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Structural Confirmation of Lymphatic Outflow from Subconjunctival Blebs of Live Humans

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Purpose: To uncover the mechanism of subconjunctival outflow in humans.

Design: Cross-sectional study.

Participants: Fifteen patients receiving subconjunctival anesthesia before intravitreal injection for routine clinical care.

Methods: Anterior segment (AS) OCT was performed in patients with various instances of conjunctival edema or subconjunctival fluid. Other patients received a subconjunctival mixture of 0.005% indocyanine green and 2% lidocaine. After subconjunctival injection of the tracer and anesthetic mixture, blebs and associated outflow pathways were imaged angiographically and the time for appearance was recorded. The pattern and structure of outflow pathways were studied using AS OCT. Angiographic and AS OCT results were compared with trabecular and conventional outflow imaging, which demonstrates veins.

Main Outcome Measures: Ocular surface lymphangiography and AS OCT images.

Results: Anterior segment OCT of the conjunctiva in a normal eye demonstrated thin nonedematous conjunctiva with absent intraconjunctival lumens or subconjunctival fluid. Patients with a history of trabeculectomy, subconjunctival drug injection, or chemosis demonstrated thickened conjunctiva and intraconjunctival luminal pathways that contained valve-like structures. Tracer-based studies in patients demonstrated blebs with irregular subconjunctival bleb-related outflow patterns that arose in a time-dependent fashion. These angiographic pathways were luminal on OCT, sausage shaped, and contained intraluminal valve-like structures. This was in contrast to trabecular and conventional outflow imaging, where pathways were classically Y-shaped, of even caliber, and lacked valve-like structures.

Conclusions: Outflow pathways were seen in patients with conjunctival edema and after subconjunctival tracer injection. These pathways were lymphatic based on pattern and structural study. Better understanding of bleb-related lymphatic outflow may lead to improved bleb-requiring glaucoma surgeries and subconjunctival drug delivery. *Ophthalmology Science 2021;1:100080 © 2021 Published by Elsevier Inc. on behalf of the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Fluid homeostasis is important for all organ systems, including the eye. This balance is achieved by using different intraocular and extraocular pathways. The eye maintains a native intraocular pressure (IOP) that, when elevated (ocular hypertension), is the primary risk factor for glaucomatous optic nerve damage.[1,2] The extraocular subconjunctival space normally exists as a closed potential space. In certain pathologic conditions (total body fluid overload[3] or ocular infection or inflammation[4]), this subconjunctival space can open. This is termed *chemosis*. Treating the underlying root cause resolves the chemosis, revealing that outflow pathways must exist to clear the fluid from the subconjunctival space and return it to its potential state.

Clinically, the subconjunctival space is important in the treatment of eye diseases. During glaucoma surgery, the success of glaucoma filtering surgeries depends on maintaining an open subconjunctival space or bleb.[5] In these cases, intraocular fluid is shunted to an extraocular subconjunctival bleb via a low-resistance pathway (tube, stent, or sclerostomy) to achieve lower IOP. Somehow, the fluid then must leave the bleb. Likewise, pharmaceutical drugs also can be injected under the conjunctiva to treat certain ocular conditions.[6,7] However, subconjunctival outflow could drain the drug away, limiting the pharmacologic effect. Understanding how fluid leaves the subconjunctival space is important for glaucoma surgery and for the development of subconjunctival drug delivery as a way to treat ocular disorders.

Competing hypotheses exist regarding how fluid leaves the subconjunctival space, natively and after surgery. The ocular surface is known to contain blood vessels and lymphatics.[8] Some investigators have attributed subconjunctival bleb outflow to lymphatics,[9] whereas others have described the use of veins.[10] To reveal which route is used, the best evidence should come from visualizing the pathways and

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then specifically isolating just those pathways for careful structural imaging and analyses.

A few case reports have observed blood (after subconjunctival hemorrhage) or injected tracer movement from under the conjunctiva in humans.[11,12] In these cases, blebs were formed, and the tracer-filled pathways displayed sausage-shaped patterns off the blebs. However, no concomitant structural evaluation of the exact pathways off the blebs were performed in these patients. With this limited knowledge, one could not conclude that these tracer-based pathways were necessarily luminal outflow routes, as opposed to irregular dissection planes caused by a tracer pushed forward under a pressure head. Also, the identity of these pathways could not be definitively determined.

Our group has experience in tracer-based studies in the conventional and trabecular pathways in the eye, as well as in subconjunctival outflow.[13] Aqueous angiography was developed to image trabecular outflow in live patients and after surgery and is known to demonstrate ocular veins.[14–16] Ocular surface lymphangiography was developed as a sister method to observe fluid flow from the subconjunctival space.[17] In porcine eyes, ocular surface lymphangiography visualized bleb-related outflow pathways and demonstrated blind end tips with semilunar valves in the direction of flow that were reminiscent of lymphatics.[17] Isolation of these exact pathways demonstrated a molecular lymphatic (PROX-1 and podoplanin positivity), but not blood vessel (CD31) identity.[17] Thus, we confirmed a lymphatic identity for nonprimate mammalian subconjunctival outflow. We now propose to image subconjunctival outflow in vivo in human patients with concurrent noninvasive structural evaluation of these subconjunctival outflow pathways.

