNV growth. In this study, spectral domain-optical coherence tomography (SD-OCT) was used to image CNV structure before and after anti-VEGF treatment.

METHODS. Out of 107 consecutive patients, a prospective CNV evaluation was possible in 78 of them. Newly diagnosed CNV (classic CNV: \( n = 16 \); occult CNV: \( n = 54 \); minimal classic CNV: \( n = 8 \)) due to AMD was imaged before and 4 weeks after anti-VEGF upload in three intravitreal injections of ranibizumab. Qualitative (structural changes) and quantitative measurements (diameter and thickness) of the CNV were obtained from the OCT images.

RESULTS. Classic CNV components were observed above the RPE/photoreceptor complex, whereas occult CNVs stayed below. Of all postoperative OCTs, 59% revealed complete dry retinal structures, 27% showed reduced edema, and 14% showed edema remaining unchanged. Mean macular thickness decreased significantly from 427 to 303 \( \mu \text{m} \) (\( P = 0.000 \)). Qualitatively, overall CNV architecture appeared to be unchanged in 78%, was reduced in thickness in 18%, and became larger in 4%. Quantitatively, in all CNV subtypes, the diameter changed in 78%, was reduced in thickness in 18%, and became larger in 4%.

CONCLUSIONS. With SD-OCT, CNV size can be two-dimensionally determined and followed up after intravitreal anti-VEGF treatment. In only 4% of CNV was enlargement observed, whereas in 78%, CNV architecture appeared qualitatively unchanged, independent of retinal edema. Quantitative measurements underlined stable CNV diameters for all subtypes but revealed significant reduction of thickness especially for classic CNV components. In this series, ranibizumab monotherapy was able to morphologically stop further CNV growth but, in most patients, did not lead to a major regression of CNV, especially of its occult components. (Invest Ophthalmol Vis Sci. 2010;51: 1671–1676) DOI:10.1167/iovs.09-4496

Age-related macular degeneration (AMD), especially its wet form characterized by an abnormal growth of choroidal neovascularization (CNV) into the subretinal space, is still the leading cause of blindness in the developed countries in people older than 50 years.1,2 Since the advent of VEGF inhibitors such as ranibizumab (Lucentis; Novartis, Basel, Switzerland), it has been possible for the first time to treat neovascular AMD and achieve a gain in visual acuity. Ranibizumab is a recombinant monoclonal antibody fragment that neutralizes all active forms of vascular endothelial growth factor (VEGF) A and has been shown in large clinical trials to improve the course of neovascular AMD significantly.3–5 It has been approved by the U.S. Food and Drug Administration for all subtypes of CNV due to AMD. Many clinicians currently treat AMD by injecting the drug three times during a period of 2 months (upload phase). After the upload phase, the patients are reexamined 4 weeks later and, depending on the clinical findings, may be given further treatment.6–8 Since the logistics of examining and treating all patients with AMD sufficiently are much enhanced by the new treatment modality of intravitreal ranibizumab, the focus today is on noninvasive and fast diagnostic tools to determine the course of treated AMD objectively and to decide properly whether retreatment is necessary. For this, the technique of optical coherence tomography (OCT) is widely approved, whereas the center of attention in clinical studies is mainly on the regression of retinal edema after therapy, as judged by OCT.7–9 With the advent of high-resolution spectral domain-optical coherence tomography (SD-OCT), a much-improved differentiation of the retinal structures and the retinal pigment epithelium (RPE) became possible, and it could be shown that thickness measurements of the retina were significantly different from those obtained by the time-domain technique.10,11 The SD-OCT provides cross-sectional retinal images with an axial resolution of approximately 7 \( \mu \text{m} \). Thus, the axial resolution is on the order of the thickness of the RPE monolayer, and—due to this high-resolution capacity—it is now possible to detect in detail the structural changes in the RPE/photoreceptor complex after different therapies.12 Regarding intravitreal ranibizumab therapy, it should therefore also be possible to image the architecture of the CNV during the course of treatment with this new OCT technology. Since it is unclear whether anti-VEGF monotherapy in AMD achieves morphologic CNV regression...
or only stops further CNV growth, it was the purpose of this study to use SD-OCT to evaluate the CNV structure before and after anti-VEGF treatment with ranibizumab.

