Published in final edited form as:

Ophthalmol Retina. 2018 September ; 2(9): 931–941. doi:10.1016/j.oret.2018.01.014.

Quantitative Evaluation of Choroidal Neovascularization under Pro Re Nata Anti–Vascular Endothelial Growth Factor Therapy with OCT Angiography

Scott M. McClintic, MD, Simon Gao, PhD, Jie Wang, MS, Ahmed Hagag, MD, Andreas K. Lauer, MD, Christina J. Flaxel, MD, Kavita Bhavsar, MD, Thomas S. Hwang, MD, David Huang, MD, PhD, Yali Jia, PhD, and Steven T. Bailey, MD

Casey Eye Institute, Oregon Health & Science University, Portland, OR 97239, USA

Abstract

Purpose—To use optical coherence tomography angiography (OCTA) derived quantitative metrics to assess the response of choroidal neovascularization to pro-re-nata (PRN) anti-endothelial growth factor (anti-VEGF) treatment in neovascular age-related macular degeneration (AMD).

Design—Prospective longitudinal cohort study

Participants—Fourteen eyes from 14 study participants with treatment-naïve neovascular AMD were enrolled.

Methods—Subjects were evaluated monthly and treated with intravitreal anti-VEGF agents under a PRN protocol for one year. At each visit, two 3×3 mm² OCTA scans were obtained. Custom image processing was applied to segment the outer retinal slab, suppress projection artifact, and automatically detect CNV. CNV membrane area (mm²) and CNV vessel area (mm²) was calculated.

Main Outcomes—Individual and mean CNV membrane area and CNV vessel area at each visit; within-visit repeatability determined by coefficient of variation.

Corresponding Author: Steven T. Bailey, MD, Casey Eye Institute, Oregon Health & Science University, 3375 SW Terwilliger Blvd, Portland, OR 97239 (bailstev@ohsu.edu).

Conflict of Interest Disclosures: Oregon Health & Science University (OHSU), Yali Jia and David Huang have a significant financial interest in Optovue, Inc., a company that may have a commercial interest in the results of this research and technology. These potential conflicts of interest have been reviewed and managed by OHSU. Scott McClintic and Steven Bailey have no financial interests.

Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Bailey, Jia.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: McClintic, Bailey, Huang, Gao.

Administrative, technical, or material support: All authors.

Study supervision: Jia, Bailey.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Results—Eight eyes had entire CNV within 3×3 mm² scanning area and had adequate image quality for CNV quantification. One case (case #2) was excluded from analysis due to the presence of a large subretinal hemorrhage overlying the CNV membrane. In the remaining cases, CNV vessel area was reduced by 39%, 50%, 43%, and 41% at months 1, 3, 6, and 12 respectively. CNV membrane area was reduced by 39%, 51%, 54%, and 45% at months 1, 3, 6, and 12. At month 6, mean change from baseline was not statistically significant for CNV vessel area, while it was statistically significant for CNV membrane area. Neither metric was significantly different compared to baseline at month 12. Individual analyses revealed each CNV had a unique response under PRN treatment. Within-visit repeatability was 7.96% (coefficient of variation) for CNV vessel area and 7.37% for CNV membrane area.

Conclusions—In this small exploratory study of CNV response to PRN anti-VEGF treatment, both CNV vessel area and membrane area were reduced compared to baseline after three months. After one year of follow-up, these reductions were no longer statistically significant. When anti-VEGF treatment was held, increasing CNV vessel area over time often resulted in exudation, but it was not possible to exactly when exudation occurs.

Introduction

Age-related macular degeneration (AMD) is a leading cause of vision loss. Neovascular AMD (NVAMD) accounts for the majority of vision loss and is characterized by the formation of choroidal neovascularization (CNV).¹ While fluorescein angiography (FA) is the gold standard for CNV diagnosis², structural optical coherence tomography (OCT) is helpful for CNV diagnosis and is an indispensable tool for monitoring response to treatment³.

Treatment of neovascular AMD with anti-vascular endothelial growth factor (VEGF) intravitreal injections given monthly is highly efficacious in preventing vision loss and improving vision in about 30% of patients over two years.⁴ However, if anti-VEGF therapy is switched to quartile dosing after a three fixed monthly loading treatments, visual acuity benefit is not as robust as fixed monthly dosing.^{5,6} Several studies have shown that by using structural OCT to guide anti-VEGF treatment decisions, pro re nata (PRN) and treat and extend (TAE) strategies can maintain the visual acuity benefits of monthly anti-VEGF treatment while reducing the number of injections needed.^{7,8} A limitation of structural OCT is that it detects exudation associated with CNV but cannot easily be used to evaluate changes in CNV size, morphologic structure or CNV blood flow. CNV area can be calculated with FA and indocyanine green angiography (ICGA), and changes in CNV area in response to anti-VEGF treatment have been demonstrated using these modalities.^{4,9} However, dye based angiography is invasive and time consuming, and it is not used routinely to help make treatment decisions.

OCT angiography (OCTA) is a new technology that detects moving red blood cells as variations in OCT amplitude, phase or complex signals over time from B-scans acquired at the same position^{10,11}. Intravenous contrast agents are not required, and scans are quickly acquired at the rate of approximately four seconds per scan. These features are desirable for both patient safety and clinic workflow. The three-dimensional images produced by OCTA

allow for visualization of individual retinal and choroidal circulations based on their relative depths, a feature not possible with traditional dye based angiography.