Methods

This study adhered to the tenets of the Declaration of Helsinki and was conducted at the Department of Ophthalmology, Doheny Eye Center, University of California, Los Angeles, and the Department of Ophthalmology, University of Alabama at Birmingham, with institutional review board approval (University of California, Los Angeles identifiers, 20-001064, 15-000083, and 15-000134; University of Alabama at Birmingham identifier, 300002967). All participants provided informed consent.

Anterior Segment OCT Findings of Participants Not Receiving Subconjunctival Tracer

Anterior segment OCT outflow imaging was performed using the Spectralis HRA+OCT (Heidelberg Engineering) or Anterion OCT (Heidelberg Engineering; Fig 1B). The Spectralis is a Food and Drug Administration-approved spectral-domain OCT device that uses an 870-nM wavelength light source.[18] All ambulatory participants were imaged with the standard Spectralis situated on a table. All aqueous angiography images for participants at the University of Alabama at Birmingham were obtained using the FLEX module, which is a Spectralis built on a flexible arm. The aqueous angiography images were obtained as previously described with indocyanine green (ICG) introduction into the anterior chamber followed by ocular surface angiographic imaging.[19] Spectralis imaging used the AS OCT module, and single line scans were obtained using a 15° scan angle (3.9-μm axial resolution and 11-μm lateral resolution; scan length, 4.5 mm). The Anterion is a CE-marked swept-source OCT.[20,21] Anterion images were obtained using the Metrics App (1310-μm wavelength; 10-μm axial resolution and 30-μm lateral resolution; scan length, 16.5 mm).

Tracer Dose-Finding and Preparation

Indocyanine green (Sigma I2633) in situ dose finding was performed in the laboratory. Indocyanine green was dissolved in water into a 2% stock solution and stored at −80° C. Indocyanine green dilutions were made using pharmaceutical grade 2% lidocaine (Hospira) to 0.4%, 0.2%, 0.1%, 0.05%, 0.01%, 0.005%, 0.001%, 0.0005%, and 0.0001% in triplicate. Five hundred microliters of each was placed in a vial and situated in front of the Spectralis HRA+OCT. The vials were imaged using the Spectralis CSLO angiographic function with the ICG capture mode (excitation wavelength, 786 nm; transmission filter set at > 800 nm). Fluorescent images were obtained using a 55° lens, 25-diopter focus, and 85 sensitivity setting. Images were opened using in-built Spectralis software (HEYEX version 1.9.10.0), downloaded as a .tiff file, and opened in Photoshop CS5 (version 12x32; Adobe) where fluorescence pixel intensity was determined in a region of interest (100 × 100-pixel box) centered on the vial.

For human participants, the ICG and lidocaine mixture was prepared using an aseptic technique over a sterile field. Briefly, 25 mg pharmaceutical grade ICG (Diagnostic Green) was dissolved in 5 ml of the manufacturer-provided solvent for a 0.5% final concentration. Then, 0.05 ml of this solution was mixed into 5 ml of 2% pharmaceutical grade lidocaine. The final 5.05-ml solution consisted of 1.98% lidocaine and 0.005% ICG.

Injection Protocol and Ocular Surface Lymphangiography

Human participants in the main part of this study were prospectively recruited from among patients requiring subconjunctival lidocaine injection for anesthesia before intravitreal drug injection for various retinal disorders, including age-related macular degeneration, cystoid macular edema, and diabetic macular edema. Exclusion criteria included a history of allergy to ICG, vision better than 20/30, age younger than 18 years, and known significant conjunctival scarring. All of these criteria were discussed in detail with the participants, including the purpose and risks of the research. Retinal specialists (P.L. or G.H.) performed the subconjunctival injections as per standard clinical practice. Participants were given 1 drop of topical 0.3% ofloxacin followed by 1% proparacaine (Bausch & Lomb) and 5% Betadine (Alcon). Under bright illumination, the retina specialist held a 1-ml syringe filled with the anesthetic and tracer mix on a 30-g needle. The bevel of the needle was used to pick up the conjunctiva and pierce into the subconjunctival space. One hundred microliters of the tracer were injected. Five participants were imaged immediately. The remaining 10 participants were imaged after a recorded period normally reserved for the anesthetic effect to take hold before intravitreal injection. Statistical comparison was made using an unpaired 2-tailed t test.