**MATERIAL AND METHODS**

**Spectral Domain-OCT**

Fundus and OCT images were acquired with a combined SD-OCT and scanning laser ophthalmoscope (SLO) imaging system (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). The system acquires en face SLO images in angiographic, autofluorescence (AF), and reflectance imaging modes, as well as cross-sectional SD-OCT images. An 870-nm super luminescent diode (SLD) is used for OCT imaging. In SD-OCT mode, the retina is scanned at 40,000 A-scans per second, presenting highly detailed images of the retinal structure. The OCT depth resolution (full width at half maximum [FWHM]) is 7 μm. The images of the SLO and OCT modes can be overlaid and then automatically and spatially co-registered. Images can also be co-registered over time for different visits of a patient. This setup was used in the present study.

**Image Acquisition**

All consecutive patients who had first-time diagnosis of neovascular AMD and were assigned to standard intravitreal ranibizumab therapy underwnt preoperative and postoperative SD-OCT examinations in a regular clinical setting. The study protocol complied with the Declaration of Helsinki. The postoperative examination took place 4 weeks after the third injection. OCTs were performed with the cross-sectional technique on six different sections; the same sections were imaged during the follow-up, to ensure matching sections for evaluation. The images were then displayed via the system software (Eye Explorer software; Heidelberg Engineering). It is possible to measure distances within the sections by using the scale bar provided by the software. For CNV evaluation, similar cuts from both images (pre- and postoperative examination) were retrospectively evaluated. The change in CNV size was qualitatively determined (smaller, same, and larger) and quantitatively measured. Thus, the maximum CNV diameter as well as the maximum CNV thickness could be determined. An example of the measurements is given in Figure 1. Also measured were the central retinal thickness (CRT), by using standard protocols of the software, and the postoperative retinal structure was qualitatively judged as (1) wet, revealing unchanged fluid conditions; (2) dry, revealing no fluid; and (3) less edema, revealing incomplete fluid regression.

Additional preoperative evaluation consisted of determination of the CNV subtype from the standard preoperative fluorescein angiography including the following three types of CNV: classic, occult, and minimal classic. Excluded were images showing significant RPE detachments or significant subretinal hemorrhages accompanying the CNV that made proper CNV evaluation derived from the OCT sections unfeasible.

Statistical analysis for paired samples was performed by t-test (SPSS for Windows 16.0; SPSS, Chicago, IL).

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** SD-OCT sections in a case of classic CNV before (left) and after (right) intravitreal ranibizumab upload. Measurements of CNV thickness and diameter (shown) were performed.

![Figure 2](https://example.com/figure2.png)

**FIGURE 2.** SD-OCT sections in cases of classic (A), occult (B), and minimal classic (C) CNV. (A, arrows) The extent of classic CNV is revealed in the diameter (both arrows at RPE level) and the thickness (single down arrow). The RPE band continues beneath the CNV formation. (B) Black arrow: the diameter of the occult CNV (Bruch’s membrane can be distinguished); white arrow: the thickness of the membrane appearing below the elevated RPE band. (C) Two parts of minimal classic CNV. Black arrow: the border of the CNV; white arrow: the thickness of the occult part. The classic CNV component above the RPE level (white arrow) is indicated (※).
RESULTS

Because of the high-resolution images, the SD-OCT technique made possible a proper assessment of CNV size. Herein, of 107 consecutive images from patients who underwent first-time ranibizumab upload due to neovascular AMD, a total of 78 pre- and postoperative proper and matching OCT sections could be evaluated. The distribution of CNV subtypes as derived from the angiographic findings was: classic ($n = 16$), occult ($n = 54$), and minimal classic ($n = 8$). OCT sections revealed classic CNV components above the RPE/photoreceptor complex, whereas occult CNV stayed below the complex (Fig. 2).

Of all the postoperative OCTs, 59% revealed completely dry retinal structures, 27% showed decreased edema, and 14% showed unchanged edema. Mean macular thickness decreased significantly from 427 to 303 $\mu m$ ($P = 0.000$).

