Novel Parameters to Assess the Severity of Corneal Neovascularization Using Anterior Segment Optical Coherence Tomography Angiography

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• PURPOSE: Assessment of anterior segment–optical coherence tomography angiography (AS-OCTA) to determine severity of corneal neovascularization (CoNV).

• DESIGN: Retrospective, cross-sectional, single-center study.

• METHODS: Patients of various CoNV etiologies were selected and classified into mild, moderate, and severe. Their AS-OCTA images were measured for CoNV anterior limit, CoNV posterior limit, CoNV thickness, CoNV depth%, CoNV vessel density, CoNV area, and CoNV volume. Further, AS-OCTA parameters were correlated to clinical parameters, such as classification, a numerical severity scale, vascular clock hours, and best-corrected visual acuity (BCVA).

• RESULTS: A total of 19 mild, 10 moderate, and 6 severe CoNV eyes were included with no significant age-gender differences. CoNV depth% and volume increased from mild to moderate (9.3 ± 1.1% to 17.7 ± 3.3%, $P = .030$, and 0.2 ± 0.1 mm^3 to 1.0 ± 0.3 mm^3, $P = .025$, respectively) and from moderate to severe CoNV (44.6 ± 5.3%, $P < .001$, and 2.0 ± 0.3 mm^3, $P = .014$, respectively). CoNV area and posterior limit increased from mild to moderate (1.7 ± 0.3 mm^2 to 4.6 ± 0.7 mm^2, $P = .001$, and 217.7 ± 16.8 μm to 349.1 ± 54.9 μm, $P = .048$, respectively), not from moderate to severe ($P = .999$ and $P = .403$, respectively). CoNV thickness increased from moderate to severe (218.2 ± 46.6 μm to 340.2 ± 8.7 μm, $P = .020$), but not from mild to moderate. CoNV area and volume showed good correlations to CoNV staging ($r = 0.703$ and $r = 0.771$, respectively; $P < .001$) and severity scale ($r = 0.794$ and $r = 0.712$, respectively; $P < .001$). CoNV area showed good correlation to clock hours ($r = 0.749$, $P < .001$). CoNV depth and volume showed good correlation to BCVA ($r = 0.744$ and $r = 0.722$, respectively; $P < .001$). CoNV anterior limit and vessel density showed no significant correlations ($P \geq .05$).

• CONCLUSIONS: Severe CoNV shows greater CoNV posterior limit, thickness, depth%, area, and volume on AS-OCTA compared to mild. CoNV volume and depth strongly correlate to BCVA. AS-OCTA provides novel, quantitative, and noninvasive parameters for assessing CoNV severity. (Am J Ophthalmol 2021;222: 206–217. © 2020 Elsevier Inc. All rights reserved.)

CORNEAL AVASCULARITY IS IMPORTANT FOR THE maintenance of corneal transparency that is critical for the preservation of vision.[1] Corneal neovascularization (CoNV) is one of the leading causes of blindness worldwide, with corneal transplantation being the primary treatment to restore vision in advanced cases.[2] CoNV can occur secondary to inflammation, infection, trauma, chemical burns, limbal stem cell deficiency, or iatrogenically, among others.[3] Topical corticosteroids[4]; immunomodulators, such as cyclosporine or tacrolimus[5]; and topical anti–vascular endothelial growth factors (VEGF)[6] have been proposed as treatment options for CoNV. However, not all patients respond to treatment, and particularly those with highly vascularized corneas and corneal scars ultimately require corneal transplantation to restore vision.[7]

To date, there are no widely accepted and standardized quantitative classifications for CoNV. Common parameters used with slit-lamp biomicroscopy are extension (number of quadrants or clock hours) and depth.[8–10] Quantitative parameters with color photography have been used to assess changes and treatment response in CoNV clinical trials with a high sensitivity and specificity.[11–14] However, the latter does not determine vessel depth and has several limitations, such as low resolution for vessel quantification, specifically for small fine vessels, imprecision of vessel border delineation with corneal scars, and the inability to assess blood flow.[15] Fluorescein and indocyanine green angiography have shown

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improved assessment of CoNV, with better resolution and vessel delineation, as well as additional advantages of blood flow direction and filling time.[15,16] Moreover, fluorescein angiography allows for quantification of vascular parameters and morphometric changes through computer-assisted image analysis that may help guide medical and surgical treatments.[15,16] However, the invasive nature of this imaging methodology with potential side effects, the inability to determine vessel depth, the time-consuming nature of this technique, and the requirement for a well-equipped and trained staff have made it challenging to implement this technology as a routine clinical tool for assessment of CoNV.[17,18]

Anterior segment–optical coherence tomography angiography (AS-OCTA) is a novel and rapidly growing imaging modality that allows for noninvasive detection of CoNV, comparable to invasive indocyanine green angiography.[19,20] AS-OCTA is capable of detecting vascular blood flow through the reflectivity and movement of red blood cells across sequential scans using low-coherence interferometry without the need for dyes. Therefore, high-density volumetric scans can be generated in a matter of seconds with a micrometer resolution of the tissue and its vasculature.[21] Nanji and associates have also shown the ability of this technology to precisely measure vessel depth in CoNV;[22] however, further studies are necessary to validate the utility of AS-OCTA in CoNV and identify the most accurate parameters, particularly how they relate to CoNV severity. Thus, we hypothesize that AS-OCTA can provide quantitative parameters for the assessment of CoNV severity. Hence, our aim was to explore novel AS-OCTA parameters and validate their correlation to clinical severity.

MATERIALS AND METHODS

• **STUDY DESIGN AND PATIENT POPULATION:** A cross-sectional, retrospective, observational study was performed on 35 eyes of 35 patients with CoNV at the Cornea Service of the New England Eye Center (NEEC), Tufts Medical Center, Boston, Massachusetts, between 2017 and 2019 seen for clinical care. This study was Health Insurance Portability and Accountability Act (HIPAA) compliant, adhered to the tenets of the Declaration of Helsinki, and was approved by the Institutional Review Board (IRB 13216)/Ethics Committee of our institution. A review of patients' clinical records with CoNV diagnosis was performed. Patients who had undergone AS-OCTA imaging for clinical care and monitoring purposes were included in the study. The exclusion criteria were ocular surgery in the 3 months prior to AS-OCTA imaging and excessive motion artifacts or poor image quality that did not allow corneal vessel visualization. Further, to accurately assess correlation of OCTA parameters to visual acuity, patients with a history of retinal detachment, moderate to severe or proliferative diabetic retinopathy, presence or under treatment for macular edema, age-related macular degeneration, or advanced glaucoma with profound visual field alterations were excluded.