Recent studies have demonstrated that OCTA is a useful technique for non-invasive CNV detection^{12–17}. CNV is identified as blood flow in the outer retinal slab, which is defined as the region between Bruch's membrane (BM) and the outer border of the outer plexiform layer (OPL)¹³. In healthy eyes, this area is devoid of blood flow. With FA, detailed discrimination of CNV architecture is impaired by leakage of fluorescein and the blockage of fluorescence by the retinal pigment epithelium (RPE); in contrast, OCTA images of CNV are not obscured by leakage or dye blockage from the RPE, allowing for superior resolution of CNV vascular branches. Structural OCT is obtained simultaneously along with the OCT angiogram, and combining color-coded cross-sectional OCTA images with structural OCT images may illustrate novel structure and blood flow relationships. CNV characterization with OCTA is analogous to histologic classification described by Gass, with type 1 CNV identified as flow beneath the RPE and type 2 as flow above the RPE in the subretinal space^{13,18}.

Several studies have reported changes in CNV morphology and changes in CNV size in response to anti-VEGF treatment^{15,18–21}. Using OCTA to develop reliable, repeatable, and quantifiable CNV metrics may facilitate clarification and practical evaluation of the relationship between changes in CNV morphology and treatment response. Before clinical implementation is possible, several challenges must be addressed. First, most techniques evaluating CNV size rely on manual contouring of the CNV lesion by the investigator, which may not be practical in a busy clinical setting. Second, automated OCT segmentation is prone to errors with retinal architecture irregularities such as those produced by intra-retinal fluid (IRF), subretinal fluid (SRF), hyper-reflective material, and pigment epithelial detachment (PED). Third, projection artifacts may interfere with precise evaluation of neovascular vessels. This may occur when superficial vessel projection artifacts are included in the outer retinal slab. Fourth, measurement repeatability has not been sufficiently evaluated, making it difficult to determine whether observed differences represent true changes. Lastly, it is not known which quantitative metrics would yield the most relevant clinical information and which would be best suited to guide anti-VEGF therapy.

We recently developed a semi-automated CNV quantification algorithm that has been applied to commercially available OCTA. This technique utilizes a semi-automated segmentation strategy to ensure that appropriate boundaries of the outer retinal/RPE slab are consistently identified and a saliency-based algorithm for automatic CNV detection and quantification.^{22,23} A prospective cohort of treatment-naïve study participants were followed under a PRN treatment regimen to determine if OCTA provides novel information that clinicians may use to develop individualized treatment strategies.

Methods

This prospective longitudinal study received approval by the Institutional Review Board at Oregon Health and Science University. Consecutive patients with treatment-naïve neovascular AMD provided informed consent to be scanned with monthly spectral domain

OCTA scans (Avanti, XR, Optovue INC.) while under PRN anti-VEGF treatment for one year. At the beginning of the study, OCT angiography had not yet obtained FDA approval. The goal of the study was to determine how OCTA quantitative features change during the first year of treatment with anti-VEGF therapy. Study participants were recruited from the retina clinics at the Casey Eye Institute (Oregon Health and Science University, Portland, OR) from September 2014 to December 2015. Visual acuity (using the Early Treatment Diabetic Retinopathy Study [ETDRS] chart), slit lamp examination, dilated fundus examination, structural spectral domain OCT (Spectralis; Heidelberg Engineering, Germany), fluorescein angiography, and OCT angiography scans were performed at baseline. Each of these evaluations, except for FA, was repeated at each subsequent follow up visit.

CNV type was classified as either “classic,” “occult,” “minimally classic,” or “retinal angiomatous proliferation,” based on interpretation of the fluorescein angiogram by two trained vitreo-retinal surgeons (SMM and STB). All study participants were treated at their initial visit with an intravitreal injection of an anti-vascular endothelial growth factor (anti-VEGF) agent in the study eye. Each participant was followed monthly with one year of follow-up. After initial treatment at baseline visit, subsequent anti-VEGF treatment was given on a *pro re nata* (PRN) basis. Treatment was administered for any one of the following conditions: reduction in ETDRS visual acuity, the presence of associated sub- or intra-retinal hemorrhage, and the presence of subretinal fluid, intra-retinal fluid, or sub-RPE fluid on spectral domain OCT (SD-OCT). The protocol was similar to the comparison of age-related macular degeneration treatment trial (CATT) and did not require three initial consecutive monthly injections.⁷

OCTA was performed using the 70 kHz RTVue-XR Avanti SD-OCT system (Optovue, Inc.; Fremont, CA), which has a wavelength spectrum centered at 840 nm and a 5 μ m full-width-half-maximum resolution in tissue. Two 3 $\times$ 3 mm scans (304 $\times$ 304 $\times$ 2 A scans) centered on the macula were obtained for each study eye at each visit. After the first scan, the participant was instructed to remove their chin from the chin rest and wait at least 30 seconds before the second scan was obtained. Angiograms were exported to the Casey Eye Reading Center for custom processing. A semi-automated segmentation algorithm separated the angiogram into an inner retinal slab, an outer retinal slab, and a choroidal slab. Accuracy of segmentation was reviewed by a grader (SSG) and corrected manually if needed. CNV was detected in the outer retinal slab, between the outer boundary of the outer plexiform layer and Bruch’s membrane. *En face* inner retinal shadow-graphic projection artifact was removed with a slab subtraction technique using a Gaussian-filtered version of the inner retinal angiogram, which was then subtracted from the outer retinal angiogram. A saliency-based method was used to automatically detect CNV.²² Only cases where the entire CNV was visible on the 3 $\times$ 3 mm scan area were included to allow consistent measuring of CNV during follow-up visit with dense OCTA scans. CNV vessel area was calculated by summing detected flow pixels and represents the total area occupied by individual neovascular vessels. Automatic contouring of the CNV allowed for calculation of CNV membrane area (mm²), a summation of all pixels within the contour. CNV membrane area is analogous to calculations of CNV area with FA and ICGA.

