Reduced Vessel Density of the Choriocapillaris during Anti-Vascular Endothelial Growth Factor Therapy for Neovascular Age-Related Macular Degeneration

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PURPOSE. To investigate macular vascular alterations by using optical coherence tomography angiography (OCTA) in patients with a history of long-term anti-vascular endothelial growth factor (VEGF) therapy for neovascular age-related macular degeneration (nAMD).

METHODS. Japanese patients with nAMD with a history of long-term anti-VEGF monotherapy at study entry were studied retrospectively. OCTA images were obtained, and the vessel densities (mm⁻²) of the superficial capillary plexus, deep capillary plexus (DCP), choriocapillaris (CC), and the plexus foveal avascular zone area (mm²) were calculated.

RESULTS. One hundred twenty-four eyes (124 patients) were included. The mean ± standard deviation follow-up period between the first and last OCTA imaging sessions was 14.5 ± 3.1 months; the duration of the anti-VEGF monotherapy before the first OCTA imaging session was 68.0 ± 23.6 months, with a mean of 3.6 ± 3.0 injections during the follow-up period. The vessel densities of the DCP and CC significantly decreased (P = 0.001 and P = 0.009, respectively) from 10.62 ± 2.72 mm⁻² and 11.84 ± 1.79 mm⁻² to 9.44 ± 2.88 mm⁻² and 11.18 ± 2.12 mm⁻². Such findings were not observed in 63 control eyes.

CONCLUSIONS. The DCP and CC deteriorate during treatment. This information may guide future treatment strategies for nAMD, such as the need to protect the capillaries to maintain visual acuity after long-term treatment. Prospective, controlled trials are required to confirm our findings.

Keywords: anti-VEGF, choriocapillaris, neovascular age-related macular degeneration, optical coherence tomography angiography, vessel density

Age-related macular degeneration (AMD) is a chronic and degenerative disease affecting the central retina. AMD is characterized by degeneration of the photoreceptors and retinal pigment epithelium (RPE), deposits between the RPE and Bruch’s membrane, and loss of the choriocapillaris (CC). The CC and RPE losses have been documented in geographic atrophy and choroidal neovascularization (CNV), and the role of CC dysfunction in AMD onset and progression has been suggested.¹⁻³ Although previous studies have shown a smaller foveal capillary plexus and an increased foveal vascular zone (FAZ) associated with age,⁴⁻⁻⁷ a recent study reported that no significant difference was seen in the density of the foveal capillary plexus and FAZ area in eyes with early and intermediate AMD compared to those in healthy eyes.⁸

The Seven-Year Observational Update of Macular Degeneration Patients Post-MARINA/ANCHOR and HORIZON Trials (SEVEN-UP) study⁹⁻¹⁰ is a multicenter, cross-sectional follow-up of the initial patients treated with ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA, USA) from the Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration (ANCHOR) trial¹¹ and the Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) trial.¹² The participants also continued ranibizumab treatment in the Open-Label Extension Trial of Ranibizumab for Choroidal Neovascularization Secondary to Age-Related Macular Degeneration (HORIZON) trial.¹³ The SEVEN-UP study assessed visual acuity (VA) and disease status 7 to 8 years after initiation of intensive ranibizumab therapy. The severity of the macular atrophy at year 7 was found to be the primary anatomic correlate in the reduced final vision, and the progression of the macular atrophy from years 2 to 7 was associated linearly with the visual decline over that period. The Comparison of AMD Treatments Trials (CATT) follow-up study,¹⁴,¹⁵ in which patients were released from the study protocol at the end of a 2-year follow-up and returned at about 5 years after initiation of treatment with either ranibizumab or bevacizumab (Avastin; Genentech Inc.), reported that the proportion of eyes with geographic atrophy increased from 20% at 2 years to 41% at 5 years. Therefore, therapeutic strategies to prevent macular atrophy are important in long-term management when anti-vascular endothelial growth factor (VEGF) agents are used to treat neovascular AMD (nAMD); however, the pathogenesis of macular atrophy in patients with treated nAMD has not been clarified.

Optical coherence tomography angiography (OCTA) is an advanced imaging technology that facilitates direct evaluation of the retinal microvasculature with depth-resolved capability. This noninvasive method using amplitude or phase decorrela-
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In patients with nAMD who had been treated with anti-VEGF therapy over the long term.