In order to compare AS-OCTA to CoNV severity, subjects were classified into mild, moderate, and severe groups, based on previously published clinical findings.[8–10,23,24] Cornea specialists referenced superficial vessels as those within the anterior one-third of the cornea and deep vessels within the posterior two-thirds of the cornea (i.e., deep stroma). Therefore, superficial CoNV involving ≤2 quadrants (equivalent to ≤6 clock hours) without deep CoNV were staged as mild (Figure 1A). CoNV with >2 quadrants of superficial vessels (>6 clock hours) or 1 quadrant of deep vessels (≤3 clock hours) were staged as moderate (Figure 2A), and CoNV with >1 quadrant of deep vessels (>3 clock hours) as severe (Figure 3A). Nineteen mild CoNV, 10 moderate CoNV, and 6 severe CoNV eyes from a total of 32 patients were included and their AS-OCTA images analyzed. One patient had moderate CoNV bilaterally and 2 patients had moderate CoNV in one eye and mild CoNV in the other eye. Further, a scoring clinical CoNV severity grading scale was performed by 2 graders (W.W.B. and N.D.K.) according to the number of quadrants, depth, and extension of the vessels.[10] A score of 1 was given to each quadrant of CoNV involved (based on an overlaying grid shown in Supplemental Figure S1). Additionally, the vessel depth of each quadrant was assessed such that superficial vessels were given a score of 1 and deep vessel a 2 for each quadrant. Lastly, the vessel extension from the limbus was assessed, in which peripheral vessels were given a score of 1, midperipheral vessel a 2, and central vessels a 3. In quadrants with more than 1 vessel, the worst vessel was scored, that is, the deepest or furthest from the limbus. Therefore, the sum of the scores per quadrant provided a continuous scale of CoNV severity [3-24].[10] The best-corrected visual acuity (BCVA) and clock hours of CoNV were obtained from the medical records to correlate to OCTA parameters. Two patients were excluded from BCVA analysis; one case had a history of retinal detachment due to proliferative diabetic retinopathy and the other a history of advanced glaucoma with significant visual field defects. The BCVA was converted to logarithm of the minimum angle of resolution (logMAR) for a continuous variable analysis, and cases with counting fingers vision and hand motion were converted to a reliable logMAR equivalent based on previously published methods in order to differentiate severity of vision within this category, that is, counting fingers at 5 m, or at 3 m, or at 1 m or hand motion.[25,26] In short, the conversion takes into account the approximated mean distance between the index and middle fingers, based on a normative database, and the distance of the measurement of vision.

• **ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY:** The spectral domain OCTA system (Avanti XR AngioVue; Optovue, Inc, Fremont, California, USA) was adapted for anterior segment by coupling the

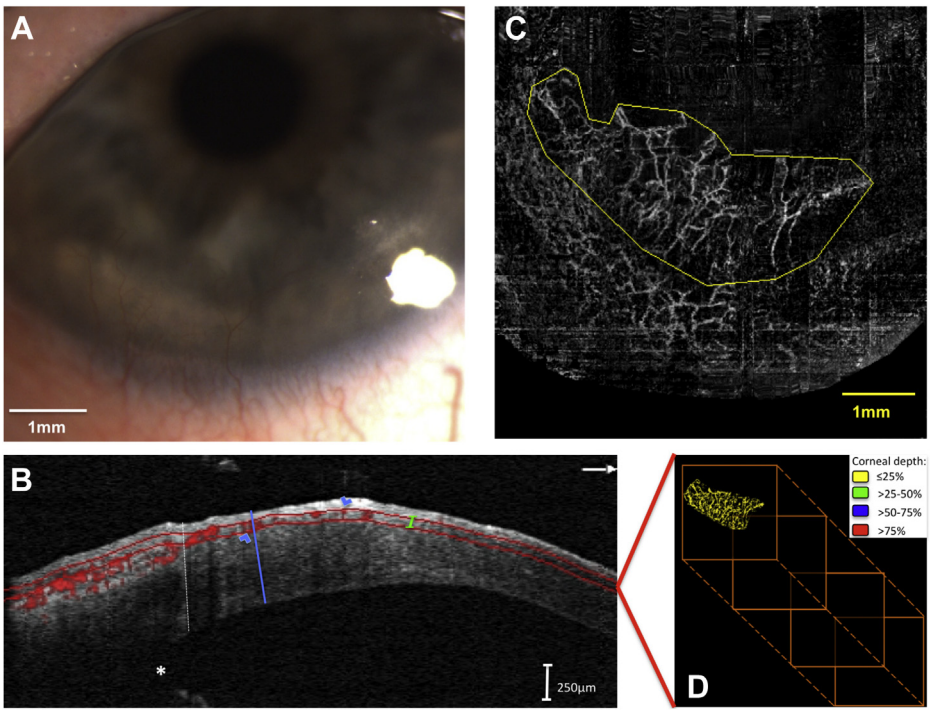

FIGURE 1. (A) Left eye slit-lamp photograph of mild CoNV patient. (B) AS-OCTA B-scan showing the sclera-cornea transition (white dashed line) and iris insertion (asterisk). Highlighted are the anterior CoNV limit (superior red line) from the most superficial vessel (superior blue arrowhead), the posterior CoNV limit (inferior red line) from the deepest corneal vessel (inferior blue arrowhead), the CoNV thickness (green line), and the corneal thickness (blue line). (C) AS-OCTA with outlined CoNV area. (D) Representation of CoNV volume at different corneal depths. AS-OCTA = anterior segment–optical coherence tomography angiography, CoNV = corneal neovascularization.

