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A SEMI-AUTOMATED QUANTITATIVE APPROACH TO CHARACTERIZE TREATMENT RESPONSE IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION: A REAL WORLD STUDY

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Abstract

Purpose—To perform a quantitative study of the vascular microstructure in actively treated choroidal neovascularization (CNV) by optical coherence tomographic angiography (OCTA).

Methods—Patients undergoing individualized anti-vascular endothelial growth factor (anti-VEGF) therapy of minimum 12 months duration were included in this cross-sectional observational study and imaged using OCTA. *En face* OCTA images were analyzed for quantitative features such as junction density, vessel length and lacunarity using validated software (Angiotool). Patients were divided into two groups depending on their individualized treatment interval: “Good responders, treated less frequently than 6 weeks” vs. “poor responders, treated every 6 weeks or more frequently”. Nonparametric testing was used to assess differences between these groups.

Results—Twenty-five eyes of 23 consecutive patients with a median 58-month history of CNV, treated by median of 34 anti-VEGF injections, were included in the analysis. There was no significant difference between any of the microvascular CNV features between the two groups ($p > 0.05$).

Conclusion—The semi-automated vessel segmentation software provides an objective and quantitative approach for CNV characterization. The consistently non-significant outcomes between the groups may provide evidence to support the “normalization hypothesis”. This would suggest that regardless of treatment interval, individualized therapy in these eyes established vessel stability.

Keywords

choroidal neovascularization; age-related macular degeneration; optical coherence tomography; optical coherence tomography angiography; lacunarity; fractal dimension

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Introduction

The introduction of intravitreal anti-vascular endothelial growth factor (anti-VEGF) treatment has led to dramatic improvements in the visual prognosis of patients with neovascular age-related macular degeneration (nAMD).¹⁻⁴ Despite the lack of consensus regarding the various therapeutic regimens used by retina specialists, the overall morphological and functional outcomes far surpass the natural course of nAMD.⁵

Monthly anti-VEGF treatment has been shown to achieve the best functional results in randomized controlled multicenter studies; however, this approach involves a significant treatment burden and an indefinite number of intravitreal injections. Furthermore, monthly treatment is cost-intensive and could play a role in the development of geographic atrophy in patients with nAMD.⁶ In order to reduce this treatment burden and associated adverse effects, pro re nata (PRN) and treat and extend (TREX) regimens have been introduced, and both have been shown to achieve comparable results to monthly treatment.⁵ Tailoring the treatment intervals for each patient, however, can be challenging and involves a long process of trial and error. Furthermore, many patients present with already treated choroidal neovascularization (CNV), having received prior anti-VEGF treatment elsewhere, a group of patients usually not represented in clinical trials.

Optical coherence tomographic angiography (OCTA), a functional extension of conventional OCT, provides high-resolution imaging of the microvasculature in CNV lesions. In contrast to dye-based angiography, OCTA does not show vessel leakage, thereby facilitating an unadulterated and detailed evaluation of CNV vascular morphology. Different CNV shapes and structural features and their response to anti-VEGF treatment have been demonstrated by OCTA.⁷⁻¹¹ Several OCTA studies have explored qualitative CNV structural features that may correlate with disease activity, but these have had conflicting results, which may, however, also be explained by different inclusion criteria.^{12, 13} In the first study, there was a significant association between CNV activity and qualitative CNV parameters on OCTA, whereas this was not the case in the latter study.

Given the contradictory results of these qualitative OCTA studies, and acknowledging the importance of identifying OCTA features that may reflect CNV lesion activity, we designed the current study to identify quantitative features of CNV microvascular structure on OCTA that could help guide clinical decision-making during individualized anti-VEGF therapy.

Methods

Study participants and clinical protocols

The study protocol and procedures adhered to the ethics tenets of the Declaration of Helsinki and were approved by Northwestern University's Institutional Review Board. Patients were recruited from the retina practice in the Department of Ophthalmology of the Feinberg School of Medicine at Northwestern University, Chicago, IL, USA. Informed consent was obtained from each participant before inclusion in this observational study. Patients were eligible if they had a diagnosis of unilateral or bilateral CNV in the setting of AMD, as well

as a course of anti-VEGF treatment of at least 12 months duration. Patients were receiving treatment either following a PRN or TREX regimen at the discretion of the treating physician. Eyes with central geographic atrophy or subretinal fibrosis, motion artifacts or poor OCTA image quality were excluded from the analysis.