**Methods**

This retrospective study followed the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act regulations. The Institutional Review Board of the Ohtsuka Eye Hospital (Sapporo, Japan) approved the study (Institutional Review Board number, 29008), and all patients provided informed consent after explanation of the study protocol and before OCTA imaging.

**Participants**

We reviewed the medical records of patients with subfoveal nAMD treated with anti-VEGF monotherapy who underwent OCTA at every visit to the Hikichi Eye Clinic from May 2016 to June 2018. At study entry, these patients had a history of treatment with anti-VEGF monotherapy for symptomatic subfoveal nAMD for more than 3 years at the Ohtsuka Eye Hospital; three consecutive monthly intravitreal injections of anti-VEGF agents (ranibizumab or aflibercept [Eylea, Regeneron Pharmaceuticals, Tarrytown, NY, USA]) were followed by a reinjection schedule based on need and, thereafter, the patients continued on anti-VEGF monotherapy at the Hikichi Eye Clinic from May 2016.

The exclusion criteria were the presence of other concomitant ocular diseases (i.e., diabetic retinopathy, glaucoma, retinal vein occlusion, or epiretinal membrane); a myopic refractive error exceeding -6 diopters; other macular abnormalities (i.e., myopic CNV, angioid streaks, or other secondary CNV); and a history of laser photocoagulation, photodynamic therapy, or vitrectomy. There were no exclusion criteria for the best-corrected VA (BCVA) at the first injection and a history of cataract surgery. Patients who had a history of switching anti-VEGF agents (e.g., from ranibizumab to aflibercept or vice versa) during the follow-up period were included. Because administration of bevacizumab is an off-label use of the drug for treatment of nAMD in Japan, patients treated with bevacizumab were not included. The subtypes of nAMD included in this study were typical AMD and polypoidal choroidal vasculopathy (PCV). Retinal angiomatous proliferation (RAP) was excluded because (1) the presence of more deteriorated retinal capillary plexus than in typical AMD caused by the presence of an intraretinal neovascular complex and early formation of retinal choroidal anastomosis and (2) RAP is a rare subtype present in only about 5% of nAMD in Japanese populations. The diagnosis of PCV was based on indocyanine green angiography (Spectralis, Heidelberg Engineering, Heidelberg, Germany), which showed a branching vascular network that terminated in polypoidal lesions. Sixty-three age-matched subjects (one eye of each patient was included) without fundus disorders served as the control group.

**Data Collection and Image Acquisition**

All patients underwent comprehensive ocular examinations including measurement of the BCVA using a Landolt ring chart, intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a preset lens, and OCTA at every visit. The OCTA images were obtained using the Zeiss Cirrus 5000 with Angioplex software (Carl Zeiss Meditec, Dublin, CA, USA), which acquires two sequential OCT scans in the same location and generates en-face OCTA images by using the optical microangiography algorithm. The $6 \times 6$ mm scans centered on the fovea were obtained repeatedly until nine OCTA cubes with sufficient image quality were obtained. The scan quality was assessed on the scan quality check screen according to acceptance criteria (i.e., clear and sharp focus, few to no artifacts [e.g., motion lines], minimal saccades [identified by horizontal misalignment of vessel segments on en-face images], and a signal strength of 7 or more). Moreover, images had to be centered on the fovea and illuminated uniformly without dark corners. If acceptable scans meeting these criteria (for all nine acquisitions) were not acquired, the eye was excluded from the analysis. En-face images of the superficial retinal capillary plexus (SCP) layer, deep retinal capillary plexus (DCP) layer, and CC were obtained using the commercial default automated segmentation boundaries and exported at a size of $1024 \times 1024$ pixels for further analyses. The boundaries of the SCP layer extended from 3 $\mu$m below the internal limiting membrane to 15 $\mu$m below the inner plexiform layer, the DCP layer boundaries were from 15 to 70 $\mu$m below the inner plexiform layer, and the CC boundaries were 20 $\mu$m below the RPE layer. The measurements of the FAF area were calculated using the nonflow function in the OCTA software. A proprietary and preliminary software, AngioExerciser V1.2.2 and OCTAVE, from Zeiss was used to extract the vessel density values from the OCTA scans. The software automatically segmented the OCTA cube data into the relevant SCP, DCP, and CC angio-slab images. The software subsequently binarized and skeletonized the images to arrive at the reported vessel “length” density in mm/mm². The authors were masked to the exact algorithm and parameters used for the processing. Although projection artifacts cause superficial vessels to appear in en-face images of structures that are below the vessel, the software automatically removes projection artifacts by subtracting them from images below the vessels.