long cornea adaptive module lens and adjusting the focus settings on the 6 × 6-mm HD Retina scan. The system acquires volumetric scans of 400 × 400 A-scans at 70,000 A-scans per second, using a light source centered on 840 nm and a bandwidth of 45 nm. Blood flow is detected through the decorrelation signal generated from the red blood cell motion through consecutive A-scans of the same location, generating a volumetric blood flow analysis. The projection-resolved algorithm used in the OCTA system is designed to suppress the projection artifacts generated from the more superficial vessels, by comparing the flow signal intensity to a superficial layer in the same location.[27] The AS-OCTA scans allowed visualization of the transition between the hyperreflective sclera and hyporeflective cornea, and vessels that extended beyond this limit were determined CoNV. Scans centered on the area of CoNV were selected and, in cases of multiple quadrant involvement where AS-OCTA could not entirely capture, the best representative scan was selected. This occurred in 2 moderate CoNV eyes with peripheral vessel involvement (as seen in Figure 2C), whereas in cases with CoNV in the central or total cornea, AS-OCTA was able to capture the CoNV (as seen in Figure 3C).

• **IMAGE ANALYSIS:** Measurements were performed by 2 masked graders (W.W.B. and N.D.K.) on de-identified images. In addition, all measurements were repeated by the same grader (W.W.B.), under the same conditions, at a different time point in order to assess the intragrader repeatability. The AS-OCTA images were measured as previously described.[28] In brief, the limbus was defined by the corneal-scleral transition, and vessels beyond the transition were analyzed. The depth limits of the CoNV (µm) were determined on the OCT device, and the corneal epithelial apex (cornea surface) was set as the zero reference. Then, the following parameters were measured: *Anterior CoNV Limit (µm)* was defined as the distance between the most superficial CoNV flow signal observed from the cornea surface (Figures 1B, 2B, and 3B) and represents the depth of the superficial vessels of CoNV. *Posterior CoNV Limit (µm)* was measured as the posterior border of the deepest CoNV flow on AS-OCTA from the surface (Figures 1B, 2B, and 3B), representing the maximum corneal depth of the CoNV vessels. *CoNV thickness (µm)* was determined as the distance between the anterior border of the most superficial CoNV and the posterior border of the deepest CoNV, representing the intracorneal maximum thickness of the CoNV (Figures 1B, 2B, and 3B). *CoNV depth%* was assessed on cross-sectional AS-OCT images, with the corneal thickness (µm) initially measured at the deepest CoNV

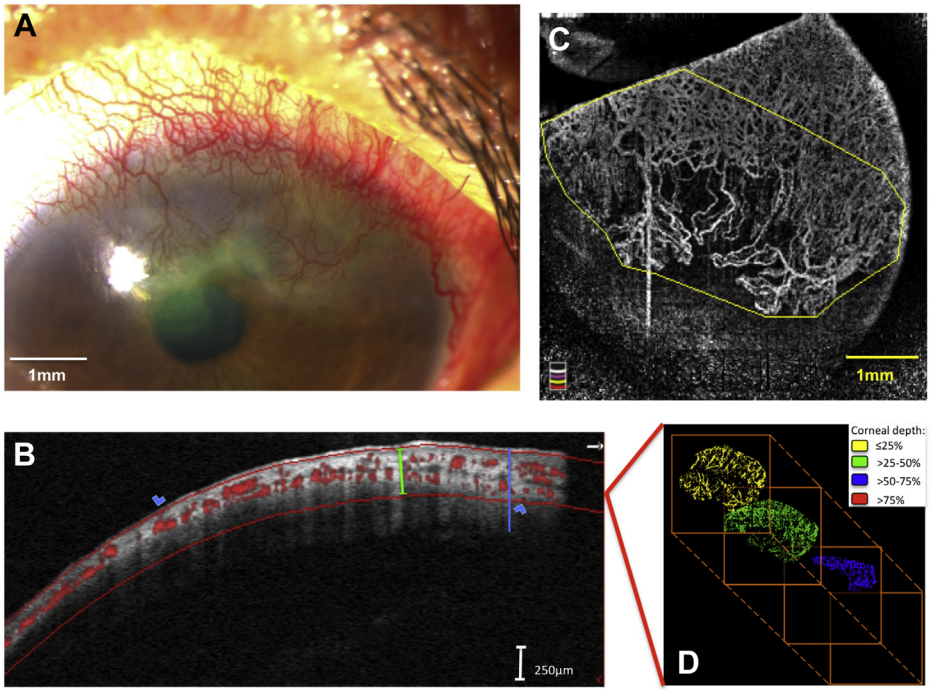

FIGURE 2. (A) Left eye slit-lamp photograph of moderate CoNV patient. (B) AS-OCTA B-scan showing the anterior CoNV limit (superior red line) from the most superficial vessel (superior blue arrowhead), the posterior CoNV limit (inferior red line) from the deepest corneal vessel (inferior blue arrowhead), the CoNV thickness (green line), and the corneal thickness (blue line). (C) AS-OCTA with outlined CoNV area. (D) Representation of CoNV volume at different corneal depths. AS-OCTA = anterior segment–optical coherence tomography angiography, CoNV = corneal neovascularization.

location. Then, the CoNV depth was established as the ratio between CoNV thickness and total corneal thickness (Figures 1B, 2B, and 3B), which represents the percentage of the corneal thickness invaded by the CoNV and accounts for the differences in corneal thickness of each patient. *CoNV area (mm^2)* was the area involved by CoNV on AS-OCTA exported images, as demonstrated in Figures 1C, 2C, and 3C, with the pixel ratio converted to millimeters based on the device specifications. The CoNV area of each image was calculated using open source FIJI software.[29] The CoNV area represented the CoNV extension onto the cornea.