Participants were then brought to the Spectralis HRA+OCT for ocular surface lymphangiography. Participants were imaged using the Anterior Segment Module with the same ICG capture mode as above in the laboratory. Anterior segment OCT was performed concurrently with ocular surface lymphangiography using the Anterior Segment Module on Scleral Mode. In angiographically positive structures, single line scans with a 15° scan angle (3.9-μm axial resolution and 11-μm lateral resolution; scan length, 4.5 mm)

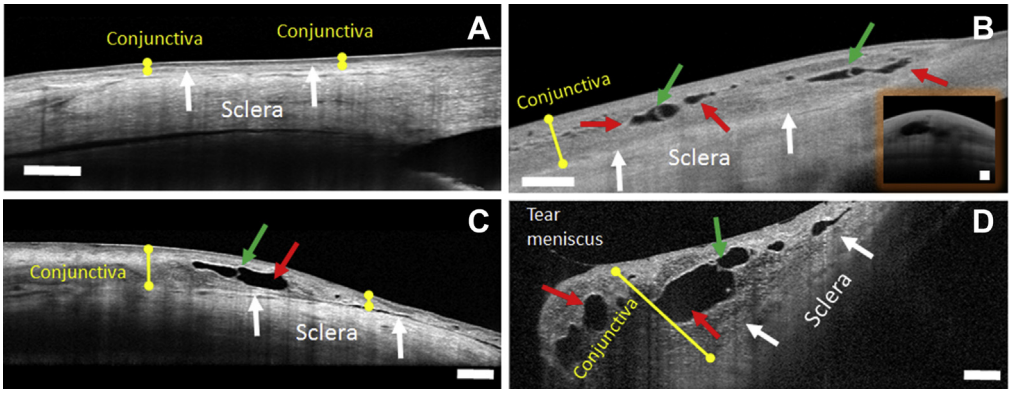

Figure 1. Anterior segment OCT images from normal eyes and eyes with edematous conjunctiva. **A**, Anterior segment OCT image showing the right eye of a healthy 43-year-old Asian man. Note the thin conjunctiva (yellow barbells) and a scleral–conjunctival border (white arrows) near the ocular surface. **B**, Anterior segment OCT image from a 71-year-old White man, who had undergone trabeculectomy in the left eye, obtained nasal to the bleb showing a thickened conjunctiva (yellow barbell) above the scleral–conjunctival border (white arrows). Outflow pathways are seen (red arrows) with valve-like structures (green arrows). Insert shows an OCT image obtained across the bleb body and bleb lumen. **C**, Anterior segment OCT image from a 79-year-old Asian man who had undergone subconjunctival lidocaine injection in the left eye before intravitreal anti–vascular endothelial growth factor injection for age-related macular degeneration. Again, thickened conjunctiva (yellow barbells) is present above the scleral–conjunctival border (white arrows). A lumen is seen (red arrow) with a valve-like structure (green arrow). **D**, Anterior segment OCT image from a 32-year-old White man with chemosis showing in the inferior left eye very thick conjunctiva (yellow barbell) above the scleral–conjunctival border (white arrows). Very dilated lumens (red arrows) are seen along with a valve-like structure (green arrow). Scale bars = 500 μm.

were obtained to assess the structure of angiographically positive pathways. Lumens were measured using in-built calipers.

Image Processing

Ocular surface lymphangiography images were downloaded using in-built Spectralis software as a .tiff file and opened in Photoshop CS5, where the images were centered and cropped on regions of interest. Spectralis comparisons between OCT and confocal angiographic ocular surface lymphangiography images were performed using in-built sliders. The accuracy of this cross-modal comparison between the Spectralis OCT and confocal imaging has been reported at approximately 14 μm, which is near the lateral resolution of the OCT itself (approximately 14 μm).[22] This is because of the Spectralis TruTrack system, where the confocal image is mapped at more than 1000 points using 1 light beam during confocal imaging and set as a reference for the second beam to acquire the OCT. This ensures accurate point-to-point correlations between the OCT and confocal images. Given such high accuracy, as an example, ophthalmic pathologic features can be followed longitudinally with confocal and OCT comparison from months to years in humans.[23]

Results

Anterior segment OCT alone demonstrated pathways that may be related to subconjunctival outflow. Anterior segment OCT in a 43-year-old healthy Asian male volunteer showed the conjunctival–scleral border, a thin conjunctiva, no clear subconjunctival space, and no intraconjunctival pathways (Fig 1A). However, AS OCT in a 71-year-old White man who had undergone trabeculectomy on a region away from the bleb showed a thickened conjunctiva with dilated intraconjunctival lumens (Fig 1B, red arrows). Valve-like structures could be seen periodically (Fig 1B, green arrows). Anterior segment OCT in a 79-year-old Asian man with age-related macular degeneration, after subconjunctival lidocaine injection for anesthesia before intravitreal anti–vascular endothelial growth factor (VEGF) injection, also demonstrated thickened conjunctiva with an intraconjunctival lumen (Fig 1C, red arrow) and a valve-like structure (Fig 1C, green arrow). Anterior segment OCT in a 32-year-old White man with chemosis showed markedly thickened conjunctiva and very dilated intraconjunctival pathways (Fig 1D, red arrows). A valve-like structure was seen within the lumen as well (Fig 1D, green arrow). Thus, conjunctiva normally is very thin with little subconjunctival fluid that can then be thickened in certain states with the appearance of clear lumens and valve-like structures.