Cross-sectional composite SD-OCT and color-coded OCT angiograms were used to classify CNV in accordance with Gass' proposed anatomical classification.²⁴ Lesions with a corresponding flow signal above Bruch's membrane and below the RPE were termed "type 1" while those with flow signal above the RPE in the subretinal space were "type 2." Lesions with a flow signal connecting the sub-RPE space into the deep capillary plexus of the inner retina was classified as type 3.²⁵

Mean change of CNV vessel area and CNV membrane area were considered statistically significant at a level of $P < 0.05$ using Wilcoxon test. For those visits with two high quality scans (signal strength index > 50), mean ($\pm$ standard deviation [SD]) values of CNV vessel area and CNV membrane area were calculated at each visit. Repeatability was determined using coefficient of variation. A second grader independently manually corrected the segmentation when needed for the baseline visits and the first visits after treatment and intergrader reproducibility was determined with coefficient of variation.

Results

Fourteen eyes from 14 study participants with treatment-naïve neovascular AMD were enrolled. Three eyes were not included in the analysis due to poor image quality that prevented quantitative assessment and three study eyes had significant portion of CNV outside the $3 \times 3 \text{ mm}^2$ scanning area. Of the eight remaining eyes, the mean age was 70 years (range 57–80) old. All eyes were treated with bevacizumab. A total of 92 visits had two high quality scans available to calculate within-visit repeatability and coefficient of variation was 7.96% for CNV vessel area and 7.37% for CNV membrane area. The inter-grader reproducibility (coefficient of variation) was 9.3% for CNV vessel area and 8.4% for CNV membrane area.

Five participants had type 1 CNV lesions and two had type 2 lesions on OCTA. The type 1 lesions on OCTA corresponded to occult lesions on FA, and the type 2 lesions on OCTA corresponded to classic CNV on FA. Additionally, one eye demonstrated abnormal vasculature associated with the deep capillary plexus (DCP) as seen on OCTA that was consistent with type 3 CNV and this corresponded to a retinal angiomatous proliferation (RAP) lesion on FA.

CNV vessel area and membrane area were calculated at each visit for each eye. Table 1 summarizes baseline characteristics as well as changes to CNV vessel area over time, and Table 2 highlights changes to CNV membrane area over time. Mean CNV vessel area and mean CNV membrane area were each calculated in seven of eight eyes. Case #2 was excluded from this analysis because the presence of subretinal hemorrhage (SRH) obscured baseline CNV vessel area and membrane area measurements. Mean baseline CNV vessel area was $0.39 \pm 0.29 \text{ mm}^2$. Mean CNV vessel area decreased to 0.29 ± 0.26 (39% reduction) at month 1 and to $0.26 \pm 0.26 \text{ mm}^2$ (50% reduction) at month 3 ($P < 0.05$ Wilcoxon test). After month 3, as more eyes were in the PRN phase of treatment, CNV reduction from baseline was 0.29 ± 0.34 (43%) and 0.29 ± 0.29 (41%) at months 6 and 12, respectively, a difference that was no longer statistically significant (Table 1).

Baseline mean CNV membrane area was $0.99 \pm 0.77 \text{ mm}^2$ decreasing to $0.74 \pm 0.65 \text{ mm}^2$ (39%), $0.64 \pm 0.61 \text{ mm}^2$ (51%), and $0.60 \pm 0.62 \text{ mm}^2$ (54%) at months 1, 3, and 6, respectively ($P < 0.05$, Wilcoxon test). At month 12, mean CNV membrane area was $0.66 \pm 0.65 \text{ mm}^2$ (45% reduction), a value no longer statistically significant.

Collectively, the general trend was a reduction in CNV vessel area over time while under PRN treatment. However, comparing individual changes in CNV vessel area over time reveals each eye demonstrated a unique response to treatment Figure 1. Individual cases categorized by number of injections provided are described.

Frequent treatment required

Three of eight eyes (Cases #2, 4, 8) had treatment at nearly every visit during the study. In two of these cases (Cases #2, 4), this was due to persistent fluid on OCT meeting re-treatment criteria. Case #2 was a type 1 CNV and the only case where CNV metrics increased in concert with resolution of subretinal hemorrhage. Baseline ICGA revealed larger CNV measuring 3.5 mm^2 compared to 1.30 mm^2 CNV membrane area measured with OCTA. This demonstrates light scatter from SRH was interfering with OCTA derived CNV vessel area and membrane area measurements (Figure 2). CNV vessel area and membrane area increased at month 1 and month 2 while under treatment due to resolution of SRH improving OCTA signal. After resolution of SRH at month 2, CNV vessel area subsequently declined with further treatment, which was required due to the presence of fluid on OCT. In case #4, a type 1 CNV was associated with persistent fluid that required frequent treatment. Over the course of one year, CNV vessel area and membrane area fluctuated from visit to visit, and at 1 year they were 29% and 27% smaller compared to baseline, respectively. One eye (Case #8) had a type 3 CNV, and, after a single treatment, all flow on OCTA and all fluid on SD-OCT had resolved (Figure 3). However, persistent intra-retinal hemorrhage was present for two months. The patient decided against PRN treatment and was treated monthly until month 12. At twelve months, no fluid was present on OCT, no flow was evident on OCTA, and all hemorrhage had resolved.

Treatment of CNV held without reactivation

Two eyes (Cases #3, 6) did not require further treatment after entering the PRN phase of treatment. In case #3 a type 1 CNV showed a 12% reduction in CNV vessel area after initial treatment. After two injections, subsequent treatment was deferred due to failure to meet re-treatment criteria, CNV vessel area fluctuated and gradually increased over time; however recurrent exudation did not develop. Case #6 was notable in that during the first six months of treatment, cross-sectional OCTA showed envelopment of the vascularized subretinal tissue by a hyper-reflective layer contiguous with the surrounding RPE (Figure 4). What initially was characterized as type 2 CNV developed the appearance of a pigment epithelial detachment (PED) and type 1 anatomy. The timing of this occurrence coincided with cessation of detectable disease activity. After extended follow-up of 24 months, no further treatment has been required and CNV vessel area and membrane area have remained stable.