Next, the images were binarized based on previously described thresholding methods.[30–32] Initially, the gray value of the corneal surface noise was subtracted from the subsequent depth scans to better isolate the CoNV flow. Then, each image was converted into a binary image using a 3-step combined process where a low global threshold was applied based on the mean gray value. Next, a morphologic closing filter was applied to connect bright particles (flow) separated by a <2-pixel radius followed by automated binarization using the Otsu local thresholding method as previously described.[33] *CoNV volume (mm^3)* was determined as the pixels representing blood flow within the previously selected CoNV area multiplied by the depth of the CoNV, as shown in Figures 1D, 2D, and 3D. The CoNV volume is a 3-dimensional parameter that accounted for the intracorneal invasion (depth) and extension. Moreover, the vessel density (%) was calculated as the ratio of pixels representing blood flow on the binarized image to the total pixels within the CoNV area, as previously determined. The vessel density represented the number of capillaries within the CoNV.

It is well known that central corneal involvement of CoNV is correlated to decreased visual acuity.[10] In order to further assess if central corneal involvement affected BCVA, eyes were stratified into peripheral CoNV, without central 3-mm-diameter involvement, and central CoNV, with central 3-mm-diameter corneal involvement, and correlated to BCVA. A total of 27 eyes of 27 patients and 8 eyes from 8 patients had peripheral and central CoNV, respectively. Moreover, to further assess if the degree of CoNV within the central cornea affected the vision, the aforementioned CoNV parameters within the central 3-mm-diameter were measured and correlated to BCVA.

• **STATISTICAL ANALYSIS:** Descriptive statistics were calculated for all parameters between the groups and values were reported as the mean ± standard error of mean. To assess for normality of distribution of the population in each parameter, Shapiro-Wilk tests were performed. In order to account for bilateral eye inclusion, generalized

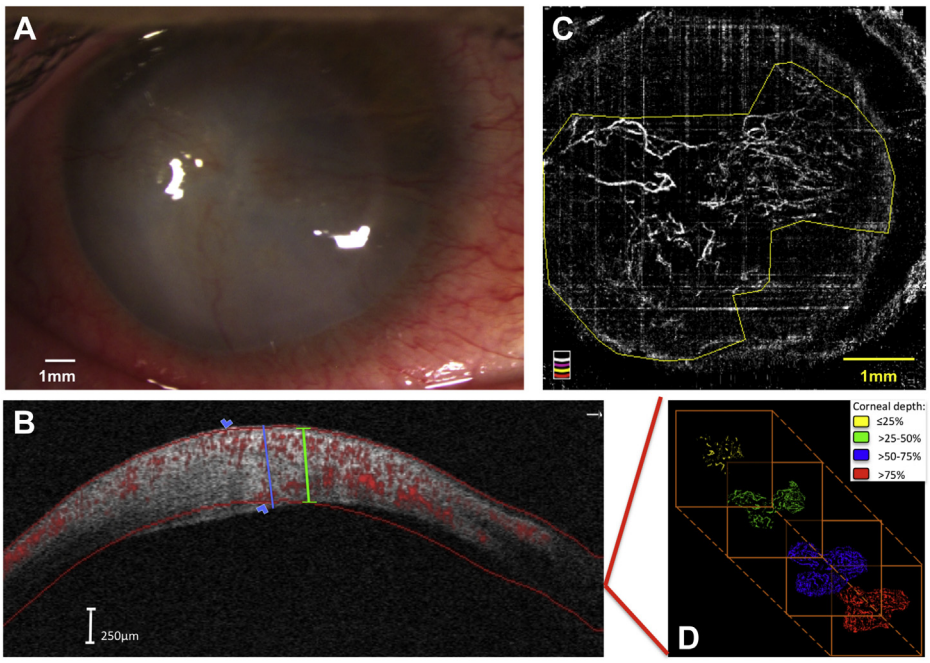

FIGURE 3. **(A) Left eye slit-lamp photograph of severe CoNV patient. (B) AS-OCTA B-scan showing the anterior CoNV limit (superior red line) from the most superficial vessel (superior blue arrowhead), the posterior CoNV limit (inferior red line) from the deepest corneal vessel (inferior blue arrowhead), the CoNV thickness (green line), and the corneal thickness (blue line). (C) AS-OCTA with outlined CoNV area. (D) Representation of CoNV volume at different corneal depths. AS-OCTA = anterior segment–optical coherence tomography angiography, CoNV = corneal neovascularization.**

estimated equations with Bonferroni corrections were performed to analyze differences of the AS-OCTA parameters, that is, anterior and posterior CoNV limits, CoNV thickness, CoNV depth, CoNV area, CoNV volume, CoNV vessel density, the age, the BCVA, and the clinical clock hours between CoNV stages. Chi-square tests were performed to assess gender distribution between groups. Mann-Whitney *U* tests were performed to determine differences in BCVA in patients with central CoNV and patients with peripheral CoNV.

Spearman correlations were used to assess correlations between AS-OCTA parameters and CoNV stages. Pearson correlations were used to compare AS-OCTA parameters with the continuous CoNV severity scale, the clinical clock hours, and the BCVA, respectively. Pearson correlations were also used to compare the central CoNV AS-OCTA parameters with BCVA. The intra- and interclass correlation coefficient of the parameters with their 95% confidence interval were performed in order to assess the intra- and intergrader repeatability, respectively. Further, Bland-Altman plots with their 95% limits of agreement were performed and 1-sample Wilcoxon signed rank tests were performed for the differences between the observers' measurements. Lastly, an intraclass correlation coefficient was performed to assess agreement between graders for the CoNV severity scores. A *P* value of less than .05 was considered statistically significant.

RESULTS

• **DEMOGRAPHICS:** The age, gender distribution, BCVA, clock hours, and CoNV severity scores between groups are shown in Table 1. There was no statistically significant difference in the age and gender distribution between groups ($P = .246$ and $P = .090$, respectively). Not surprisingly, there was a significant progressive increase in CoNV severity scores, clinical clock hours, and BCVA (logMAR) with increased severity in CoNV staging (all *P* values $<.001$). The specific etiologic distribution between groups is shown in Table 2. The most common CoNV category was limbal stem cell deficiency, representing a total of 19 cases (54%), followed by infectious causes, with a total of 14 cases (40%).