To study subconjunctival outflow, we proposed using indocyanine green (ICG). Indocyanine green is a Food and Drug Administration-approved tracer that is used routinely and safely during medical imaging. However, the choice of tracer concentration can differ when imaging different parts of the body. First, fluorophores can exhibit unusual behaviors such as the inner-filter effect,[24,25] where high concentrations can result in the fluorescent emission itself exciting nearby fluorophores. In this case, no fluorescent emission escapes and the solution appears dark. Second, the imaging depth and opacity of overlying tissue can influence the desired concentration because of fluorescent signal scattering. Therefore, an in situ dose-finding study was performed in the laboratory. Indocyanine green of varying concentrations (0.4%, 0.2%, 0.1%, 0.05%, 0.01%, 0.005%, 0.001%, 0.0005%, and 0.0001%) was imaged in front of the Spectralis HRA+OCT using the same settings as for human participants. Fluorescent intensity was measured (Fig 2A), demonstrating low emission at high concentrations (inner-filter effect), but high emissions at lower concentrations. Focusing on lower concentrations (Fig 2B; 0.0001%–0.01%), the highest emission was at 0.001% with a drop-off on each side. The final

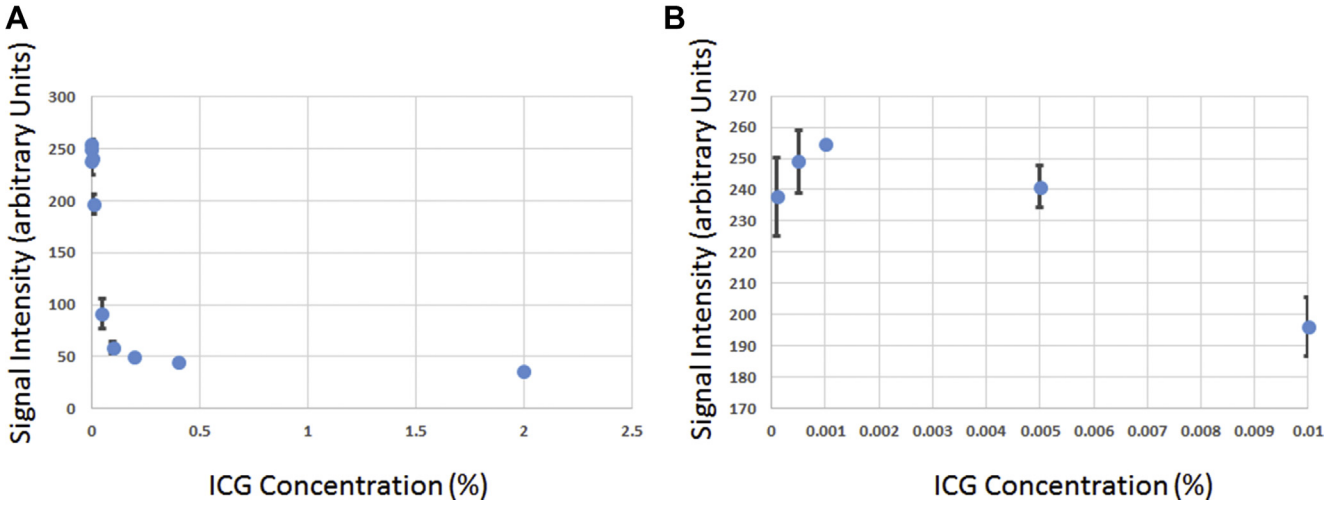

Figure 2. Graphs showing indocyanine green (ICG) dose finding for human patient ocular surface lymphangiography. **A**, Indocyanine green was mixed at various concentrations and the eye was imaged using the Spectralis HRA+OCT, the same camera used for human participants. High ICG concentration resulted in little or no signal, consistent with an inner filter effect. **B**, Data from only 0.01% to 0.0001% ICG concentration. The 0.005% concentration was chosen for human participants in the study. Data shown as mean ± standard error of the mean.

concentration of 0.005% was chosen because any minimal subconjunctival fluid could only serve to dilute the tracer to a higher emission point and to help overcome signal scatter from overlying conjunctival tissue.

Human participants who needed subconjunctival lidocaine anesthetic before intravitreal injection of anti-VEGF therapy during routine clinical care were then recruited. Indocyanine green was mixed into the lidocaine (final solution, 1.98% lidocaine and 0.005% ICG) using an aseptic technique and given as a single subconjunctival injection for (1) anesthesia before the intravitreal injection and (2) tracer for the subconjunctival outflow imaging. No systemic or local adverse reactions were observed after the subconjunctival injection of ICG and lidocaine mixture. Five eyes from 5 participants (3 men and 2 women) were imaged initially with mean age of 74.2 ± 4.8 years. Subconjunctival blebs arose after injection in all participants; however, no outflow pathways were observed when imaged immediately after injection (data not shown).