Treatment held and re-treatment subsequently required

In three eyes (Cases #1, 5, 7), treatment was held and then restarted when re-treatment criteria was met at a later date. In Case #1, a type 1 CNV, reduced CNV vessel area and membrane area was associated with three monthly bevacizumab injections, at which time injections were held. At month 5 and 6, CNV vessel area increased, while there was minimal change in CNV membrane area (Figure 5). During this time, structural OCT showed no recurrence of SRF or IRF and an injection was not given (Figure 6). Evaluation at month 6 showed a further increase in CNV vessel area as well as the presence of recurrent SRF on OCT. These events suggest that changes seen on OCTA may precede fluid recurrences as seen on structural OCT. In this example, CNV vessel area increased prior to fluid while there was minimal change in CNV membrane area, suggesting CNV vessel area may be a more sensitive metric. Two additional bevacizumab injections were administered (months 6 and 7), and no further signs of disease activity were seen over the study period. Between months 10 and 11, CNV vessel area and CNV membrane area decreased spontaneously without treatment and this trend was maintained during subsequent visits. During this period where spontaneous reduction in flow was detected with OCTA, there were no systemic health changes nor changes in medications, and blood pressure remained stable.

In Case #7, a type 2 CNV, after three monthly injections all CNV flow became immeasurable with OCTA and all fluid on structural OCT resolved. At months 3 and 4, treatment was not indicated. At month 5, new visual symptoms developed and new subretinal hyper-reflective material (SHRM) was seen on structural OCT. Neovascular vessels associated with the SHRM were detected on OCTA, and both CNV vessel area and membrane area began to increase. After one injection, symptoms improved and the area of SHRM resolved. With OCTA, CNV flow continued to be detected at months 6, 7, and 8; however, treatment was not indicated based on absence of fluid on structural OCT. At month 9, recurrence of both SRF and SHRM was noted on structural OCT. Treatment was required at month 9, but unfortunately the study participant did not have time for an OCTA study on that day. After this visit, CNV vessel area and membrane area continued to increase slightly in subsequent visits without the need for retreatment.

Case #5 illustrates a situation where treatment with anti-VEGF did not seem to affect type 1 CNV. Minimal change was noted in this type 1 CNV after initial treatment. At month 3, the CNV vessel area and membrane area decreased; however, treatment was administered because of a small amount of IRF present on structural OCT. After this treatment, CNV vessel area appeared to increase in subsequent visits. Then, after a spontaneous reduction in CNV flow metrics at month 5, flow gradually increased over the next six visits with treatment needed at the final visit of the study. In this case, anti-VEGF treatment may have had more effect on vascular permeability and less effect on the CNV flow that was detected by OCTA.

Discussion

This is the first prospective case series we are aware of that uses OCTA to monitor quantitative flow changes in treatment-naïve CNV during a year of anti-VEGF treatment under PRN protocol. At follow-up visit months 1 and 3, most eyes had reductions in both

CNV vessel area and membrane area in response to anti-VEGF treatment, and the mean vessel and membrane areas were significantly reduced. At each of these time points, the magnitude of the reduction of both CNV vessel area and membrane area were greater than the calculated within-visit coefficients of variation, suggesting that measured reductions in CNV flow are real and are not related solely to variations in scan quality. While several other studies have used OCTA to demonstrate reductions in CNV flow in treatment-naïve eyes, these studies had variable follow-up intervals and did not follow a pre-specified treatment protocol.^{15,16,18,20,26} Prior studies using FA-based measurements found that CNV size remained stable after one year of monthly anti-VEGF treatment²⁷ and that CNV size increased slightly when switched to a PRN treatment protocol.⁷ In our study, CNV membrane area and vessel area were reduced at one year in most eyes, and the mean reductions were 45% and 41%, respectively. It is not surprising that FA and OCTA results do not correlate well since CNV size measurements with FA are dependent on leakage and staining patterns that may be influenced by the morphology of surrounding tissues, such as fibrosis or detachment of the pigment epithelium. In contrast, OCTA detects moving red blood cells to calculate quantitative flow metrics, and changes in blood flow may be more sensitive to anti-VEGF treatment.

The presence of CNV exudation on structural OCT (as indicated by intra- or sub-retinal fluid) is commonly used as a surrogate for CNV activity and guides administration of anti-VEGF therapy. It has previously been shown that CNV surface area measurements with ICGA can be predictive of exudation on OCT.²⁸ In the current series, OCTA showed each case to have a unique response (Figure 1) to anti-VEGF treatment, and increases in CNV vessel area and membrane area did not always correlate with presence of exudation. A comparison of cases #1 and #5, both type 1 CNV, provides an example. In case #1, a relatively rapid increase in CNV vessel area of 25% between months 4 and 5 followed by an increase of 18% between months 5 and 6 preceded the recurrence of SRF and the need for treatment, suggesting that increased vessel area may be associated with exudation. In case #5, between months 2 and 3, CNV vessel area decreased spontaneously by 46% without anti-VEGF treatment. Then, despite further reduction in vessel area at month 4, a small amount of IRF developed and treatment was recommended. After treatment, CNV vessel area fluctuated and gradually increased; however, treatment was not indicated until month 12. Comparing these two cases highlights that changes OCTA derived CNV vessel area may be predictive of exudation in some, but not all cases of CNV. Because anti-VEGF affects both angiogenesis and vascular permeability, and it is possible that for some CNV, such as in case #5, anti-VEGF treatment is more effective at reducing vascular permeability than reducing CNV blood flow. In other cases, anti-VEGF may have a greater effect on inhibiting angiogenesis and thereby reducing flow within the CNV, such as in case #1.