Qualitatively, the overall CNV architecture appeared to be unchanged in 78%, reduced in thickness in 18%, and larger in 4% ($n = 3$). Quantitatively, the overall mean diameter of the CNV lesion changed significantly from 2813 $\mu m$ before surgery to 2804 $\mu m$ after surgery ($P = 0.626$); however, the overall thickness of the lesion decreased significantly from 205 to 175 $\mu m$ (reduction rate: 15%; $P = 0.000$). Five cases of classic ($n = 2$) and occult ($n = 2$) CNV, as well as one case of minimal classic CNV were exemplary of the course of structural CNV changes during ranibizumab upload (Figs. 3–7).

The changes in CNV morphology are summarized in Table 1. Regarding the three CNV subtypes, no significant changes in mean CNV diameter were observed in each subtype. The diameter of classic CNV lesions changed from 2499 to 2485 $\mu m$ ($P = 0.390$); the diameter of occult CNV changed from 2904 to 2921 $\mu m$ ($P = 0.405$); and finally, the diameter of minimal classic CNV changed from 2833 to 2656, an insignificant reduction (reduction rate, 6%; $P = 0.092$). The impact of postoperatively apparent retinal edema on CNV diameter changes was as follows: If retinal structure was completely dry ($n = 46$), CNV diameter reduction was small (2658–2611 $\mu m$; reduction rate: 2%) but significant ($P = 0.020$). In cases with nonreduced subretinal and/or retinal edema ($n = 11$), CNV diameter enlarged insignificantly from 3119 to 3222 $\mu m$ (enlargement rate: 3%; $P = 0.224$). In 21 cases presenting reduction of edema, only small changes were observed (2993–3010 $\mu m$; $P = 0.523$).

Regarding lesion thickness in the three CNV subtypes, reduction was enhanced, especially in CNVs with classic components ($n = 25$; 252 to 197 $\mu m$; $P = 0.000$; reduction rate, 22%), whereas the reduction was smaller but also significant in occult CNV (183 to 164 $\mu m$; $P = 0.003$; reduction rate, 10%). The impact of postoperatively apparent retinal edema on CNV thickness changes was as follows: If retinal structure was completely dry, CNV thickness decreased significantly from 187 to 152 $\mu m$ (reduction rate: 19%; $P = 0.000$). In cases of nonreduced subretinal and/or retinal edema ($n = 11$) thickness changed insignificantly from 247 to 241 $\mu m$ (enlargement rate: 3%; $P = 0.224$). In cases with less retinal fluid, CNV thickness reduction was also significant (222 to 187 $\mu m$; reduction rate, 16%; $P = 0.018$).
When evaluating the CNV lesion size (diameter and thickness) in accordance with CNV subtype and grade of postoperative retinal edema, we observed significant changes in terms of CNV size reduction for thickness but not for diameter in classic and occult CNV, if retinal structures appeared to be dry (Table 1). According to the table, no significant changes were observed in CNV diameter in all cases and in thickness, if edema was still apparent.

DISCUSSION

There is still an ongoing debate about whether intravitreal anti-VEGF treatment can lead to CNV regression. Despite the fact that this new treatment modality is undoubtedly very successful in improving or preserving vision in neovascular AMD, it became clear that the intravitreal injection has to be repeated in many patients to maintain the intraocular anti-VEGF effect and that this effect may mainly be derived from the regression of sub- and intraretinal edema, whereas no possible stabilizing long-term effects—especially on the architecture of CNV membranes—have been evaluated so far.

It is widely accepted that the anti-VEGF effect maintains only a certain steady state between the agent and the VEGF molecules during the time of its presence in the eye (~4 weeks in the nonvitrectomized eye), preventing further CNV growth. Therefore, the goal of the current research was to find solutions for maintaining the therapeutic effect for longer duration and as a consequence reducing the frequency of anti-VEGF therapy.

A complete regression of CNV structure may be important for such a long-term stabilizing effect. However, it is thought that anti-VEGF is not capable of inducing regression of the pathologic tissue growth of CNV. In contrast, photodynamic therapy (PDT) leads to selective vessel occlusion of the CNV; therefore, fibrovascular tissue regression is assumed for this treatment modality. Such CNV regression may lead to better nutrition and maintenance of the photoreceptors in the long term, because no abnormal CNV tissue blocks the physiologic connection between the RPE and photoreceptors or the RPE and choroid. The combination of anti-VEGF and another potential therapy as, for example, the PDT or as a newer modality an anti-PDGF-B treatment also covering mural cell regulation may be enhanced over current anti-VEGF alone in achieving vessel regression.