Optical coherence tomographic angiography protocol

For OCT angiographic imaging, a commercial spectral-domain optical coherence tomography (SD-OCT) device (RTVue- XR Avanti; Optovue, Inc., Fremont, CA) was used. The OCT device uses a probing light beam with a center wavelength of 840nm and a full-width half maximum of 45 nm. The axial scan rate is 70,000 A-scans per second. For detection of blood flow, two consecutive B-scans are acquired at the same position in order to differentiate between static tissue and particles with high signal intensity fluctuation (caused by blood flow in blood vessels). The OCTA device uses split-spectrum amplitude-decorrelation angiography (SSADA) to improve the performance of the vessel segmentation algorithm.¹⁴ OCTA volume scans measuring 3×3mm (304 × 304 pixels), centered on the CNV membrane were acquired in approximately 3 seconds. In cases where the CNV membrane exceeded the field of view of 3×3mm, a montage of multiple volume scans was generated using i2k Retina Pro (Version 3.0.0; DualAlign, LLC, Clifton Park, NY, USA) and used for further analysis of the vasculature.

Post-processing and qualitative evaluation of images

Depending on the location of the CNV membrane, either the outer retinal (OR) or the choriocapillaris (CC) slab was used in order to best encompass the whole neovascular complex and the slab thickness manually adjusted using B-scan information. In order to remove projection artifacts originating from the superficial retinal capillary plexus (SCP) onto the choriocapillaris slab, a protocol was applied for all CC slabs using FIJI software (ImageJ 2.0.0, imagej.net) prior to quantitative vessel analysis.¹⁵ The CC *en face* angiographic slab was multiplied with the color-inverted SCP *en face* image, resulting in an *en face* CC slab without flow signal from the SCP (see Figure 1). Qualitative evaluation was performed by two graders (AAF, PKR) independently. If disagreement occurred, open adjudication between the two graders was performed to reach consensus.

We evaluated several previously described qualitative features of CNV activity including CNV shape, branching, the presence of anastomoses and vessel loops, morphology of vessel termini and the presence of a hypo-intense halo around the lesion.¹²

Quantitative Measurements

After artifact removal, the CNV lesion was manually delineated in FIJI for quantitative evaluation. In cases with ill-defined lesion borders, the B-scan information was used in combination with the *en face* OCTA scans to delineate the boundaries of the CNV (see Figures 2 and 3).

For the quantitative analysis, we used a semi-automated, validated, open source software (Angiotool 0.5a, <https://ccrod.cancer.gov/confluence/display/ROB2>), to detect and calculate several morphological and spatial parameters of the vascular network.¹⁶ The software

identifies the vascular configuration based on user-defined parameters such as vessel diameter and intensity. Furthermore, particles can be added or removed from the segmented vascular network based on user-defined size threshold. After optimizing the parameters that best delineate the vascular structure, the program segments, skeletonizes and analyzes the explant area, vessel area, vessel density, total number of junctions, branching index, total vessel length, average vessel length and lacunarity. For a detailed explanation of each of these parameters we refer to a recent publication by Zudaire et al.¹⁶ In brief, the explant area is equivalent to the total area of the manually segmented CNV complex. The vessel area is equivalent to the total area within the CNV complex showing flow signal on OCTA imaging. The vessel percentage area is the percentage of the total CNV lesion showing flow signal and can be derived from the lesion area and vessel area. Total vessel length is calculated as the sum of all vessels, whereby a vessel is defined as the distance between two junctions or between a junction and an endpoint. The junction density is calculated as the number of junctions normalized per unit area. Lacunarity is a measure of lesion inhomogeneity with higher values reflecting more inhomogenous vascular structure. Two quantitative comparative analyses were performed. For both analyses, the patients were divided into two groups: “Good responders” vs. “poor responders”, based on their individualized treatment intervals required to maintain a “dry retina”. A dry retina was defined as absence of subretinal or intraretinal fluid per SD-OCT.

Interval analysis one—“Good response” to treatment was defined as treatment interval of 12 weeks or more, while “poor response” was defined as interval of four weeks or less to maintain a dry retina.

Interval analysis two—“Good response” to treatment was defined as treatment interval of more than six weeks, while “poor response” was defined as treatment interval of six weeks or less.