Prior work has demonstrated multimodal imaging can be used to classify CNV as type 1, type 2, or type 3, and that this classification may be clinically meaningful in the anti-VEGF era.²⁵ OCTA has the advantage of being able to classify lesion type by detecting depth of flow in relation to RPE on cross-sectional OCTA and this information can be used to directly compare treatment response between CNV types. In this study, one month after the first treatment, type 1 CNV had a CNV vessel area reduction ranging from 4% to 23%, while the two cases with type 2 CNV had larger magnitude reductions of 51% and 60%. The small

number of type 2 eyes in this series limits any firm conclusions that type 2 eyes are more responsive to anti-VEGF treatment, however these findings are agreeable to those reported by Coscas et al., who used OCTA to show that type 2 CNV had a greater reduction with treatment compared to type 1 CNV.¹⁸ Furthermore, our results for type 1 treatment response were similar to those reported by Querques, et al, who found reduced CNV surface area with ICGA at one year while under PRN anti-VEGF treatment; however, this was not statistically significant compared to baseline.²⁹ In our study, the lone type 3 CNV (case #8) had complete resolution of intra-retinal fluid on structural OCT and flow on OCTA after a single treatment. The type 3 CNV was small, and the robust treatment response may have been due to the relatively early initiation of anti-VEGF.^{30–32} In this case, ongoing treatment was given because the intra-retinal hemorrhage persisted for several months, and it was then continued because of the patient's preference. Larger studies are needed to validate if the different CNV subtypes based on OCTA can be predictive of unique treatment response with anti-VEGF therapy. Finally, case #6 (Figure 3) is an example of a retinal pigment epithelial cells enveloping a type 2 CNV resulting in an apparent type 1 CNV, an event Gas had postulated based on histology.³³ Identifying this phenotype with structural OCT along with stable CNV vessel area over time with OCTA may help clinicians identify CNV that are at low risk for developing exudation without anti-VEGF treatment.

With OCTA, multiple different metrics are available for CNV for quantification. In this study, we used vessel area and membrane area. Both CNV vessel area and membrane area had a similar within-visit repeatability and inter-grader reproducibility. In most of the presented cases, each metric yielded similar results. CNV vessel area was reduced by 39%, 50%, 43%, and 41% at months 1, 3, 6, and 12 respectively. CNV membrane area was reduced by 39%, 51%, 54%, and 45% at months 1, 3, 6, and 12. At month 6, mean change from baseline was not statistically significant for CNV vessel area, while it was statistically significant for CNV membrane area. This suggests that these parameters are not entirely interchangeable. An example of the difference of vessel area and membrane area is highlighted in case #1. During the months 4–6, that preceded the development of SRF while treatment was held CNV vessel area increased sharply, while CNV membrane area remained relatively stable (Figure 5). Membrane area reflects the overall footprint of the membrane and does not account for changes within the footprint. Vessel area can better detect vascular changes within the footprint such as branches enlarging or shrinking, or changes in caliber of vessels. This suggests CNV vessel area may better correlate with CNV activity compared to membrane area. Because it is easy to apply multiple quantitative analyses to the same scan, larger studies will be able to compare the usefulness of different quantitative metrics.

Meaningful quantitative metrics derived from OCTA require high quality scans and steps to address segmentation errors and projection artifact. At each visit, two scans were obtained. Ninety-two visits had two high-quality scans, and averaged CNV vessel area and membrane were used. If only one high quality scan was available (i.e., there were excessive motion artifacts in one scan but not both), only the higher quality scan was used for analysis. Coefficient of variation calculation showed good within-visit repeatability for both CNV vessel area and membrane. For each scan, at each visit, segmentation was reviewed throughout the entire volume, and manual segmentation was used to correct errors. Manual segmentation correction is a time-consuming process, but it is imperative for ensuring

accuracy since quantitative metrics are derived from *en face* images and inaccurate segmentation will lead to misleading metrics. However, the process of manual segmentation itself introduces variability evidenced by the inter-grader reproducibility of 9.3% for CNV vessel area and 8.4% for CNV membrane area. Projection artifact was addressed using a slab subtraction technique that mitigates, but does not eliminate, projection artifact. Slab subtraction method was also demonstrated to be effective for CNV detection by other groups.²⁸ Newer projection artifact removal techniques have been shown to reduce artifact without attenuating the CNV signal, and these will be tested in future studies³⁴. Finally, an automated, saliency-based detection method was used that has been shown to be less variable than manual contouring.²²

There are several limitations to this work that limit generalizations that can be made to real world clinical practice. The most notable limitation is the small sample size of this study. For example, at one year, CNV vessel area and membrane area were reduced by over 40%, however this was not statistically significant due limited statistical power. Retinal vessel density values have been shown to correlate with SSI, and while all scans included in the analysis had a SSI greater than 50, that does not exclude the possibility of that variation in SSI at different visits may have affected CNV vessel density measurements^{36,37}. Also, because scans were obtained monthly, potential variations in the metrics between visits remain unknown. Lumbroso, et al. noted dramatic reductions in CNV vessel area measurements within the first 10 days after treatment with subsequent reperfusion of CNV later on.³⁵ These changes would have been missed in the present study; however, their clinical relevance is also unclear. Finally, while the extensive post image processing is helpful to achieve meaningful quantitative data, it is time consuming and unrealistic for this to be achieved in a busy clinical practice. As OCTA technology and automated image processing improves, reliable quantitative metrics should become available on commercial machines.