Given that anecdotal observation in the literature raised the variable of time,[12] 10 eyes from another 10 participants (4 men and 6 women) with mean age of 75.6 ± 3.1 years (mean ± SEM) were imaged again. In this case, we waited the time normally reserved after subconjunctival injection for the anesthetic effect to take hold prior to intravitreal injection before imaging. This timing was variable because of the time required for routine clinical workflow. Subconjunctival blebs were visualized again in all participants, but now 7 of 10 participants demonstrated clear visible outflow pathways (Figs 3–5). To determine if timing was the issue, blebs that showed no pathways (n = 8) were noted to have been imaged at an earlier time (5.63 ± 2.98 minutes after injection) compared with blebs that showed pathways (n = 7; 16.43 ± 1.93 minutes after injection; $P = 0.01$). For all participants receiving a tracer injection, the prior number of recorded intravitreal injections for those eyes in our clinics was 16 ± 3.5.

The bleb-related outflow pathways appeared variegated or sausage-shaped, with thicker regions alternating with thinner regions (Fig 3A–D). The pathways were also varied with different patterns, including right angles, U-turns, and some Y-shaped branching. The subconjunctival outflow patterns were in contrast to trabecular or conventional outflow, which was imaged using aqueous angiography (Fig 3E, F). Aqueous angiography is known to demonstrate the postlimbal intrascleral venous plexus as well as aqueous and episcleral veins, as opposed to lymphatics. These pathways were more regular, of consistent caliber throughout, and web-like or with primarily classic Y-shaped extension patterns. Therefore, subconjunctival outflow pathways were clearly qualitatively different from trabecular or conventional aqueous humor outflow pathways in the eye that are venous.[14–16]

To study the structure of the subconjunctival outflow pathways, concurrent AS OCT was performed. Anterior segment OCT cross-sections of subconjunctival outflow pathways clearly demonstrated lumens that matched with the angiographic patterns (Fig 4A, B). Some outflow pathways were complex with a pattern of interwoven lumens. Careful observation of these pathways could distinguish different routes that correlated to different lumens on AS OCT (Fig 4C–F). Cross-sectional luminal sizes were measured from 3 pathways obtained from each participant who showed bleb-related outflow pathways. The diameters were highly variable, from 31 to 203 μm (average, 92.5 ± 20.2 μm), which was consistent with the sausage-shaped and variegated nature of these outflow pathways.

To help identify the subconjunctival outflow pathways, structural features were studied further using AS OCT. Subconjunctival outflow pathway lumens were noted to