Reproducibility

To test for reproducibility of the program, we compared the results of the Angiotool analysis using two approaches. The first approach used the same set of preset parameters for all study eyes. The second approach used individualized parameters set by the reader for each eye, so the vessels would be optimally segmented as suggested by the developers.¹⁶

Statistics

For statistical analysis, IBM SPSS statistics version 21 (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA) was used. To test for reproducibility of the quantitative analysis, we calculated the intraclass correlation coefficient (ICC) for lacunarity, which was the main outcome measure of this study. Independent samples Mann-Whitney U test was used to assess significant differences in quantitative findings between the good and poor responder groups. Descriptive statistics and Chi-square test were used for qualitative findings. A p-value of <0.05 was considered statistically significant.

Results

Twenty-five eyes of twenty-three consecutive patients (12 female, 11 male) with a median age of 76.5 years (range: 54-97 years) were included in the analysis. Median duration from initiation of therapy for nAMD to OCTA examination was 58 months (range: 12-112 months), with a median number of 34 anti-VEGF injections (range: 5-124). Sixteen eyes had been diagnosed with type-1 CNV (vascularized PED), four eyes with type-2 CNV (subretinal), one eye with a type-3 (retinal angiomatous proliferation) and four eyes with a type-4 (polypoidal) lesion as assessed by baseline fluorescein angiography and SD-OCT. Eight out of the 25 study eyes had received anti-VEGF injections at outside institutions prior to presentation at Northwestern. The median treatment interval for all study eyes was four weeks (range: 2-6 weeks) in the “poor responder” and 11 weeks (range 9-184 weeks) in the “good responder” group. There was no significant difference between good and poor responders in terms of duration of treatment before the date of the OCTA examination ($p=0.152$).

Results of the qualitative analysis are presented in table 1.

Fourteen eyes (7 good responders and 7 poor responders) met the criteria to be included in interval analysis one. There was no significant difference in any of the analyzed parameters. Twenty-five eyes (13 good responders and 12 poor responders) were included in interval analysis two. There was no significant difference in any of the analyzed parameters.

A detailed description of the quantitatively analyzed microvascular parameters is reported in table 2. The reproducibility for lacunarity showed excellent repeatability with $ICC=0.890$. Therefore we only report the individualized analysis approach.

Discussion

The study described herein was designed to identify imaging biomarkers for the response to anti-VEGF therapy in previously treated nAMD eyes. OCTA provided an elegant means for imaging due to its high resolution and the lack of dye leakage obscuring the microvascular network. In order to reflect a real world situation, only patients with prior anti-VEGF treatment and stable individualized therapy plan were included. In addition to a qualitative evaluation, we performed a quantitative analysis and neither showed any significant difference between “good” and “poor” responders on OCTA.

In a mixed population of previously treated and treatment-naïve eyes, Coscas et al. reported a significant association between CNV activity and qualitative CNV parameters on OCTA such as a seafan or lacy wheel shape, the presence of tiny capillaries, anastomoses and vessel loops, a peripheral arcade, and a hypo-intense halo around the lesion.¹² In their study, they compared traditional multimodal imaging with OCTA and showed that CNV lesions that required treatment showed more of the mentioned findings than lesions not requiring treatment. Unlike our study, where individual qualitative parameters were compared between the good and poor responder group, Coscas et al. implemented a system, where the presence of three qualitative features was required to classify a lesion as active on OCTA. Furthermore, we did not include treatment-naïve eyes in our study, which also may explain

the different outcomes. Miere et al., using OCTA, investigated active vs. inactive CNV with a disease duration similar to ours (minimum duration of CNV of 12 months and an average 44-month history of CNV prior to inclusion), with the only exception that their study required the presence of subretinal fibrosis for inclusion.¹³ Their analysis of microvascular patterns such as pruned vascular tree, vascular loop, tangled network, dark halo and large flow void revealed no significant difference between the two groups. Using a quantitative approach, we have now investigated a similar patient cohort and confirmed the results of this latter study.