• **AS-OCTA PARAMETERS IN CORNEAL NEOVASCULARIZATION:** The AS-OCTA parameters are shown in Table 3 and their pairwise comparisons in Figure 4. The severe CoNV group showed greater CoNV posterior limit (430.8 ± 18.5 µm, $P < .001$), CoNV thickness (340.2 ± 8.7 µm, $P < .001$), CoNV depth% ($44.6 \pm 5.3\%$, $P < .001$), CoNV area (5.5 ± 0.7 mm^2, $P < .001$), and CoNV volume (2.0 ± 0.3 mm^3, $P < .001$) when compared to mild CoNV (217.7 ± 16.8 µm, 135.4 ± 15.7 µm, $9.3\% \pm 1.1\%$, 1.7 ± 0.3 mm^2, and 0.2 ± 0.1 mm^3, respectively). The severe CoNV group also showed greater CoNV

TABLE 1. Demographics of Patients With Corneal Neovascularization

	Mild Stage	Moderate Stage	Severe Stage	*P* Value
Age, y	56.7 ± 4.6	48.5 ± 6.1	47.8 ± 5.0	.246[a]
Gender: males/females, n	6/13	7/3	4/2	.090[b]
CoNV Severity Scale [3-24]	**3.2 ± 0.2**	**11.5 ± 1.7**	**17 ± 1.9**	**<.001**[a]
Clock hours	**2.1 ± 0.3**	**7.5 ± 1.2**	**9.3 ± 1.5**	**<.001**[a]
BCVA, logMAR	**0.15 ± 0.04**	**0.50 ± 0.12**	**1.97 ± 0.28**	**<.001**[a]
Total n	19	10	6	

BCVA = best-corrected visual acuity, CoNV = corneal neovascularization. $P < .01$ in bold.
[a]Generalized estimated equations with Bonferroni correction.
[b]Chi-Square

TABLE 2. Etiologic Distributions of Corneal Neovascularization

Etiologies	Mild, n (%)	Moderate, n (%)	Severe, n (%)	Total, n (%)
Infectious				14 (40)
Viral keratitis	6 (32)	2 (20)	1 (17)	
Bacterial keratitis	—	1 (10)	1 (17)	
Fungal keratitis	1 (5)	—	—	
Parasitic keratitis	—	—	2 (33)	
Neurotrophic keratopathy	1 (5)	1 (10)		2 (6)
Limbal stem cell deficiency				19 (54)
Allergic keratoconjunctivitis	3 (16)	2 (20)	—	
Dry eye disease	3 (16)	—	—	
Stevens-Johnson syndrome	—	1 (10)	2 (33.0)	
Contact lens wear	4 (21)	2 (20)	—	
Idiopathic	—	1 (10)	—	
Iatrogenic	1 (5)	—	—	
Total eyes, n	19 (100)	10 (100)	6 (100)	35 (100)

thickness ($P = .020$), CoNV depth% ($P < .001$), and CoNV volume ($P = .014$) when compared to moderate CoNV (218.2 ± 46.6 μm, 17.7 ± 3.3%, and 4.6 ± 0.7 mm^3, respectively). The moderate CoNV group showed a greater CoNV posterior limit (349.1 ± 54.9 μm, $P = .048$), CoNV depth% (17.7 ± 3.3%, $P = .030$), CoNV area (4.6 ± 0.7 mm^2, $P = .001$), and CoNV volume (1.0 ± 0.3 mm^3, $P = .025$) compared to the mild CoNV group. Therefore, both the CoNV depth% and CoNV volume showed significant increase with increased severity. In the moderate CoNV group, AS-OCTA showed increased CoNV area CoNV volume, CoNV depth%, and CoNV posterior limit, but not CoNV thickness, when compared to mild stage. In contrast, in the severe CoNV group, AS-OCTA showed increased CoNV depth%, CoNV volume, and CoNV thickness, but not CoNV area and CoNV posterior limit, compared with the moderate CoNV group. In comparison to CoNV posterior limit and CoNV thickness, the CoNV depth% better represented the CoNV stages by accounting for the individual corneal thickness. The CoNV anterior limit and vessel density showed no statistical significance between CoNV groups ($P \geq .05$).

When stratifying by etiology, the herpetic keratitis patients (8 herpes simplex cases and 1 herpes zoster in the early group) showed significantly deeper anterior CoNV limit when compared to nonherpetic cases (171.4 ± 139.5 μm and 77.2 ± 24.2 μm, respectively; $P = .042$). The herpetic keratitis cases showed no significant differences of the posterior CoNV limit (315.6 ± 185.9 μm, $P = .893$), CoNV thickness (143.6 ± 82.4 μm, $P = .166$), CoNV depth% (13.3 ± 12.4%, $P = .406$), corneal thickness (749.3 ± 25.6 μm, $P = .428$), CoNV area (3.192 ± 1.740 mm^2, $P = .787$), CoNV volume (0.844 ± 1.064 mm^3, $P = .589$), and CoNV vessel density (30.9% ± 5.5%, $P = .368$). Therefore, CoNV seems to originate deeper in the cornea (ie, anterior stroma) in herpetic keratitis cases.

• **CORRELATIONS TO VISUAL ACUITY:** The correlations between AS-OCTA parameters and CoNV staging,

TABLE 3. AS-OCTA Parameters in Patients With Corneal Neovascularization

Parameter	Mild Stage	Moderate Stage	Severe Stage	*P* Value
CoNV area, mm^2	**1.7 ± 0.3**	**4.6 ± 0.7**	**5.5 ± 0.7**	**<.001**[a]
CoNV volume, mm^3	**0.2 ± 0.1**	**1.0 ± 0.3**	**2.0 ± 0.3**	**<.001**[a]
Anterior CoNV limit, μm	82.0 ± 6.2	130.4 ± 43.7	90.6 ± 19.0	.471[a]
Posterior CoNV limit, μm	**217.7 ± 16.8**	**349.1 ± 54.9**	**430.8 ± 18.5**	**<.001**[a]
CoNV thickness, μm	**135.4 ± 15.7**	**218.2 ± 46.6**	**340.2 ± 8.7**	**<.001**[a]
CoNV depth, %	**9.3 ± 1.1**	**17.7 ± 3.3**	**44.6 ± 5.3**	**<.001**[a]
CoNV vessel density, %	31.8 ± 6.1	30.5 ± 4.8	29.1 ± 4.0	.458[a]
Total n	19	10	6	

AS-OCTA = anterior segment–optical coherence tomography angiography, CoNV = corneal neovascularization. $P < .01$ in bold.
[a]Generalized estimated equations with Bonferroni correction.