Two independent graders (T.H. and M.A.) reviewed the images, and poor-quality images were excluded based on evidence of (1) poor fixation, including double vessel patterns, motion artifacts, and the presence of blink artifacts more than 5 pixels wide; (2) medium opacity and/or exudative changes, marked by shadowing or obscuration of the vessel signal in the field of view or a signal strength index less than 50 with reference to the corresponding structural OCT scan; and (3) segmentation errors in the outline of the vascular networks. Thus, the baseline and last examinations of this study were the examinations at which the first and the last OCTA images were adopted for analyses during this study.

**Evaluation of Macular Atrophy**

Color fundus photography and OCT were used to assess macular atrophy at the baseline and last examinations. Macular atrophy was defined according to the criteria proposed by the Classification of Atrophy Meetings program as follows: (1) a region of hypertransmission of at least 250 $\mu$m in diameter in any lateral dimension; (2) a zone of attenuation or disruption of
the RPE of at least 250 μm in diameter; and (3) evidence of overlying photoreceptor degeneration, including loss of the interdigitation zone, ellipsoid zone, and external limiting membrane and thinning of the outer nuclear layer. Any images with scrolled RPE or other signs of an RPE tear were excluded.

### Statistical Analysis

Statistical analyses were performed using the EZR statistical software (Saitama Medical Centre, Jichi Medical University, Japan) and Excel software (Microsoft, Redmond, WA, USA). Comparisons of the characteristics between the patients and controls or of the patients by the criteria were carried out using the Mann-Whitney U test, χ² test, and Wilcoxon rank sum test. To consider multiplicity issues in statistical analyses when comparing the vascular densities and FAZ area between baseline and the last examinations of the patients, the Tukey-Kramer procedure was used. The BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) for statistical purposes. P < 0.05 was considered significant.

### Results

The medical records of 137 patients satisfied the current study criteria. Seven (5.1%) patients with RAP were excluded from the study due to the small sample size, as described in the Methods section. The quality of the OCTA images obtained from six (4.4%) patients was not sufficiently high to analyze automatically or manually. Thus, 124 eyes of 124 patients were included in this study. If both eyes satisfied the study criteria, the eye with the longer history of treatment for nAMD was included in this study.

### Table 1. Comparisons of Characteristics Between 124 Patients with nAMD and 63 Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, Mean ± SD, y</th>
<th>Men, n (%)</th>
<th>Duration of Anti-VEGF Therapy, Mean ± SD, Months</th>
<th>Follow-up Period, Mean ± SD, Months</th>
<th>Injections During Follow-up at Study Entry, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 63)</td>
<td>75.5 ± 8.7</td>
<td>40 (63.5)</td>
<td>-</td>
<td>16.4 ± 5.3</td>
<td>-</td>
</tr>
<tr>
<td>nAMD (n = 124)</td>
<td>76.5 ± 8.7</td>
<td>73 (58.9)</td>
<td>58.0 ± 23.6</td>
<td>14.8 ± 3.1</td>
<td>3.6 ± 3.0</td>
</tr>
<tr>
<td>P value</td>
<td>0.463*</td>
<td>0.541†</td>
<td></td>
<td>0.015*</td>
<td></td>
</tr>
</tbody>
</table>

Bold values represent statistical significance.

* Mann-Whitney U test.
† χ² test.

The baseline clinical characteristics of 124 eyes of the 124 Japanese patients included in this study are shown in Table 1. The mean (± standard deviation [SD]) age was 76.5 ± 8.7 years, and 73 (58.9%) were men. Of the 124 eyes, 64 (51.6%) eyes had typical AMD and 60 (48.4%) had PCV. A mean of 3.6 ± 3.0 injections was administered during the follow-up period (14.8 ± 3.1 months). The previous treatment period at study entry was a mean of 58.0 ± 23.6 months. The mean baseline logMAR BCVA was 0.39 ± 0.42 (20/50 Snellen VA), which was maintained at the end of this study (0.32 ± 0.40) (20/40 Snellen VA). The mean (± SD) age of the 63 control patients was 75.5 ± 8.7 years, 40 (63.5%) were men, and the interval between the first and the last OCTA was 14.5 ± 5.3 months; all values were similar to those in the nAMD groups. The control patients were followed for a significantly (P = 0.015) longer period than the patients with nAMD.