Unlike these previous studies, we found that many CNV lesions in our study did not fit any of the previously described shapes such as a seafan, lacy wheel or dead tree shape (see Figures 1 and 3). Furthermore, qualitative assessments of the shape, presence of anastomoses and loops or peripheral arcade, were very subjective in nature, making it difficult to reach consensus between readers. Therefore, we focused our study on a quantitative, automated and more objective approach. The Angiotool software offers semi-automated analysis of several morphological and spatial vessel parameters, providing quantitative information about vessel density, branching pattern and uniformity of the CNV microvasculature. A detailed description of the software and the evaluated parameters was published by Zudaire et al¹⁶. In brief, the junction density, calculated as the number of junctions normalized per unit area, can be interpreted as a measure of sprouting activity of the neovascular complex. Lacunarity represents a measure of vessel non-uniformity where higher values reflect inhomogeneity and lower values reflect a more homogenous vascular structure.¹⁷ For the purpose of this study we only assessed reproducibility of the lacunarity, which showed excellent reproducibility. Other measures need to be evaluated in future studies of CNV. Lacunarity was of particular interest, as it has been suggested as a surrogate parameter for vascular changes in allantoic membranes treated with angiostatic drugs.^{16, 18} As observed previously by OCTA imaging, microvascular features of CNV membranes undergo a transformation when treated with anti-VEGF agents, resulting in loss of smaller vessels and a “strengthening” of the larger trunk vessels.^{7, 8} This qualitative observation would translate to (objectively quantifiable) higher lacunarity or “gapiness”, with less homogeneously filled space within the CNV lesion after anti-VEGF treatment. We would expect CNV membranes with more exudative activity (poor responders) to show lower lacunarity values than CNV membranes with less exudative activity (good responders). Furthermore, a difference in the junction density corresponding to a decrease in sprouting activity could be expected. Overall, however, our results showed consistently non-significant differences in all of the tested parameters between the “good” and “poor” responders. These findings, while counter-intuitive at first glance, fit well in the context of the “normalization hypothesis” that was established in angiogenesis research in oncology.¹⁹ The normalization hypothesis states that newly formed blood vessels in tumors are abnormal in shape, but acquire a more normal shape when treated with anti-angiogenic agents. In this “normalization window”, pro-angiogenic and anti-angiogenic factors are kept in a delicate balance. The normalized blood vessels are characterized by a greater coverage with pericytes, are less leaky, less tortuous and less dilated.¹⁹ If anti-angiogenic treatment is intensified, the new blood vessels become degenerative and inadequate for oxygen delivery. Based on our findings, we hypothesize that regardless of the interval or approach (PRN vs

TREX regimen), individualized treatment in nAMD results in a normalization of the CNV vasculature, which could explain the lack of significant difference between the “good” and “poor” responder groups.

Patients included in our study had been treated with a median of 34 anti-VEGF injections prior to study inclusion and an individualized treatment regimen had already been established. We postulate that these patients were receiving sufficient anti-VEGF treatment to maintain a non-exudative state, without triggering vessel collapse. Once these lesions have achieved a therapeutic equilibrium between anti- and pro-angiogenic factors, we would expect the vessels to appear similar on OCTA in all treated eyes. This hypothesis could also explain the finding that geographic atrophy during anti-VEGF therapy is more likely to occur in eyes receiving monthly treatment than those treated PRN.⁶ This could mean that in patients with CNV lesions particularly susceptible to anti-VEGF treatment, sustained monthly treatment could result in excessive pruning of vessels, causing hypoxia and, subsequently, RPE and photoreceptor death.^{19, 20} Future OCTA studies will be important to validate our findings and confirm signs of CNV quiescence. These studies may allow clinicians to use OCTA as a marker of vessel stability and optimum anti-VEGF therapeutic dosage.

The authors are aware of limitations of this study including the limited number of participants. Previous treatment of study eyes with differing types and total number of anti-VEGF injections was a potential confounding factor. About one third of the patients included in this study (eight out of 25) had been treated at a different institution prior to first presentation, a scenario frequently encountered in clinical practice and often not accounted for in randomized clinical trials. Clinicians have to adapt to this circumstance and make decisions based on the “snapshot” obtained by clinical and OCT-examination at a single patient visit. The signal roll-off in the choroid of the spectral-domain based OCTA device used in our study was limited. Swept-source based OCTA machines operating at longer wavelengths could offer improved image quality and deeper penetration.

We believe that utilization of Angiotool to objectively and quantitatively evaluate CNV microvascular structure is an important strength of our study. The quantitative approach presented here illustrates a method to precisely assess even subtle differences, which might be challenging to incorporate in a solely qualitative analysis. Quantitative analysis of treatment-naïve CNV using this approach could help to identify benchmark values for active vs normalized vasculature in the future. Defining target lacunarity or junction density could be an objective way of following and managing patients with CNV. Another strength is that this study was designed to reflect a real life scenario, taking into account that most patients have already been started on anti-VEGF treatment.

In conclusion, we present a quantitative study of CNV microstructure based on OCTA imaging. There was no significant difference between eyes that required a short treatment interval and eyes that could be managed using longer treatment intervals. These results, reflecting a real-world scenario of study participants with a history of previous treatment, can be explained by vessel normalization, whereby individualized anti-VEGF treatment (regardless of treatment interval) achieved a “dynamic stability” between anti- and pro-

angiogenic factors. Given our study population, our findings do not rule out the possibility that treatment-naïve eyes may show significant baseline differences in microvascular patterns on OCTA that may be predictive of future treatment response. This is an important question that should be explored in future studies.