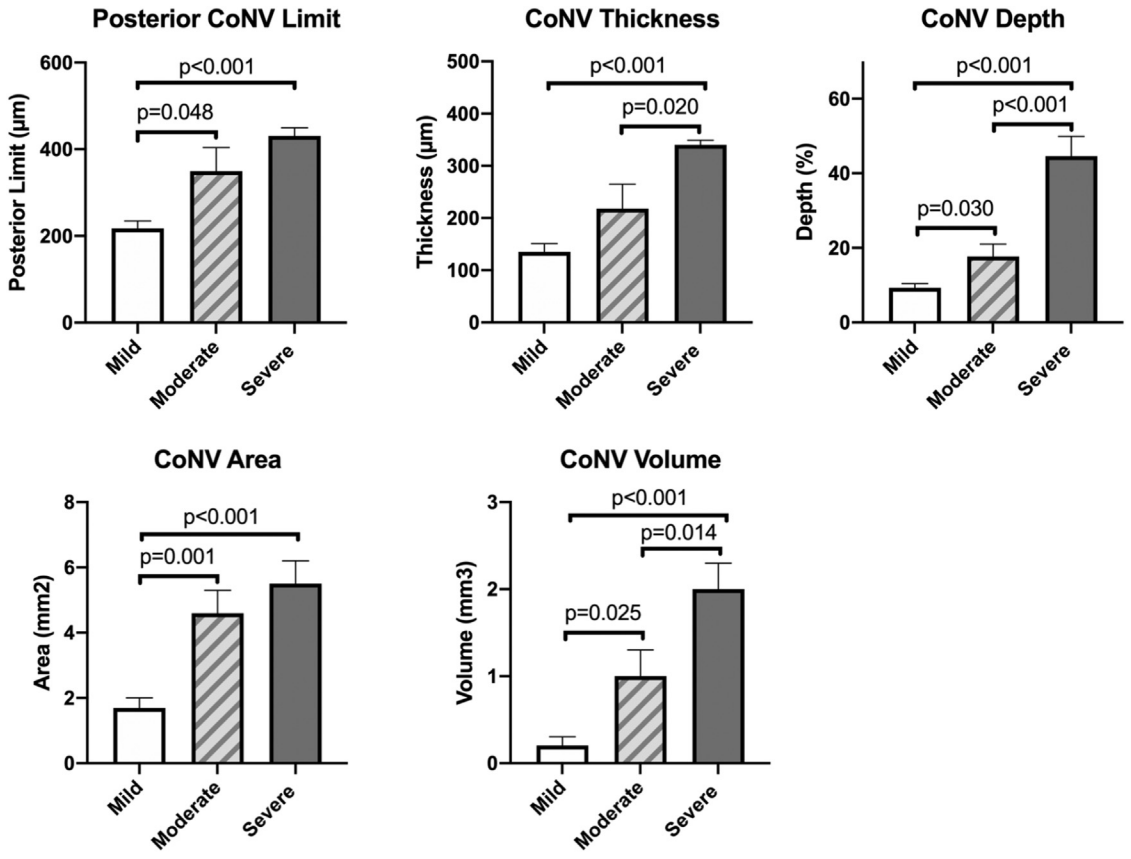

FIGURE 4. Pairwise comparisons with Bonferroni corrections. CoNV = corneal neovascularization.

severity scale, clock hours, and BCVA are shown in Table 4. For the CoNV stages, CoNV volume showed the strongest correlation ($r = 0.771$, $P < .001$), followed by CoNV area ($r = 0.703$, $P < .001$). For the CoNV severity scale, the CoNV area showed the strongest correlation ($r = 0.794$, $P < .001$), followed by the CoNV volume ($r = 0.712$, $P < .001$). In addition, the CoNV clock hours showed the strongest correlation with CoNV area ($r = 0.749$, $P < .001$), followed by CoNV volume ($r = 0.580$, $P = .001$). Lastly, both the CoNV depth% and CoNV volume showed a strong correlation with BCVA ($r = 0.744$ and $r = 0.722$, respectively; $P < .001$). The CoNV anterior limit and vessel density showed no significant correlations to CoNV staging,

TABLE 4. Correlations of AS-OCTA Parameters With Clinical Findings

Clinical Findings	CoNV Area	CoNV Volume	CoNV Depth%	CoNV Thickness	Posterior CoNV Limit	BCVA, logMAR
CoNV stages[a]	0.703	0.771	0.699	0.627	0.592	0.797
P value	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CoNV severity scale	0.794	0.712	0.666	0.481	0.352	0.737
P value	**<.001**	**<.001**	**<.001**	**.005**	**.045**	**<.001**
Vascular clock hours	0.749	0.580	0.499	0.385	0.160	0.500
P value	**<.001**	**.001**	**.006**	**.039**	.407	**.007**
BCVA, logMAR	0.598	0.722	0.744	0.520	0.558	—
P value	**<.001**	**<.001**	**<.001**	**.002**	**.001**	—

AS-OCTA = anterior segment–optical coherence tomography angiography, BCVA = best-corrected visual acuity, CoNV = corneal neovascularization. $P < .05$ in bold.

[a]Spearman correlations.

severity scale, clock hours, or BCVA. Furthermore, the presence of central CoNV resulted in a significant decrease in BCVA compared to peripheral CoNV (logMAR 1.64 ± 0.81 and 0.24 ± 0.27, respectively; $P < .001$). However, the AS-OCTA parameters within the 3-mm diameter of the central cornea showed no significant correlations with BCVA ($P \geq .05$), as shown in Supplementary Table S1. Put differently, the presence of central CoNV significantly affected the vision, but was not further affected by the degree of CoNV within the central cornea.

