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Optical Coherence Tomography Angiography for Anterior Segment Vasculature Imaging

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Purpose: To evaluate the application of an optical coherence tomography angiography (OCTA) system adapted for the assessment of anterior segment vasculature.

Design: Cross-sectional, observational study.

Participants: Consecutive subjects with normal eyes on slit-lamp clinical examination and patients with abnormal corneal neovascularization.

Methods: All scans were performed using a commercially available AngioVue OCTA system (Optovue, Inc., Fremont, CA) using an anterior segment lens adapter and the split-spectrum amplitude decorrelation angiography algorithm. Each subject underwent scans from 4 quadrants (superior, inferior, nasal, and temporal) in each eye by 2 trained, independent operators.

Main Outcome Measures: Analysis of signal strength, image quality, and reproducibility of corneal vascular measurements was performed.

Results: In our study of 20 normal subjects (10 men, 10 women; mean age, 25.3±7.8 years), we found good repeatability (κ coefficient, 0.76) for image quality score and good interobserver agreement for vasculature measurements (intraclass coefficient, 0.94). After optimization of the angiography scan protocol, vascular measurements within the regions of interest were compared in the superior versus inferior quadrants (mean vascular loops, 3.34±1.16 vs. 3.12 ± 0.90 [$P = 0.768$]; segment-to-loop ratio, 4.18±0.71 vs. 4.32±0.87 [$P = 0.129$]; fractal dimension [D_f] value, 1.78±0.06 vs. 1.78±0.06 [$P = 0.94$]; vascular loop area, 25.9±14.5 vs. 25.9±10.7 × 10^{-3} mm^2 [$P = 0.21$]) and nasal versus temporal quadrant (mean vascular loops, 2.89±0.98 vs. 3.57±0.99 [$P < 0.001$]; segment-to-loop ratio, 3.94±0.69 vs. 4.55±0.78 [$P = 0.897$]; D_f value, 1.78±0.06 vs. 1.77±0.06 [$P = 0.14$]; vascular loop area, 29.7±15.7 vs. 22.1±7.1 × 10^{-3} mm^2 [$P = 0.38$]. We then used the established OCTA scanning protocol to visualize abnormal vasculature successfully in 5 patients with various corneal pathologic features, including graft-associated neovascularization, postherpetic keratitis scarring, lipid keratopathy, and limbal stem cell deficiency.

Conclusions: This preliminary study describes a method for acquiring OCTA images of the cornea and limbal vasculature with substantial consistency. This technique may be useful for the objective evaluation of corneal neovascularization in the future. *Ophthalmology 2015;■:1–8 © 2015 by the American Academy of Ophthalmology.*

Since its first in vivo use for the retina, optical coherence tomography (OCT) imaging has revolutionized our ability to evaluate the eye and its structures on a microscopic level.[1] It also has been established as a useful tool in providing rapid, noncontact evaluation of the cornea and anterior segment.[2,3] Further technological developments also have increased the imaging capabilities of OCT in terms of speed, image resolution, and, more recently, evaluation of vascular flow.[4] These new OCT imaging techniques have been described for noninvasive evaluation of vessels within the retina and optic disc.[4,5] As opposed to Doppler OCT techniques, which depend on axial flow of blood, OCT angiography (OCTA) techniques visualize vessels via motion contrast imaging of erythrocyte movement across sequential B-scans.[4]

Currently, assessment of the corneal and anterior segment vasculature is constrained to slit-lamp photography or angiography techniques using fluorescein or indocyanine green.[6] Semiautomatic quantitative techniques to analyze photographic images have been described,[7] with standardized methods of quantification of corneal neovascularization established and used in several clinical trials.[8–10] However, underestimation of poorly visible blood vessels may occur, especially in the presence of dense corneal scars,[11] whereas invasive angiography techniques expose patients to potential adverse reactions.[6] Thus, methods of evaluating abnormal corneal neovascularization remain important, given its prevalence and potential sight-threatening effects,[12,13] where visual loss may ensue from associated corneal edema, scarring, and lipid deposition.[14] As new antiangiogenic treatments for various corneal pathologic features with associated vascular changes are being developed,[15] a recent roundtable expert review

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identified the development of new imaging techniques for the evaluation of corneal neovascularization as an important, unmet need.[16]

Therefore, we conducted a proof-of-concept study to evaluate the feasibility of using an OCTA device intended for retinal vessel imaging for the corneal and limbal vasculature. Although this imaging technique has been used to evaluate retinal vascular pathologic features such as choroidal neovascularization,[17] to our knowledge, its role has not been reported for use in the anterior segment at the time of this publication. We then used the derived OCTA scanning protocol to evaluate and describe quantitatively abnormal corneal neovascularization in patients with various corneal pathologic features in a preliminary clinical study.