A pro ne rata (PRN) injection schedule was followed during the study in 79 (63.7%) eyes and a proactive injection schedule in 45 (36.3%) eyes, that is a treat-and-extend schedule in 25 (20.2%) eyes and a fixed injection schedule (e.g., bimonthly or every 3- or 4-month injections) in 20 (16.1%) eyes. During this study, the anti-VEGF agent was changed from ranibizumab to aflibercept in three (2.4%) eyes and vice versa in three (2.4%) eyes. Ranibizumab or aflibercept alone was continued in 88 (71.0%) and 30 (24.2%) eyes, respectively (Table 2).

The vessel densities and FAZ area measurements in eyes with nAMD and controls are shown in Table 3. In eyes with nAMD, the vessel densities of the DCP and CC decreased significantly (P = 0.016 and P = 0.029, respectively) from 10.62 ± 2.72 mm⁻¹ and 11.84 ± 1.79 mm⁻¹ at baseline to 9.44 ± 2.88 mm⁻¹ and 11.18 ± 2.12 mm⁻¹ at the last examination, whereas no apparent difference was found in the FAZ area and...
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The CC decreased during the study period and CC dropout (Fig., panel F) was observed at the last examination.

Regarding the analysis of each CNV type, significant decreases in the vessel densities in the DCP and CC were found in eyes with typical AMD but not in those with PCV. No significant differences were found in the vessel density of the SCP or FAZ areas between baseline and the last examination in eyes with both typical AMD and PCV (Table 4). The characteristics listed in Table 2 were similar in both groups with the exception of age.

In eyes treated with the PRN injection schedule and the proactive injection schedule, the vessel densities in the DCP decreased significantly (P = 0.031 and P = 0.046, respectively) during the follow-up period, whereas no apparent difference was found in the vessel density of the SCP and the CC and the FAZ area between baseline and the last examination (Table 4). The number of injections of anti-VEGF agent during this study was assumed to be significantly (P < 0.001) higher in eyes treated with the proactive injection schedule than in the eyes treated with the PRN injection schedule, but no apparent differences between the two injection schedules were found in age, sex distribution, duration of the history of anti-VEGF therapy at entry into this study, and the follow-up period of this study (Table 2).

In eyes treated with aflibercept alone, the vessel densities in the DCP and CC decreased significantly (P = 0.035 and P = 0.047, respectively) from 11.07 ± 2.58 mm⁻¹ and 11.99 ± 1.62 mm⁻¹ at baseline to 10.8 ± 2.55 mm⁻¹ and 10.90 ± 2.28 mm⁻¹ at the last examination, whereas in eyes treated with ranibizumab alone, the vessel densities in the CC showed no apparent decrease (P = 0.056) at the last examination compared to baseline. The vessel density of the DCP decreased significantly (P = 0.031) (Table 4). The number of injections of anti-VEGF agent during this study was significantly (P < 0.001) higher in eyes treated with aflibercept than in eyes treated with ranibizumab, but no apparent differences between the two anti-VEGF agents were found in age, sex distribution,

### TABLE 3. Comparisons of Vascular Densities and FAZ Area Between Baseline and Last Examinations in 124 Eyes with nAMD and 63 Eyes in Controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Last Examination</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 63)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular density (mm⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>14.72 ± 0.58</td>
<td>14.60 ± 0.56</td>
<td>0.485</td>
</tr>
<tr>
<td>DCP</td>
<td>10.92 ± 2.60</td>
<td>10.49 ± 2.73</td>
<td>0.392</td>
</tr>
<tr>
<td>CC</td>
<td>12.55 ± 2.18</td>
<td>12.34 ± 1.68</td>
<td>0.633</td>
</tr>
<tr>
<td>FAZ area (mm²)</td>
<td>0.24 ± 0.13</td>
<td>0.25 ± 0.16</td>
<td>0.761</td>
</tr>
<tr>
<td>nAMD (n = 124)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular density (mm⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>14.46 ± 0.67</td>
<td>14.38 ± 1.37</td>
<td>0.595</td>
</tr>
<tr>
<td>DCP</td>
<td>10.62 ± 2.72</td>
<td>9.44 ± 2.88</td>
<td>0.016</td>
</tr>
<tr>
<td>CC</td>
<td>11.84 ± 1.79</td>
<td>11.18 ± 2.12</td>
<td>0.029</td>
</tr>
<tr>
<td>FAZ area (mm²)</td>
<td>0.23 ± 0.18</td>
<td>0.26 ± 0.22</td>
<td>0.253</td>
</tr>
</tbody>
</table>