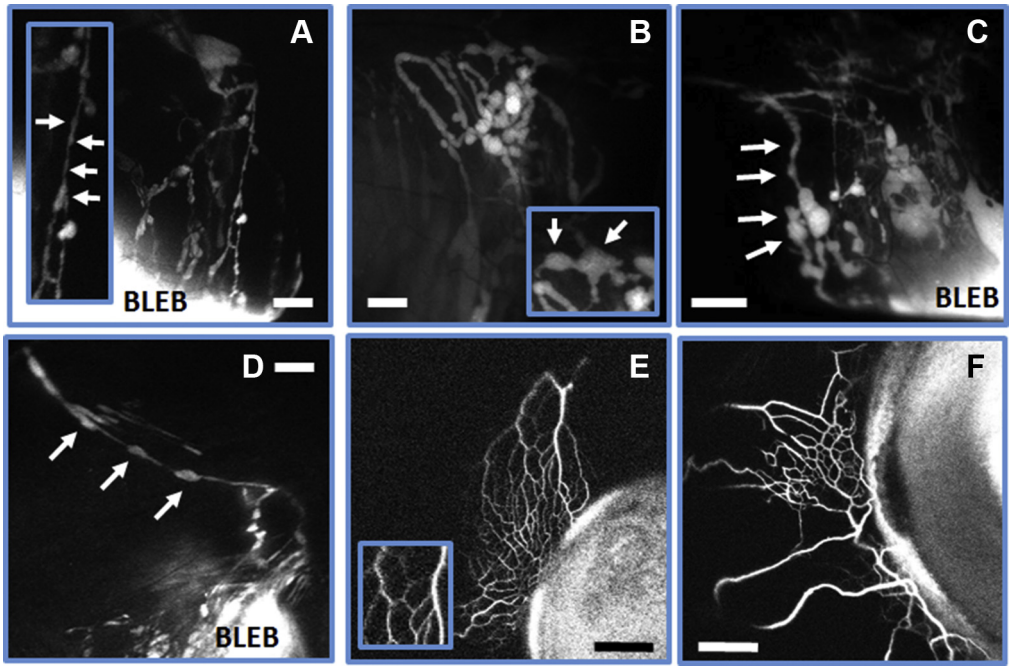

Figure 3. Ocular surface lymphangiography images obtained after subconjunctival injection. **A**–**D**, Patients requiring subconjunctival lidocaine injection before intravitreal drug treatment received lidocaine mixed with indocyanine green. **A**, **C**, **D**, Initial bleb is visualized clearly. **B**, Bleb is below and to the left of the image. **A**–**D**, Irregular pathways are seen with intermittent dilations (white arrows) alternating with thin regions along the pathways. **E**, **F**, This is in contrast to aqueous angiography, where tracer is placed in the anterior chamber to visualize trabecular and conventional outflow that uses scleral and episcleral veins: (**E**) left eye of a 61-year-old Asian women and (**F**) left eye of a 54-year-old Hispanic man. In these patients, the pathways are more organized as a web-like mesh in addition to Y-shaped patterns. They appeared even-calibered throughout. Scale bars = 1 mm.

contain valve-like structures (Fig 5). Invagination points on the angiographic outflow pathways (Fig 5A, D, G, J, white arrows) matched valve-like structures on OCT (Fig 5C, F, I, L, white arrows). Some appeared bicuspid, whereas others appeared as single leaflets. As a point of comparison, aqueous angiography imaged trabecular or conventional outflow veins were uniform in caliber and lacked valve-like structures (Fig 5M–O, P–R).

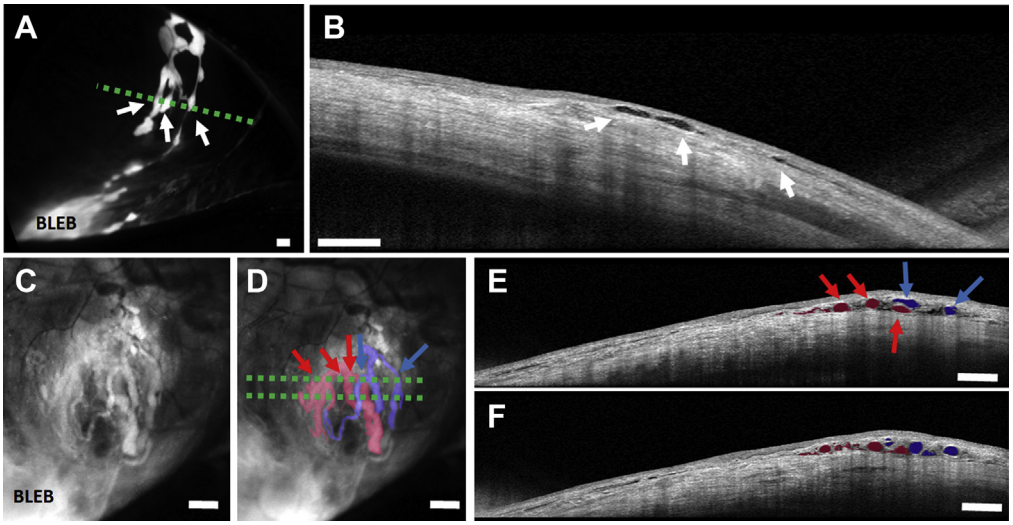

Figure 4. Images showing bleb-related outflow pathway structural evaluation. **A**, Ocular surface lymphangiography image showing irregular pathways off a bleb. **B**, Anterior segment (AS) OCT of this region (green dotted line in (**A**)) demonstrating lumens that match between the angiographic image and OCT image (white arrows). **C**, Complex bleb-related outflow pathways can be elicited by ocular surface lymphangiography. **D**, Careful observation allowed delineation of interwoven pathways (red and blue). **E**, **F**, These pathways could be identified as lumens on AS OCT (the OCT in (**E**) represents the top B-scan green dotted line in (**D**), and the OCT in (**F**) represents the bottom B-scan green dotted line in (**D**)). Scale bars = 500 μm.