The study also has its strengths. It was prospectively designed, and quantitative changes in CNV flow patterns were tracked over time while under a strict protocol of PRN anti-VEGF treatment. The sole exception to this occurred in case #8 where the patient decided in the middle of the study to forego the PRN protocol in favor of monthly treatment. Study coordinators were also used to contact patients and arrange for consistent intervals between visits and treatments. Great care was taken to ensure that quantitative OCTA metrics were as accurate as possible by applying manual segmentation correction, reducing the impact of artifacts, and evaluating the within-visit repeatability to reduce the chance that observed changes were due to expected scan-to-scan variation. Lastly, this is the first report comparing the quantitative metrics of CNV vessel area with CNV membrane area, showing agreement in most but not all visits (fig. 4). This study suggests that CNV vessel area may be a more sensitive metric for detecting changes CNV blood flow compared to CNV membrane area.

Conclusion

This small case series used OCTA to demonstrate a reduction in CNV vessel area and membrane area in response to the first three months of PRN anti-VEGF treatment. After one

year of PRN treatment, CNV vessel area and membrane area were reduced, however it was not statistically significant compared to baseline. Longitudinal follow-up revealed that each CNV had a unique response to anti-VEGF treatment as well as variable changes in CNV vessel area when anti-VEGF treatment was deferred. Increasing CNV vessel area tended to correlate with future exudation, but was less useful in predicting exactly when exudation develops. Future studies comparing fixed monthly treatment, with PRN protocol or treat and extend protocol would be useful to better evaluate how anti-VEGF treatment affects blood flow in CNV measured with OCTA and if OCTA can be useful to predict when exudation will develop.

Acknowledgments

This work was supported by grant R01 EY024544, R01EY027833, DP3 DK104397, P30 EY010572 from the National Institutes of Health (Bethesda, MD), and by unrestricted departmental funding from Research to Prevent Blindness (New York, NY).

References

1. Ambati J, Ambati BK, Yoo SH, et al. Age-Related Macular Degeneration: Etiology, Pathogenesis, and Therapeutic Strategies. *Survey of Ophthalmology*. 2003; 48:257–293. [PubMed: 12745003]
2. Mokwa NF, Keane PA, Kirchhof B, et al. Grading of Age-Related Macular Degeneration: Comparison between Color Fundus Photography, Fluorescein Angiography, and Spectral Domain Optical Coherence Tomography. *Journal of Ophthalmology*. 2013; 2013:1–6.
3. Rosenfeld PJ. Optical Coherence Tomography and the Development of Antiangiogenic Therapies in Neovascular Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*. 2016; 57:OCT14–26. [PubMed: 27409464]
4. Brown DM, Kaiser PK, Michels M, et al. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med*. 2006; 355:1432–1444. [PubMed: 17021319]
5. Schmidt-Erfurth U, Eldem B, Guymer R, et al. Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration: the EXCITE study. *Ophthalmology*. 2011; 118:831–839. [PubMed: 21146229]
6. Abraham P, Yue H, Wilson L. Randomized, double-masked, sham-controlled trial of ranibizumab for neovascular age-related macular degeneration: PIER study year 2. *American Journal of Ophthalmology*. 2010; 150:315–324e1. [PubMed: 20598667]
7. Martin DF, Maguire MG, et al. CATT Research Group. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2011; 364:1897–1908. [PubMed: 21526923]
8. Wykoff CC, Croft DE, Brown DM, et al. Prospective Trial of Treat-and-Extend versus Monthly Dosing for Neovascular Age-Related Macular Degeneration: TREX-AMD 1-Year Results. *Ophthalmology*. 2015; 122:2514–2522. [PubMed: 26391465]
9. Rush RB, Rush SW, Aragon AV, Ysasaga JE. Predictability of Recurrent Exudation and Subretinal Hemorrhaging in Neovascular Age-Related Macular Degeneration With Indocyanine Green Angiography. *Ophthalmic Surg Lasers Imaging Retina*. 2015; 46:718–723. [PubMed: 26247452]
10. Jia Y, Tan O, Tokayer J, et al. Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. *Opt Express*. 2012; 20:4710–4725. [PubMed: 22418228]
11. Motaghianezam R, Fraser S. Logarithmic intensity and speckle-based motion contrast methods for human retinal vasculature visualization using swept source optical coherence tomography. *Biomed Opt Express*. 2012; 3:503–521. [PubMed: 22435098]
12. Jia Y, Bailey ST, Hwang TS, et al. Quantitative optical coherence tomography angiography of vascular abnormalities in the living human eye. *Proc Natl Acad Sci USA*. 2015; 112:E2395–402. [PubMed: 25897021]