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Summary statement

In this study we used optical coherence tomographic angiography to quantitatively analyze microvascular characteristics of choroidal neovascularization with poor and good response to treatment.

Figure 1.

Optical coherence tomographic angiographic *en face* images showing the choriocapillaris slab (a), the inverted superficial retinal capillary plexus (b) and the artifact-removed choriocapillaris slab (c) after multiplication with the inverted superficial retinal capillary plexus. Removal of projection artifacts allows for better visualization and more accurate quantitative assessment of the neovascular component of the angiogram.

Figure 2.

En face optical coherence tomographic angiography (OCTA) slab of the choriocapillaris (a), the manually delineated choroidal neovascular lesion before (b) and after quantitative analysis (c). The vascular network is highlighted in green, automatically detected vessels are highlighted by red lines and vessel junctions are highlighted by blue spots in (c). The OCTA B-scan (d) shows a vascularized retinal pigment epithelial detachment with red spots indicating flow signal.

Figure 3.

En face optical coherence tomographic angiography (OCTA) slab of the choriocapillaris (a), the manually delineated choroidal neovascular lesion before (b) and after quantitative analysis (c). The vascular network is highlighted in green, automatically detected vessels are highlighted by red lines and vessel junctions are highlighted by blue spots in (c). The B-scan (d) shows a vascularized retinal pigment epithelial (RPE) detachment with red flow signal between Bruch's membrane and the RPE.

Table 1

Qualitative evaluation of the CNV lesions according to interval analysis two

	Interval analysis one			Interval analysis two		
	Good responders (n=7)	Poor responders (n=7)	p-value	Good responders (n=13)	Poor responders (n=12)	p-value
Shape (“seafan” or “lacy wheel”) detected (n)	3 (43%)	1 (14%)	0.237	5 (38%)	2 (17%)	0.225
Anastomoses and vessel loops (peripheral arcade) (n)	6 (86%)	6 (86%)	1.0	12 (92%)	10 (83%)	0.476
Branching (small capillaries)	1 (14%)	2 (29%)	0.515	3 (23%)	4 (33%)	0.568
Vessel termini (peripheral arcade)	4 (57%)	1 (14%)	0.094	6 (46%)	2 (17%)	0.114
Peri-lesion halo	3 (43%)	5 (71%)	0.280	7 (54%)	7 (58%)	0.821

Numbers of eyes presenting the respective qualitative findings for the good and poor responder groups. Interval analysis one: poor response = treatment every four weeks or more frequent, good response = treatment every 12 weeks or less frequent. Interval analysis two: poor response = treatment every six weeks or more frequent, good response = treatment less frequent than every six weeks. Pearson Chi-square test was used to assess significant differences between the two groups.

Table 2

Quantitative analyses (Interval analyses one and two) of the microvasculature in CNV lesions of good responders vs. poor responders.

	Interval analysis one			Interval analysis two		
	Good responders (n=7)	Poor responders (n=7)	p-value	Good responders (n=13)	Poor responders (n=12)	p-value
Lesion area (mm²)	1.32	1.00	0.805	1.32	1.66	0.728
Vessels area (mm²)	0.63	0.39	0.535	0.63	0.54	0.979
Vessel percentage area (%)	51.11	37.80	0.097	46.16	39.51	0.611
Number of junctions	85	48	0.456	85	64	0.936
Junctions density	0.000622	0.000440	0.383	0.000456	0.000451	0.894
Total vessel length (mm)	14.06	8.83	0.620	14.06	12.31	0.894
Average vessel length (mm)	2.19	1.39	0.209	1.42	1.20	0.894
Number of end points	40	38	0.805	40	63	0.611
Average lacunarity	0.20	0.22	0.259	0.20	0.23	0.650

Median quantitative features of the good responder and poor responder group and p-values calculated by the Mann-Whitney U test are presented. The vessel percentage area is the percentage of the total choroidal neovascular lesion showing flow signal in optical coherence tomographic angiography and can be derived from the lesion area and vessel area. Total vessel length is calculated as the sum of all vessels, whereby a vessel is defined as the distance between two junctions or between a junction and an endpoint. The junction density is calculated as the number of junctions normalized per unit area. Lacunarity is a measure of lesion inhomogeneity with higher values reflecting more inhomogenous vascular structure.