* Tukey-Kramer procedure.

Bold values represent statistical significance.

The vessel density of the SCP between baseline and the last examination. The eyes of the control patients had no apparent changes in the vessel densities of the SCP, DCP, and CC and the FAZ area between baseline and the last examination (Table 3).

The Figure shows OCTA en-face images (6 × 6 mm) of the SCP, DCP, and CC layers at baseline and the last examination obtained from the left eye of a 72-year-old man, who had a 5-year follow-up history of anti-VEGF therapy for nAMD and received three aflibercept intravitreal injections during the 14-month follow-up period of this study. The decimal VA was 0.8 (20/25 in Snellen chart) at baseline and slightly decreased to 0.6 (20/32 in Snellen chart) at the last examination. Although the vessel densities of the SCP and DCP and FAZ area were unchanged during the follow-up period, the vessel density of the CC decreased during the study period and CC dropout (arrow) is seen at the last examination (F).

**FIGURE.** OCTA en-face images (6 × 6 mm) of the SCP layer, DCP layer, and CC layer at baseline (A–C) and the last examination (D–F). The left eye of a 72-year-old man, who had a 5-year follow-up history of anti-VEGF therapy for nAMD and received three aflibercept intravitreal injections during the 14-month follow-up period. The vessel densities (mm⁻¹) of the SCP, DCP, and CC and the FAZ area (mm²) were 14.46, 14.38, 11.84, and 0.23 at baseline, and 14.38, 11.18, 0.26 at the last examination, respectively. The vessel density of the CC decreased during the study period, and CC dropout (arrow) is seen at the last examination (F).
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**TABLE 4.** Comparisons of Vascular Densities and FAZ Area Between Baseline and Last Examinations in Patients by Criteria

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Last Examination</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNV type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical AMD (n = 64)</td>
<td>Vascular density (mm⁻¹)</td>
<td>14.47 ± 0.64</td>
<td>14.40 ± 1.80</td>
</tr>
<tr>
<td>C</td>
<td>DCP</td>
<td>CC</td>
<td>FAZ area (mm²)</td>
</tr>
<tr>
<td>10.92 ± 2.74</td>
<td>11.99 ± 1.59</td>
<td>0.23 ± 0.18</td>
<td>0.24 ± 0.19</td>
</tr>
<tr>
<td>9.05 ± 2.90</td>
<td>10.84 ± 2.07</td>
<td>0.25 ± 0.24</td>
<td>0.28 ± 0.19</td>
</tr>
<tr>
<td>Treatment schedule</td>
<td>Vascular density (mm⁻¹)</td>
<td>14.46 ± 0.70</td>
<td>14.47 ± 0.67</td>
</tr>
<tr>
<td>PRN injection (n = 79)</td>
<td>SCP</td>
<td>DCP</td>
<td>CC</td>
</tr>
<tr>
<td>10.29 ± 2.69</td>
<td>11.69 ± 1.98</td>
<td>0.24 ± 0.19</td>
<td>0.27 ± 0.20</td>
</tr>
<tr>
<td>9.90 ± 2.81</td>
<td>11.56 ± 2.12</td>
<td>0.28 ± 0.19</td>
<td>0.24 ± 0.19</td>
</tr>
<tr>
<td>Proactive injection (n = 45)</td>
<td>Vascular density (mm⁻¹)</td>
<td>14.30 ± 0.68</td>
<td>14.38 ± 0.58</td>
</tr>
<tr>
<td>SCP</td>
<td>DCP</td>
<td>CC</td>
<td>FAZ area (mm²)</td>
</tr>
<tr>
<td>10.43 ± 2.43</td>
<td>11.78 ± 1.43</td>
<td>0.21 ± 0.17</td>
<td>0.26 ± 0.24</td>
</tr>
<tr>
<td>9.34 ± 2.76</td>
<td>11.08 ± 2.26</td>
<td>0.26 ± 0.24</td>
<td>0.24 ± 0.19</td>
</tr>
<tr>
<td>Anti-VEGF agents</td>
<td>Vascular density (mm⁻¹)</td>
<td>14.42 ± 0.69</td>
<td>14.33 ± 1.50</td>
</tr>
<tr>
<td>Ranibizumab alone (n = 88)</td>
<td>SCP</td>
<td>DCP</td>
<td>CC</td>
</tr>
<tr>
<td>10.54 ± 2.73</td>
<td>11.77 ± 1.84</td>
<td>0.25 ± 0.18</td>
<td>0.27 ± 0.22</td>
</tr>
<tr>
<td>9.51 ± 2.92</td>
<td>11.23 ± 2.07</td>
<td>0.27 ± 0.22</td>
<td>0.24 ± 0.19</td>
</tr>
<tr>
<td>Aflibercept alone (n = 30)</td>
<td>Vascular density (mm⁻¹)</td>
<td>14.51 ± 0.64</td>
<td>14.58 ± 0.65</td>
</tr>
<tr>
<td>SCP</td>
<td>DCP</td>
<td>CC</td>
<td>FAZ area (mm²)</td>
</tr>
<tr>
<td>11.07 ± 2.58</td>
<td>11.99 ± 1.62</td>
<td>0.22 ± 0.18</td>
<td>0.24 ± 0.20</td>
</tr>
<tr>
<td>10.08 ± 2.55</td>
<td>10.90 ± 2.28</td>
<td>0.24 ± 0.20</td>
<td>0.20 ± 0.19</td>
</tr>
</tbody>
</table>