13. Jia Y, Bailey ST, Wilson DJ, et al. Quantitative optical coherence tomography angiography of choroidal neovascularization in age-related macular degeneration. *Ophthalmology*. 2014; 121:1435–1444. [PubMed: 24679442]
14. Moulton E, Choi W, Waheed NK, et al. Ultrahigh-Speed Swept-Source OCT Angiography in Exudative AMD. *Ophthalmic Surg Lasers Imaging Retina*. 2014; 45:496–505. [PubMed: 25423628]
15. Kuehlewein L, Bansal M, Lenis TL, et al. Optical Coherence Tomography Angiography of Type 1 Neovascularization in Age-Related Macular Degeneration. *American Journal of Ophthalmology*. 2015; 160:739–48e2. [PubMed: 26164826]
16. de Carlo BATE PhD MABFM, BA, ATC, et al. Spectral-Domain Optical Coherence Tomography Angiography of Choroidal Neovascularization. *Ophthalmology*. 2015; 122:1–11. [PubMed: 25542536]
17. Coscas GJ, Lupidi M, Coscas F, et al. OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY VERSUS TRADITIONAL MULTIMODAL IMAGING IN ASSESSING THE ACTIVITY OF EXUDATIVE AGE-RELATED MACULAR DEGENERATION: A New Diagnostic Challenge. *Retina (Philadelphia, Pa)*. 2015; 35:2219–2228.
18. Coscas G, Lupidi M, Coscas F, et al. Optical coherence tomography angiography during follow-up: qualitative and quantitative analysis of mixed type I and II choroidal neovascularization after vascular endothelial growth factor trap therapy. *Ophthalmic Res*. 2015; 54:57–63. [PubMed: 26201877]
19. Spaide RF. Optical Coherence Tomography Angiography Signs of Vascular Abnormalization With Antiangiogenic Therapy for Choroidal Neovascularization. *American Journal of Ophthalmology*. 2015; 160:6–16. [PubMed: 25887628]
20. Kuehlewein L, Sadda SR, Sarraf D. OCT angiography and sequential quantitative analysis of type 2 neovascularization after ranibizumab therapy. *Eye (Lond)*. 2015; 29:932–935. [PubMed: 25976641]
21. Liang MC, De Carlo TE, Bauman CR, et al. CORRELATION OF SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY AND CLINICAL ACTIVITY IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION. *Retina (Philadelphia, Pa)*. 2016:1.
22. Liu L, Gao SS, Bailey ST, et al. Automated choroidal neovascularization detection algorithm for optical coherence tomography angiography. *Biomed Opt Express, BOE*. 2015; 6:3564–3576. [PubMed: 26417524]
23. Zhang M, Wang J, Pechauer AD, et al. Advanced image processing for optical coherence tomographic angiography of macular diseases. *Biomed Opt Express, BOE*. 2015; 6:4661–4675. [PubMed: 26713185]
24. Gass JDM. *Stereoscopic Atlas of Macular Diseases*. Mosby Incorporated; 1996.
25. Freund KB, Zweifel SA, Engelbert M. Do we need a new classification for choroidal neovascularization in age-related macular degeneration? *Retina (Philadelphia, Pa)*. 2010; 30:1333–1349.
26. Muakkassa NW, Chin AT, de Carlo T, et al. CHARACTERIZING THE EFFECT OF ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY ON TREATMENT-NAIVE CHOROIDAL NEOVASCULARIZATION USING OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY. *Retina (Philadelphia, Pa)*. 2015; 35:2252–2259.
27. Kaiser PK, Blodi BA, Shapiro H, Acharya NR. Angiographic and Optical Coherence Tomographic Results of the MARINA Study of Ranibizumab in Neovascular Age-Related Macular Degeneration. *Ophthalmology*. 2007; 114:1868–1875e4. [PubMed: 17628683]
28. Rush RB, Rush SW. Predictability of Recalcitrance in Neovascular Age-Related Macular Degeneration With Indocyanine Green Angiography. *Asia Pac J Ophthalmol (Phila)*. 2015; 4:187–190. [PubMed: 26147016]
29. Querques G, Tran THC, Forte R, et al. Anatomic response of occult choroidal neovascularization to intravitreal ranibizumab: a study by indocyanine green angiography. *Greases Arch Clin Exp Ophthalmol*. 2012; 250:479–484.

30. Tan ACS, Dansingani KK, Yannuzzi LA, et al. TYPE 3 NEOVASCULARIZATION IMAGED WITH CROSS-SECTIONAL AND EN FACE OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY. *Retina* (Philadelphia, Pa). 2017; 37:234–246.
31. Yannuzzi LA, Freund KB, Takahashi BS. Review of retinal angiomatous proliferation or type 3 neovascularization. *Retina*. 2008; 28:375–384. [PubMed: 18327130]
32. Miere A, Querques G, Semoun O, et al. OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY CHANGES IN EARLY TYPE 3 NEOVASCULARIZATION AFTER ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR TREATMENT. *Retina*. 2017:1.
33. Gass JD. Biomicroscopic and histopathologic considerations regarding the feasibility of surgical excision of subfoveal neovascular membranes. *Trans Am Ophthalmol Soc*. 1994; 92:91–111. discussion 111–6. [PubMed: 7533960]
34. Zhang M, Hwang TS, Campbell JP, et al. Projection-resolved optical coherence tomographic angiography. *Biomed Opt Express*, BOE. 2016; 7:816–828. [PubMed: 27231591]
35. Lumbroso B, Rispoli M, Savastano MC, et al. Optical Coherence Tomography Angiography Study of Choroidal Neovascularization Early Response after Treatment. *Dev Ophthalmol*. 2016; 56:77–85. [PubMed: 27022967]

Figure 1.

Graphical representations of changes in CNV vessel area over time for Cases #1–8. Red arrows indicate time points where anti-VEGF treatment was administered. Error bars indicate standard deviation.

Figure 2.

Case #2. A: Color fundus photograph at baseline showing drusen, pigmentary changes, and subretinal hemorrhage (SRH). B: Late phase indocyanine green angiogram at baseline showing hyperfluorescent plaque consistent with a choroidal neovascular membrane (CNV). There is partial obscuration of the temporal border of the membrane due to blockage from SRH. C: Baseline outer retinal OCTA revealed CNV; however, SRH obstructs portions of OCTA signal. D: Outer retinal OCTA at month 3 after consecutive monthly anti-VEGF treatments. CNV becomes more visible as SRH resolves and has similar appearance to baseline ICGA.

Figure 3.

Case #8. Top row (A1–A4) baseline OCTA of type 3 CNV. En face outer retinal slab showing to type 3 lesions (A1). Inner retinal slab with retinal vessels in purple and type 3 CNV highlighted with pink (A2). Cross-sectional OCTA showing blood flow (yellow) of superior (A3) type 3 CNV (corresponds to red line in A2) and of inferior (A4) type 3 CNV (corresponds to red line in A2). Bottom row B1–B4 one month after bevacizumab treatment. Resolution of type 3 CNV in outer retinal slab (B1) and inner retinal slab (B2). Cross-sectional OCTA showing loss of detected blood flow and resolution of pigment epithelial detachment.