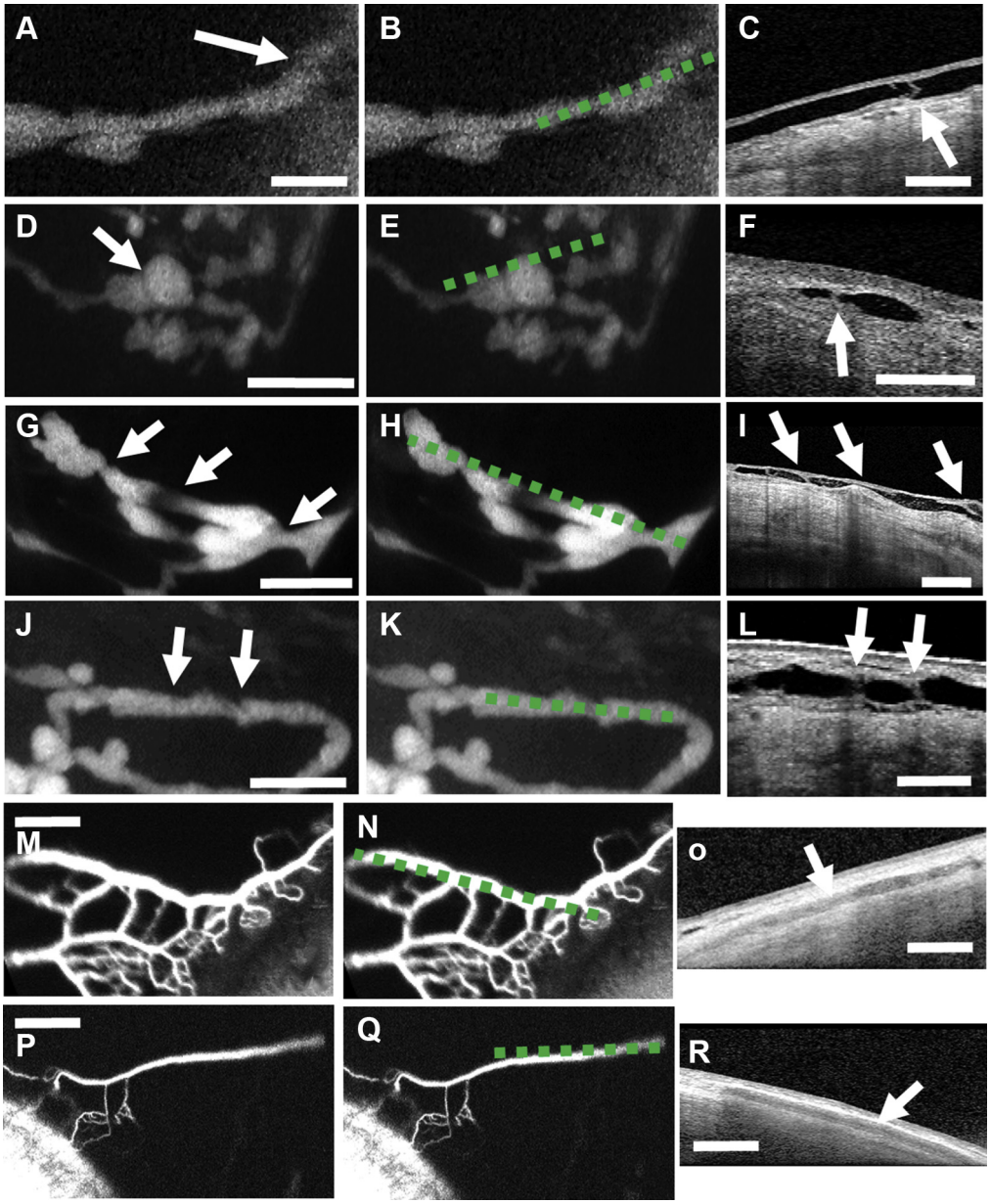

Figure 5. Images showing bleb-related outflow pathways with valve-like structures. **A**, **D**, **G**, **J**, Ocular surface lymphangiography images demonstrating irregular pathways with intermittent dilations alternating with thinner areas (white arrows). **B**, **E**, **H**, **K**, Ocular surface lymphangiography images showing where OCT B-scans were placed along these pathways (green dotted lines). **C**, **F**, **I**, **L**, Corresponding OCT B-scans demonstrating structures that appeared as semilunar valves, single leaflet valves, or septae (white arrows). **M**–**R**, Aqueous angiography with OCT B-scans on trabecular outflow pathways demonstrating episcleral vein luminal pathways (white arrows) that were uniform in diameter and devoid of valves: (**M**–**O**) left eye of a 67-year-old Asian man and (**P**–**R**) right eye of a 73-year-old White man). Scale bars = 1 mm.

Discussion

The present study demonstrated the structural basis of subconjunctival outflow in live human eyes and supports a lymphatic (as opposed to blood vessel) outflow hypothesis. The morphologic features of these visualized outflow pathways strongly suggest that they are lymphatic vessels, given their irregular contour with intermittent dilation or saccules and because of their luminal diameters, which are of similar size (approximately 100-μm diameter) to previously measured porcine subconjunctival lymphatic outflow pathways (100–200-μm diameter).[17] Finally, the presence of valve-like structures or septae further supports the lymphatic hypothesis.[26,27] This is all in contrast to aqueous angiography imaging (known to label aqueous humor outflow pathway as blood vessels, as opposed to

lymphatics), which shows typical Y-shaped[16] patterns and demonstrates OCT lumens that are uniform in caliber and without valve-like structures.

Valves are a typical hallmark of lymphatic pathways. Using similar methods as in this study, our group previously showed the presence of clear and consistent semilunar valves in porcine conjunctiva.[17] The current study showed that human eyes are slightly different. Classic semilunar or bicuspid valves were not always seen in human conjunctiva. In some cases, single partitions were seen. This is why we described the structures as "valve-like" instead of as definitive valves. A few reasons may explain the different appearance. First, if the OCT optical section is not obtained exactly longitudinal through the outflow pathway, a bicuspid structure could appear skewed because of an oblique imaging plane. Structural imaging is challenging in live participants who can move. Finally, lymphatic valve variability has been reported.[28] Valves containing 1 to 5 leaflets have been described in mammals.[29–31] In the case of unicuspid valves, the structure can appear more like single septae. Overall, our data show that lymphatic valvular structure is present in human eyes, but these structures appear to be more variable in nature compared with classic lymphatic presentations.