• **REPEATABILITY AND REPRODUCIBILITY OF AS-OCTA PARAMETERS:** The anterior and posterior CoNV limit, the CoNV thickness, corneal thickness, and the CoNV depth% showed excellent intragrader repeatability of 0.993 (95% CI: 0.986-0.997), 0.995 (95% CI: 0.989-0.997), 0.993 (95% CI: 0.986-0.997), 0.970 (95% CI: 0.939-0.985), and 0.995 (95% CI: 0.989-0.997), respectively. The same parameters showed excellent intergrader reproducibility of 0.981 (95% CI: 0.961-0.991), 0.982 (95% CI: 0.964-0.991), 0.971 (95% CI: 0.942-0.985), 0.944 (95% CI: 0.890-0.972), and 0.981 (95% CI: 0.961-0.991), respectively. The area selected for the CoNV also showed excellent intragrader repeatability (0.996, 95% CI: 0.992-0.998) and intergrader reproducibility (0.985, 95% CI: 0.970-0.993) with no significant differences between them ($P = .916$ and $P = .273$, respectively); therefore, the selected area from one grader was used for the CoNV area, volume, and vessel density measurements. There was no significant bias for all measurements, including grader areas, and the Bland-Altman plots of the intra- and intergrader repeatability showed high agreement and low variability of measurements within the 95% limits of agreement as demonstrated in Supplemental Figures S2 and S3, respectively. Lastly, there was an excellent agreement between graders (0.916, 95% CI 0.807-0.965) for the CoNV severity scale.

DISCUSSION

CONV ASSESSMENT IS IMPORTANT TO NOT ONLY DETERmine severity of the underlying disease, but also for management and treatment response. Initial studies by Ang and associates have shown that OCTA adapted for the anterior segment is capable of imaging CoNV is comparable to indocyanine green angiography.[19,20] A more recent study by Nanji and associates further explored the 3-dimensional feature of AS-OCTA, by assessing the depth of vessels in the cornea, which correlated with the subjective depth perceived by the clinician.[22] Herein, we expand the application of AS-OCTA in assessing CoNV and present novel and specific objective parameters for the assessment of CoNV, particularly CoNV area and volume. In addition to detailed analytical validation,[34] including the intra- and intergrader repeatability of each AS-OCTA CoNV parameter, we provide biological validation of the AS-OCTA parameters by correlating the OCTA parameters to several clinical staging parameters and to visual acuity in order to determine CoNV severity.

Our work shows that from mild to moderate CoNV stages, there is a greater increase in AS-OCTA parameters that represent CoNV extension (CoNV area) and to a lesser degree intracorneal invasion (CoNV posterior limit, CoNV depth%, CoNV volume) but not the maximum CoNV thickness. In contrast, from moderate to severe CoNV stages, intracorneal invasion parameters (i.e., CoNV depth%, CoNV volume, and CoNV thickness) are increased with no significant difference in extension (CoNV area). Therefore, our study highlights that both CoNV extension and intracorneal invasion play important

roles in the severity of CoNV and should be analyzed in conjunction, when assessing patients with CoNV. Future prospective, multicenter studies are warranted to further validate the AS-OCTA CoNV parameters proposed herein. Because the diagnosis of CoNV can be assessed through slit-lamp biomicroscopy, the role of AS-OCTA as a sole diagnostic tool may be limited in this context. However, given the unique capacity of generating high-resolution volumetric scans noninvasively, which was not possible with previous modalities, we believe AS-OCTA is potentially useful for disease monitoring, assessing prognosis of corneal transplant rejection, managing treatment, and measuring therapeutic efficacy to treatment modalities.

Overall, this study shows that CoNV area, CoNV volume, CoNV depth%, CoNV thickness, and CoNV posterior limit correlate well to clinical classifications. By analyzing the volumetric scans, we were able to determine the CoNV volume and depth%, which correlate strongly with visual acuity. The latter also shows a good correlation with both the CoNV clinical classification and severity scale. Further, for the clinical assessment in which the CoNV extension is mostly accounted for, such as vascular clock hours and the CoNV severity scale, the AS-OCTA CoNV area parameter shows the strongest correlation. Interestingly, when assessing severity only by clinical CoNV extension (clock hours), the correlation to BCVA was moderate ($r < 0.600$). The BCVA showed good correlation to CoNV stages ($r = 0.797$) and similar correlations to AS-OCTA CoNV volume and depth% ($r = 0.722$ and $r = 0.744$, respectively). The advantage of the latter is their capability of providing a continuous quantitative measure of CoNV severity, rather than a 3-category classification system. Moreover, AS-OCTA CoNV depth shows a stronger correlation to BCVA than the continuous clinical severity scale, further highlighting its clinical application. Although the aforementioned AS-OCTA parameters were useful to stage CoNV severity, their implications on the BCVA after treatment warrants future prospective studies. Furthermore, it is important to highlight that the vessel density reported herein directly reflected the capillary density within the CoNV area, which was however not associated with the staging or severity of the disease. This measurement is not comparable to other studies that measured vessel density over the entire cornea with digitalized slit-lamp photographs, which is currently not technically feasible with OCTA.[11–14] Therefore, future studies combining images of the entire cornea are necessary to directly compare vessel density to the previous studies.