Figure 4.

Case #6. Top row (A–C): images obtained at baseline. Bottom row (D–F): images obtained after 6 monthly injections. A: Early FA obtained at baseline showing a choroidal neovascular membrane (CNV). B: OCTA obtained at baseline showing flow in outer retinal space consistent with CNV (yellow). C: Cross sectional composite OCT at baseline (corresponds to location of green dashed line in frame B) showing type 2 configuration of CNV. D: Color fundus photo at 6 months showing pigmented subretinal lesion. E: OCTA obtained at 6 months showing reduced CNV flow (yellow). F: Cross sectional composite OCT at 6 months (corresponds to location of green dashed line in frame E) showing RPE-envelopment of the CNV, which now has an apparent type 1 appearance. OCTA and composite OCT legend: purple=inner retinal flow signal, yellow=outer retinal flow signal, red=choroidal flow signal.

Figure 5.

Case #1. Outer retinal OCTAs (A, B) after removal of projection artifact showing the flow metrics of CNV vessel area (A) and membrane area (B). C and D: Graphical representations of changes in CNV vessel area (B) and membrane area (D) over time for Case #1. Red arrows indicate time points where anti-VEGF treatment was administered. At month 6, re-treatment criteria were met, and the in the preceding visits (yellow shade) CNV vessel area increased to baseline value while CNV membrane area remained stable, suggesting that CNV vessel area and membrane area may not always coincide with one another. Error bars indicate standard deviation.

Figure 6.

Case #1. Outer retinal OCTAs and corresponding cross-sectional composite OCTs at baseline and during follow up under PRN anti-VEGF treatment. Monthly injections were given over the first 3 visits (blue arrow). CNV vessel area decreased over the first three injections, and no treatment was indicated at month 4 due to resolution of subretinal fluid (SRF). CNV vessel area increased during month 5; however, no fluid was present and treatment was not provided. At month 6, CNV vessel area continued to enlarge to baseline level, and SRF recurred while treatment was held, demonstrating that OCTA can detect increasing CNV vessel area in the absence of exudation. There is dramatic spontaneous reduction in CNV size without treatment between months 10 and 11. Green dashed lines correspond to locations of composite OCTs at each time point. Composite OCT legend: purple=inner retinal flow signal, yellow=outer retinal flow signal, red=choroidal flow signal.

Table 1

CNV vessel area in response to PRN anti-VEGF treatment

Case #	Age	Initial visual acuity	FA lesion type	OCTA lesion type	Initial CNV vessel area (mm ²)	CNV vessel area (mm ²) after 1 mo	% Change from Baseline	CNV vessel area (mm ²) after 3 mo	% Change from Baseline	CNV vessel area (mm ²) after 6 mo	% Change from Baseline	CNV vessel area (mm ²) after ~12 mo	% Change from Baseline
1	69	20/25	Occult	Type 1	0.33	0.26	-21	0.21	-38	0.34	3	0.08	-75
2 ^a	68	20/50	Occult	Type 1	0.62	0.85	37	1.32	112	1.43	130	0.73	18
3	80	20/50	Occult	Type 1	0.83	0.74	-12	0.75	-10	0.99	18	0.65	-22
4 ^a	65	20/20	Occult	Type 1	0.19	0.14	-23	0.14	-27	0.12	-36	0.14	-27
5	77	20/20	Occult	Type 1	0.52	0.50	-4	0.33	-37	0.31	-40	0.70	35
6	57	20/80	Classic	Type 2	0.65	0.32	-51	0.40	-38	0.29	-55	0.38	-41
7	66	20/50	Classic	Type 2	0.17	0.07	-60	0	-100	0.01	-92	0.07	-57
8	76	20/80+6	RAP	Type 3	0.02	0	-100	0	-100	0	-100	0	-100
Avg ^b					0.39 ± 0.29	0.29 ± 0.26 [*]	-39 ± 34	0.26 ± 0.26 [*]	-50 ± 36	0.29 ± 0.34	-43 ± 44	0.29 ± 0.29	-41 ± 43

^a Indicates participant was treated at every visit.^b Average (mean ± standard deviation) values do not include case 2.^{*} P<0.05 when compared to baseline (Wilcoxon test), does not include case 2

Table 2

CNV membrane area in response to PRN anti-VEGF treatment

Case #	Initial CNV membrane area (mm ²)	CNV membrane area (mm ²) after 1 mo	% Change from Baseline	CNV membrane area (mm ²) after 3 mo	% Change from Baseline	CNV membrane area (mm ²) after 6 mo	% Change from Baseline	CNV membrane area (mm ²) after ~12 mo	% Change from Baseline
1	1.00	0.85	-15	0.83	-18	0.80	-20	0.32	-68
2 ^a	1.30	1.72	33	2.57	98	3.00	131	2.04	57
3	2.23	1.83	-18	1.68	-24	1.76	-21	1.32	-41
4 ^a	0.38	0.32	-16	0.25	-36	0.22	-42	0.27	-29
5	1.48	1.26	-15	0.99	-33	0.85	-43	1.75	18
6	1.40	0.75	-47	0.73	-48	0.55	-61	0.72	-49
7	0.40	0.14	-65	0	-100	0.03	-92	0.21	-48
8	0.06	0	-100	0	-100	0	-100	0	-100
Avg ^{a,b}	0.99 ± 0.77	0.74 ± 0.65 [*]	-39 ± 33	0.64 ± 0.61 [*]	-51 ± 35	0.60 ± 0.62 [*]	-54 ± 32	0.66 ± 0.65	-45 ± 36

^aIndicates participant was treated at every visit.^bAverage (mean ± standard deviation) values do not include case 2.^{*}P<0.05 when compared to baseline (Wilcoxon test), does not include case 2