This study has several limitations. The sample size is small. Only structural and no molecular studies were performed because tissue could not be acquired from these participants for histologic examination. Also, the live human participants in this study did not have completely normal conjunctiva. In the main study, inclusion criteria included history of retinal disease requiring subconjunctival anesthetic injection before intravitreal drug injection. By far, most patients had a diagnosis of age-related macular degeneration requiring anti-VEGF therapy. Most patients had a history of multiple injections, and true quantification of past injections was not possible because most participants had a history intravitreal injections before seeking treatment at our clinics. Therefore, all participants in the main study had some level of ocular surface scarring from a history of prior repeat injections. This may have resulted in atypical subconjunctival outflow or lymphatic structure such as for the valves. This also may have impacted timing in this study. Unlike in porcine imaging, where outflow pathways appeared almost instantaneously after injection,[17] in this study, bleb-related outflow pathways appeared only after waiting a longer period after injection.

The clinical importance of studying subconjunctival lymphatics is in understanding certain disease states as well as in the treatment of some ocular diseases. Subconjunctival lymphatic pathologic features are seen in conjunctival lymphangiectasia, where patients demonstrate dilated lymphatics with chronic bulbar chemosis and ocular surface irritation.[32] For treating eye diseases, subconjunctival lymphatics may be leveraged to improve therapies. For example, glaucoma surgical blebs are essentially focal regions of chemosis intended to be permanent and provide a low-resistance outflow pathway to lower IOP. In this case, greater subconjunctival outflow is desirable. Trabeculectomy research has shown greater IOP reduction in cases where the bleb shows more tracer-based lymphatic-like outflow pathways.[33] Failed trabeculectomies are devoid of lymphatics.[34] Separately, subconjunctival drug injection has also been described for treating various ocular diseases, such as scleritis.[6] However, this route has not gained widespread acceptance (e.g., compared with intravitreal injection) for many reasons. Ocular drug penetration is a problem, and concern also exists that the drug depot does not last.[35] For example, subconjunctival injection of antibiotics and steroids to form blebs are often made at the end of eye surgery, and clinical experience indicates that they typically are not seen during the first postoperative visit. The outflow pathways shown in this study clearly explain this observation and demonstrate where the pharmaceutical agents exit. Therefore, material egress from the subconjunctival space limits the subconjunctival location as a drug delivery target.

One potential future therapeutic strategy resides in the manipulation of subconjunctival lymphatics. If greater outflow is desired from glaucoma surgical blebs, then greater subconjunctival lymphatic presence may be beneficial. Alternatively, if lesser outflow is desired for subconjunctival drug delivery, then lesser subconjunctival lymphatic presence may be beneficial. Lymphatics are regulated by VEGFs.[36] Type A and B VEGFs are well known to promote blood vessel and, to a lesser extent, lymphatic growth,[37] to be involved in ocular neovascularization, and to represent the targets of anti-VEGF therapy used for conditions such as age-related macular degeneration[38] and neovascular glaucoma.[39] In this study, anti-VEGF type A was used in the participants, and this could have impacted subconjunctival lymphatics, although the risk is small because the injection was aimed intraocularly at the vitreous as opposed to the subconjunctival space, which is extraocular. Type C VEGF[40] also promotes lymphatic growth, and certain mutations (VEGFC-C156S)[41] are particularly specific for lymphatics. Therefore, molecular pathways exist to modify lymphatics. Preliminarily, we reported that VEGF type C can increase the subconjunctival lymphatic number and branching in a mouse model.[42] Separately, antimetabolites (mitomycin C and 5-fluorouracil) used in glaucoma surgery to limit scarring have been hypothesized to damage subconjunctival lymphatics.[43] In the same mouse model, these antimetabolites decreased subconjunctival lymphatic number and branching as well.[42] Thus, lymphatic manipulation is a prime target for exploring improved ocular therapeutics in the future.

In conclusion, subconjunctival lymphatics have long been known to exist, and this article ties lymphatics to subconjunctival outflow in humans. This was studied in the past in nonprimate mammals[17,44] and nonhuman primates.[9] In humans, although outflow studies had been performed,[11,12] more precise anatomic and structural evaluation supporting a lymphatic identity is provided herein. Future steps involve exploring lymphatics as a way to potentially improve ocular therapeutics.

Footnotes and Disclosures

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Author Contributions:

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Abbreviations and Acronyms:
AS = anterior segment; **ICG** = indocyanine green; **IOP** = intraocular pressure; **VEGF** = vascular endothelial growth factor.

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