*Bold values represent statistical significance.
* Tukey-Kramer procedure.

**TABLE 5.** Relation Between Baseline Macular Atrophy and Vessel Densities in CC

<table>
<thead>
<tr>
<th>Macular Atrophy</th>
<th>Baseline</th>
<th>Last Examination</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macular atrophy at baseline</td>
<td>Present (54 eyes)</td>
<td>11.46 ± 1.88</td>
<td>10.47 ± 2.29</td>
</tr>
<tr>
<td>Absent (70 eyes)</td>
<td>12.14 ± 1.67</td>
<td>11.75 ± 1.80</td>
<td>0.179</td>
</tr>
<tr>
<td>Macular atrophy at last examination</td>
<td>Present (19 eyes)</td>
<td>12.39 ± 1.43</td>
<td>11.51 ± 1.63</td>
</tr>
<tr>
<td>Absent (51 eyes)</td>
<td>12.04 ± 1.75</td>
<td>11.84 ± 1.88</td>
<td>0.576</td>
</tr>
</tbody>
</table>

*Wilcoxon rank sum test.
†Mann-Whitney U test.

**Discussion**

Using quantitative analysis, we showed that nAMD with a long-term history of anti-VEGF therapy was characterized by decreased vascular densities in the DCP and CC during continuous anti-VEGF therapy, whereas such changes were not found in the inner retinal capillaries. Furthermore, the decreased vascular densities were pronounced in eyes with typical AMD but not found in eyes with PCV during this study. Compared to the controls, these capillary alterations were specific to eyes with nAMD.

Although definitive conclusions regarding the pathogenesis of the decreased vascular density in the CC in patients with treated nAMD cannot be drawn from this study, some hypotheses have been forwarded. McLeod et al. reported in a histologic study that CC dropout occurred in three wet AMD specimens before nCNV developed, resulting in a 50% decrease in the vascular area compared with aged control eyes. The theory that the choroidal vasculature plays a driving role in nAMD development is well established. The presence of CC atrophy near the CNV has been reported. Thus, the pathology of nAMD itself may be a possible reason for the decreased vascular density in the CC observed in the current study.