CoNV occurs as a result of an imbalance of pro- and antiangiogenic factors in the cornea, leading to pathologic invasion of the pericorneal vessels into the cornea.[35,36] There is evidence suggesting that proangiogenic factors, which are mainly expressed in the epithelium in normal corneas, may diffuse into the stroma, depending on the severity of injury, subsequently initiating an inflammatory cascade that triggers angiogenesis.[37] Likewise, limbal stem cells and their niche have been shown to play an important role in maintaining the antiangiogenic balance of the cornea.[38,39] Therefore, damage to the niche, either by inflammatory causes or by chemical or mechanical traumas, can disrupt the angiogenic barrier, which explains why CoNV is an important hallmark in limbal stem cell deficiency.[40] Conversely, severe inflammation, such as during acute infectious keratitis, or untreated chronic inflammation, such as with allergic keratoconjunctivitis or contact lens wear, are known risk factors for disruption of the corneal homeostasis and can consequently recruit deeper stromal neovessels into the cornea.[37] In a recent study by our group on AS-OCTA assessing LSCD patients, CoNV depth showed a strong correlation to BCVA.[28] Similarly, in this study, severe CoNV shows deeper corneal vessel infiltration with greater CoNV depth% and CoNV volume, compared with both mild and moderate CoNV stages. In addition, the latter strongly correlates to BCVA, suggesting that the degree of CoNV invasion corresponds to worse visual outcomes. The results of this study thus support the association of deeper CoNV intracorneal invasion with the severity of CoNV, regardless of the cause.

In herpes simplex keratitis, the presence of CoNV alone can be a sign of disease severity and viral replication.[41] Conversely, recent animal experiments indicate that ocular herpes simplex virus type 1 can induce corneal angiogenesis and lymphangiogenesis, even after resolution of acute disease.[42] Postinfectious continuous production of proinflammatory cytokines and angiogenic factors by keratinocytes, macrophages, or polymorphonuclear cells of the stromal cornea has been demonstrated to contribute to the development of CoNV.[42,43] Interestingly, our study showed that in the herpetic CoNV cases, the anterior limit was deeper than nonherpetic cases at the level of the anterior stroma. Additionally, 2 cases in this group with a diagnosis of herpetic keratitis with intact epithelium and no acute inflammation presented with only deep stromal neovessels (CoNV anterior limit = 370.0 ± 45.2 μm). Therefore, although CoNV alone is unlikely to provide an etiologic diagnosis given that it is a feature common to many diseases, it can shed some light on different pathophysiological characteristics of CoNV, such as those highlighted in herpetic cases. Particularly in the aforementioned disease, AS-OCTA may serve as an important monitoring tool for CoNV because progression is often asymptomatic and detected late, when outcomes are worst and visual axis is involved. Furthermore, it is important to highlight that AS-OCTA is not able to detect ghost vessels, identifying only active CoNV with blood flow. This should be taken into account in cases with regression and inactive CoNV, where ghost vessels are more prevalent.

In corneal transplantation, the overall extension of CoNV and the number of deep stromal vessels in the host bed are risk factors for graft rejection and reports show a decreased graft survival at 4 years with each additional CoNV quadrant involved, or with the number of

vessels that invade the cornea beyond the graft-host junction.[9,23,44–46] In fact, Hill has reported that increased CoNV actually decreases the chance of reverting a corneal graft rejection.[47] In this context, AS-OCTA offers a unique noninvasive method to assess the severity of CoNV as it can precisely determine the vessel depth and could potentially be used as a preoperative predictor in corneal transplantation. Our study suggests that CoNV volume and the CoNV depth%, which provide quantitative measures for the host bed vascularization, are possible candidates for outcome measures in corneal transplantation, because they show significant differences in all CoNV stages with the highest correlations to BCVA. Another advantage is the noninvasive nature that allows consecutive scans during follow-up to monitor CoNV progression.[48] Moreover, isolated treatment of CoNV with anti-VEGF or immunomodulatory therapies or fine-needle thermal cauterization of CoNV has not shown significant long-term BCVA improvement with treatment-related reduction of CoNV.[49] However, the latter have been proposed as neoadjuvant treatment to increase the chances for corneal graft survival in high-risk corneal transplantation.[12,13,24] A recent study suggests increased graft survival rates in high-risk corneal transplants using a combined preoperative angiostatic treatments with fine-needle thermal cauterization and subconjunctival bevacizumab injection.[50] In such cases, AS-OCTA CoNV parameters are promising to assess treatment response in angiostatic therapies and help guide location of cauterization. Future prospective studies are warranted to determine their role as such.

The main limitation of this study is its inherent retrospective nature and relatively small number of subjects in each subcategory. AS-OCTA is a novel and emerging technique for CoNV, where its technological development specifically for the anterior segment will facilitate further application in larger prospective studies to confirm these findings. Nevertheless, to our knowledge, this study provides the largest comparison of AS-OCTA with CoNV severity to date and important bases for necessary future studies to establish the proposed CoNV parameters and better understand their exact role for clinical practice. Another limitation is the limited field of view (6-mm frame) that requires combined scans in large CoNV involving more than 2 quadrants of the cornea (>6 clock hours). However, even in such cases, the 6 × 6-mm-frame AS-OCTA was able to capture the majority of the CoNV in the central cornea within a single scan. Nevertheless, further technological advances for AS-OCT devices will allow automated combined imaging or larger frame scan acquisitions with high resolution and fast acquisition time to help establish AS-OCTA parameters as endpoints for clinical trials.[51] One clear advantage of AS-OCTA over slit-lamp photography is the higher resolution and capability of detecting capillaries even within opaque corneas, as highlighted in other studies.[19,20,22] Further, fluorescent dye angiography to assess CoNV was not available in this study for comparison to the AS-OCTA findings. Even though AS-OCTA has reported lower precision in detecting smaller vessels in CoNV compared to indocyanine green angiography,[52] AS-OCTA has a micrometer resolution with the advantage of being noninvasive, without the advent of dye leakage, and can additionally provide the precise depth of the vessel in the cornea. Therefore, additional prospective studies comparing AS-OCTA to fluorescent dye angiography and to digitalized slit-lamp photography are warranted to confirm these novel parameters for CoNV assessment.

In conclusion, CoNV volume and CoNV depth% increase gradually from mild to moderate to severe CoNV stages, as measured by AS-OCTA. Conversely, CoNV area increases only from mild to moderate CoNV stages, and CoNV maximum thickness increases only from moderate to severe CoNV stages. Furthermore, CoNV volume and CoNV area correlate strongly with CoNV clinical staging, whereas CoNV depth% and CoNV volume show the strongest correlation to BCVA. Thus, AS-OCTA is a promising, novel, quantitative, and noninvasive tool to assess CoNV severity in the clinical setting.

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