Methods

In the first phase of the study, to establish the OCTA scanning protocol for the anterior segment, we performed OCTA in 20 subjects with no ocular history and a normal slit-lamp examination at the Moorfields Eye Hospital from October 1, 2014, through December 31, 2014. Our study followed the principles of the Declaration of Helsinki, with ethics approval obtained from the local institutional review board. All subjects underwent imaging using the split-spectrum amplitude decorrelation angiography algorithm on the on the AngioVue OCTA system (Optovue, Inc., Fremont, CA) intended for retina imaging (AngioRetina mode), but with the anterior segment optical adaptor lens. Each scan was performed with axial resolution of 5 μm and a beam width of 22 μm, with a light source centered on 840 nm. The instrument captures consecutive B-scans containing 304×304 A-scans at 70 000 scans per second in a slow transverse direction, which constructs a 3-dimensional scan cube in approximately 3 to 4 seconds.[4] Because the default focus of the system is for the retina, the autofocus function was turned off, and manual adjustments to the XYZ focal lengths had to be made until the vessels of interest were seen clearly in focus on the camera image. Because this often led to the anterior segment lens adapter being just 2 to 4 cm from the subject's anterior corneal surface, care had to be taken to prevent contact with the eye. For the proof-of-concept phase of the study, all subjects underwent OCTA scans (6×6-mm volume cubes) in 4 quadrants of the cornea limbus (superior, inferior, temporal, and nasal) in both eyes by 2 independent trained operators.

Image Analysis

The best scans were processed automatically to reduce motion artifacts such as transverse saccadic and residual axial motion by the internal software (ReVue version 2014.2.0.15; Optovue, Inc.). Next, images were exported from the system as a portable network graphics image file into ImageJ 1.38X software (National Institutes of Health, Bethesda, MD) for analysis, similar to a previously described method for quantifying corneal neovascularization.[18] In brief, we identified the regions of interest (ROIs) in each scan by labeling 5 contiguous squares (100 pixels; 850 μm) spanning a circumferential arc length of approximately 4 mm, aligned along the circumference of the anterior border of the corneal limbus (Fig 1). We then used a selective filter to highlight the linear structures such as blood vessels and to reduce the surrounding noise to export the resulting vascular tree as a binary image for further processing, similar to a previously described method.[19] Each ROI was assessed for the number of vascular loops (as illustrated in Fig 1),[6] vessel segments (previously defined as the section between 2 branch points or a terminal point),[19] fractal dimension (D_f) value, and the area enclosed by each vascular loop (square millimeters).[19,20] The quality of the scan images were assessed using the signal strength index and image quality score using a recognized system, that is, 0 to 4 (0, no vessel discernible; 1, poor vessel delineation; 2, good vessel delineation; 3, very good vessel delineation; 4, excellent vessel delineation) on 2 scans per quadrant, performed by 2 independent, masked assessors.[20] We then used the scan protocol as derived above to perform OCTA imaging in 5 patients with various corneal pathologic features, namely, corneal neovascularization in a corneal graft, pterygium, postherpetic keratitis scar, lipid keratopathy, and limbal stem cell deficiency.

Statistical Analysis

To evaluate the scan protocol described, we analyzed all scan images obtained for repeatability, image quality, and vascular measurements between quadrants. We calculated the κ coefficient value for the repeatability of scans using the image quality score, where $\kappa \leq 0.2$ was considered slight, $\kappa = 0.21$ to 0.40 was considered fair, $\kappa = 0.41$ to 0.6 was considered moderate, $\kappa = 0.61$ to 0.8 was considered substantial, and $\kappa = 0.81$ to 1.0 was considered almost perfect in agreement.[21] The intraclass coefficient (ICC) was calculated for vascular measurements performed by both independent assessors, evaluating the mean number of vascular loops and segment-to-loop ratios in the ROI of the scans in each quadrant. Statistical analysis also included descriptive statistics, where the mean ± standard deviation was calculated for the continuous variables and compared using the Mann−Whitney *U* test. A *P* value less than 0.05 was considered statistically significant for comparisons between 2 quadrants. Statistical Package for the Social Sciences software version 17.0 (SPSS, Inc., Chicago, IL) was used to analyze the data.

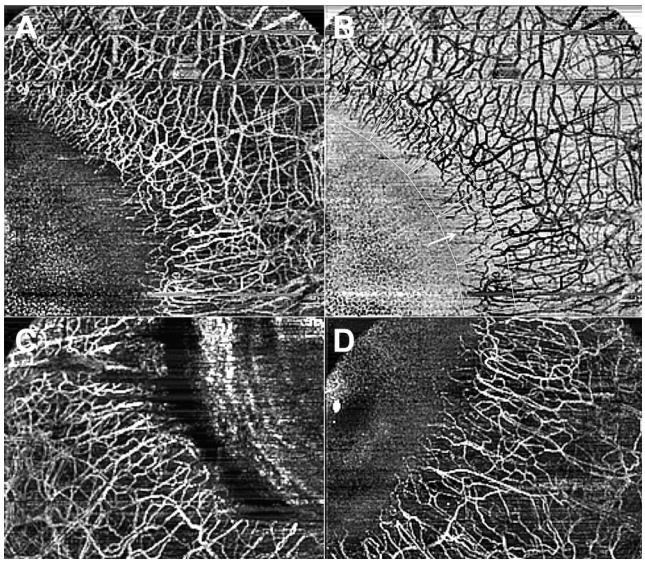

Figure 1. Optical coherence tomography angiograms of the cornea and anterior segment. **A**, **C**, **D**, Examples of whole-depth, split-spectrum amplitude decorrelation optical coherence tomography angiograms with en face maximum projection of the corneolimbal vasculature in various quadrants of the cornea. **B**, The binary image was processed with a selective filter and regions of interest along the corneal vascular arcade using a method previously described; the white arrow points to an example of a marginal corneal vascular loop.[12]