A second possibility is that anti-VEGF therapy may promote decreased vascular density in the CC by counteracting the role of constitutively produced VEGF in neuronal or vascular maintenance. Peters and associates reported that a significant loss of CC endothelial cell fenestrations was present as early as 24 hours after intravitreal injection of bevacizumab, a humanized monoclonal anti-VEGF antibody, in primate eyes and became even more pronounced up to day 4. On day 14 after the injection, its effect in closing endothelial cell fenestrations was mainly diminished, but there were still significantly fewer fenestrations than in untreated eyes. Saint-Geniez and associates reported that the absence of soluble VEGF isoforms in mice led to age-dependent degenerative changes in the RPE-CC complex that recapitulate the classical features of dry AMD, which supported that RPE-derived VEGF is essential for maintenance of the CC. Thus, those authors recommended that chronic use of anti-VEGF therapies in the eye should be monitored closely to evaluate the deterioration in the RPE-CC complex. However, the authors also speculated that the relatively short intravitreal half-life of both ranibizumab and bevacizumab indicated that VEGF neutralization is neither total nor chronic and, thus, may achieve neutralization of the VEGF involved in pathologic vessel growth without affecting the endogenous levels.

Third, the decreased vascular density in the CC may be secondary to RPE deterioration because the RPE produces and secretes a variety of growth factors, including VEGF, which play a role in maintaining the CC. RPE cells secrete VEGF toward the CC, and VEGF receptors in humans are expressed on the choroidal endothelium facing the RPE layer.
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...and may accelerate the decreased vascular density in the CC.

Another hypothesis is secondary to the exudative changes themselves; episodic leakage over a period of years that subject the macula to fluid and blood, and the increased intravitreal pressure and CNV outgrowth and contraction, and ischemic and inflammatory effects should be considered. In fact, the current study showed apparent deterioration of the DCP but not the SCP, which suggested that episodic leakage from the CNV and/or a weakened RPE barrier over a period of years may contribute to the decreased vascular density in the outer retina. The number of injections was 3.6 ± 3.0 during the follow-up period (14.8 ± 3.1 months) in this real-world, long-term follow-up study. Especially in 79 (63.7%) eyes treated with a PRN treatment schedule, the mean number of injections during the follow-up period was 2.2. Such minimal intervention may indicate under-intervention and insufficient treatment to control the exudative changes. In the SEVEN-UP study,39 macular atrophy progressed in the context of very low anti-VEGF injection frequency and, thus, is seen as a nonspecific result of cumulative damage from exudative episodes over the patients’ disease course. Evidence from the CATT study for using anti-VEGF therapy on a PRN basis showed favorable outcomes with a much higher frequency of use in the range of 6.9 (ranibizumab) to 7.7 (bevacizumab) injections annually in the initial years of exudation.14 However, our finding of apparent deterioration of the DCP but not the SCP may be interpreted another way. The SCP is supplied by the central retinal artery and comprised of larger arteries, arterioles, capillaries, venules, and vein vessels, whereas the DCP is supplied by vertical anastomoses from the SCP.51 Such anatomic features in these two layers may be associated with different responses to anti-VEGF therapy (i.e., the SCP may be unaffected because it contains the major retinal blood vessels and fewer capillaries). In several studies of anti-VEGF agents used to treat diabetic macular edema and retinal vein occlusion, no deterioration of the macular retinal capillaries attributable to these agents was seen.51–55

Kuroda and associates56 reported that the nAMD subtype affected the development of RPE atrophy during ranibizumab treatment. The incidence of newly developed RPE atrophy was lower in PCV than in typical AMD, whereas the progression of the RPE atrophic area was faster in typical AMD than in PCV. In the current study, decreased vascular density in the CC was significantly greater in eyes with typical AMD than those with PCV. PCV recently was suggested to be a pachychoroid-driven disease and a pathology of developing CNV that differs from typical AMD.57–59 The VEGF concentration in the aqueous humor was higher in eyes with PCV than that in normal controls but seemed much lower than in eyes with typical nAMD.60–62 Freund and associates62,63 hypothesized that PCV often develops from long-standing type 1 CNV and speculated that PCV may occur when type 1 CNV fails to erode through the overlying RPE monolayer with polyps developing gradually over months to years. The RPE may be resistant to erosion in certain ethnicities, such as African-American, Hispanic, and Asian, making PCV more common in these individuals. Thus, RPE atrophy would be less likely to occur in PCV. Differential progression of RPE atrophy and decreased vascular density in the CC between typical AMD and PCV may be related to the differing pathologies of the two clinical entities.