Using the SD-OCT, it was now possible to clearly differentiate the CNV structure from the physiologic retinal structure and to follow the course of CNV architecture during anti-VEGF therapies. Therefore, it was feasible that the status of CNV regression induced by anti-VEGF therapy could be determined by judging both the diameter and the thickness of the lesion.

The CNV lesion’s location in relation to the RPE level as well as its size were two-dimensionally determined qualitatively and quantitatively. Qualitatively, the size appeared to be unchanged in most cases (~80%) independent of accompanying

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The CNV lesion’s location in relation to the RPE level as well as its size were two-dimensionally determined qualitatively and quantitatively. Qualitatively, the size appeared to be unchanged in most cases (~80%) independent of accompanying
retinal edema. Also quantitatively, the diameter did not become smaller from therapy but stayed stable regardless of CNV subtype. This result correlates with those in another study in which SD-OCT segmentation techniques were used to determine CNV area and showed a nonsignificant postoperative reduction from $6.0 \pm 3.0$ to $5.0 \pm 3.1$ mm$^2$ at month 3 ($P = 0.115^{11}$). However; in contrast, overall CNV thickness reduced significantly in our study. Especially in classic CNV components, which revealed the greatest extent of CNV thickness, the largest thickness reduction was observed (22%); however, despite this significant tissue reduction, the major portion of CNV—above the RPE level—persisted. This observation was also true of occult CNV, in which thickness reduction was only 10% and thus most of the CNV tissue, beneath the RPE level, persisted.

Regarding CNV diameter, no significant reduction was found for each of the subtypes, regardless of retinal edema after therapy. There were some enlargement in diameter (3%) in cases of persistent fluid; however, these changes were not significant. In contrast, regarding the CNV thickness, the status of postoperative retinal edema had in fact some influence on CNV reduction. Significant reduction was observed, if the retina appeared to be completely dry after treatment, whereas this was not the case if edema only improved or persisted (Table 1).

Thus, it can be concluded that ranibizumab monotherapy, as also suggested by Jo et al., does not induce significant CNV regression in clinical therapy. Major parts of all CNV subtypes appeared to be stable, and only some thickness reduction, depending on the retinal edema status was observable. Therefore, ranibizumab seemed to have only a small impact on the CNV structure if a tendency toward resolution of the edema is obvious; however, the impact was clinically negligible.

In summary, SD-OCT can be used to determine the location of the CNV in relation to the level of the RPE, giving an impression of classic and occult CNV components. Anti-VEGF monotherapy with ranibizumab does not achieve clinically significant regression of CNV. Combination treatment with anti-VEGF and, for example, PDT or anti-PDGF, may be an adequate approach for achieving CNV regression. Both regimens are currently under investigation and the results may show an enhanced stabilizing effect on neovascular AMD.

Changes of diameters and thicknesses are expressed in micrometers. Regarding minimal classic CNV, sample size was only large enough in the dry type for proper calculation.

### Table 1. Dependence of CNV Size (Diameter and Thickness) According to CNV Subtype and Postoperative Classification of Retinal Edema

<table>
<thead>
<tr>
<th>CNV Type</th>
<th>Diameter</th>
<th>Thickness</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Wet</td>
<td>Less Edema</td>
</tr>
<tr>
<td>Classic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>10</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Change</td>
<td>2430 to 2404</td>
<td>3558 to 3589</td>
<td>2141 to 2136</td>
</tr>
<tr>
<td>$P$</td>
<td>0.218</td>
<td>0.759</td>
<td>0.816</td>
</tr>
<tr>
<td>Occult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>29</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Change</td>
<td>2761 to 2734</td>
<td>3022 to 3140</td>
<td>3097 to 3136</td>
</tr>
<tr>
<td>$P$</td>
<td>0.103</td>
<td>0.252</td>
<td>0.200</td>
</tr>
<tr>
<td>Minimal classic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>2560 to 2392</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>0.160</td>
<td></td>
<td></td>
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</table>

* Significant changes were observed in CNV thickness reduction in classic and occult CNV (nearly significant in minimal classic CNV) only if the postoperative retinal structure was dry.
References

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