Several types of artifacts may affect the OCTA measurements.44,45 Although we excluded images with severe distortion, the possibility of a measurement error due to other forms of artifacts, including segmentation, motion, and projection artifacts, should be considered. High-speed eye tracking and faster scan speeds have reduced motion artifacts, and advanced software can neutralize projection artifacts. The exudative materials distort the retinal contours, and the segmentation scan does not follow the curvature of the exudative materials, which can cause segmentation errors on the OCTA images. The software used in this study performed an automated segmentation, and the average number of vessel densities; however, the knowledge of decreased vascular density in the CC during anti-VEGF treatment should be unchanged despite that the analysis was performed using OCTA images with less exudative changes.

In this study, decreased vascular densities in the DCP and CC during continuous anti-VEGF therapy were more apparent in eyes treated with aflibercept alone than in eyes treated with ranibizumab alone. In rabbits, the vitreous half-life of aflibercept is 94.1 hours (3.92 days).64 This is shorter than that of bevacizumab (6.99 days) but longer than that of ranibizumab (2.51 days). Aflibercept is a soluble decoy receptor that binds VEGF-A and placental growth factor with a greater affinity than the body’s native receptors, and the drug also has a significantly higher affinity (94-fold) for VEGF-A165 than that of ranibizumab or bevacizumab.57 Julien and associates48 administered intravitreal injections of 0.5 mg of ranibizumab or 2 mg of aflibercept to four cynomolgus monkeys and reported that the drugs reduced the lumen of the CC in the same manner. However, hemolysis and microangiopathy were more pronounced after the aflibercept injection, whereas formation of protein complexes, RPE hypertrophy, and cell death were seen only after the aflibercept injections. The investigators speculated that the fragment crystallizable region of antibody of aflibercept may cause unpredictable reactions with other molecules and cells. Whether aflibercept has a stronger affect on the DCP and CC remains unclear from this study because more vitreous injections of aflibercept were administered during this study than that for eyes treated with ranibizumab.

The decreased vascular density in the CC have significant clinical implications in nAMD treatment, in that the outer retinal layers do not have a vascular supply and the choroid is the major source of oxygen and nutrients for the RPE-photoreceptor layer.2,49 Therefore, a reduced CC supply can lead to deterioration of the RPE-photoreceptor layer, resulting in macular atrophy. However, the RPE in the regions of decreased vascular density in the CC are presumably hypoxic, which may result in increased VEGF production by the RPE and stimulation of CNV and, consequently, the need for continuous anti-VEGF therapy for a longer term. Munk et al.50 found macular atrophy in 73.5% of subjects treated with a real-life treat-and-extend schedule, during which patients received an average of eight annual injections over a mean of 6.2 years. The lower prevalence of macular atrophy in this study than in the SEVEN-UP study5,65 was explained by the higher number of treatments and suggested undertreatment rather than overtreatment as a possible cause of formation and growth of macular atrophy. In the current study, the proactive treatment schedule was associated with decreased vascular density of the CC but the PRN schedule was not. In eyes with basal macular atrophy, the baseline vessel density of the CC...
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was significantly lower than in eyes without, which was further apparent at the last examination. The treatment strategy of low invasiveness of the CC may require long-term therapy for nAMD.

The limitations of the current study included its retrospective nature, the relatively small number of patients, and the absence of a correlation between the decreased vascular density in the CC and development of macular atrophy. All study participants continued the anti-VEGF monotherapy. Selection bias must be considered in this study. However, because long-term follow-up is difficult in ordinary clinics, bias tends to be present in such a study. This is a scenario in an ordinary clinic. Another limitation of this study was that the axial length was not measured, which may cause an error in any lateral measurement, especially when comparing different groups of eyes. However, it should not play a role when the changes in the same eye are investigated. Furthermore, although a correlation between the SCP and DCP had been reported in normal eyes and early AMD,51,52 which may possibly affect the statistical results, we did not consider this issue in the statistical analysis in the current study.

To the best of our knowledge, this is the first retrospective study to report changes in the CC during anti-VEGF therapy in nAMD. Despite the limitations of this retrospective study, the fact that the vascular density in the CC decreased during long-term anti-VEGF therapy for nAMD should be considered during the long-term follow-up period of these patients. Therapeutic strategies may curtail visual loss over the long term. Prospective, multicenter controlled trials are required to confirm our findings.

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