Canine glaucoma constitutes a group of diseases inducing degeneration of the retinal ganglion cells and optic nerve, with elevated intraocular pressure (IOP) being a predominant risk factor.\(^1,2\) Regardless of the proposed mechanisms or classification of this disease, uncontrolled or even managed glaucoma almost always progresses to blindness.\(^1-3\) However, no radical therapy to date has emerged; limited methods for lowering IOP, reducing the production of aqueous humor, increasing its outflow, or creating a bypass can be performed medically or surgically. Independent of the employed method, early diagnosis and therapeutic interventions are essential to preserve visual function and mitigate pain associated with elevated IOP.\(^3,4\)
Surgical procedures that divert aqueous humor from the anterior chamber are considered when IOP becomes unresponsive to medical treatment. For draining aqueous humor, various devices with different materials, sizes, and functions have been applied to patients with canine glaucoma. While there have been diverse propositions regarding the location for shunting the aqueous humor, most devices have primarily targeted the subconjunctival space recently, including the Ahmed glaucoma valve (AGV). The drained aqueous humor is absorbed into the surrounding vasculature, accompanied by the formation of a filtering bleb. One of the most significant complications of such shunting surgery is the fibrosis of the bleb, which can obstruct aqueous absorption, leading to insufficient or failed reduction of the IOP.

Ultrasound biomicroscopy (UBM) using a 50-MHz transducer has a resolution of approximately 20 to 50 μm, which is almost 10 times higher than that of standard ultrasonography (10-MHz transducer, approx 200-μm resolution). UBM enables the visualization of the ocular anterior segment with penetrability of up to 5 mm. In veterinary ophthalmology, UBM has been used for analyzing the corneal and scleral thickness, measuring the anterior chamber depth, comparing the iridocorneal angle, iris and ciliary body, and visualizing cystic or neoplastic changes in the iris and ciliary body. In addition to these applications, UBM is extensively used in human medicine to evaluate blebs formed in the subconjunctival space following filtering or shunting surgery. This technique is beneficial for assessing the internal morphology of blebs, particularly regarding understanding the relationship between bleb fibrosis and its functional outcomes.

Shunting surgery for glaucoma has been practiced in veterinary medicine since the 1980s; however, prognostic research regarding postoperative blebs is limited. Therefore, this study was conducted to observe and assess the characteristics of blebs formed following AGV surgery in dogs using UBM, while analyzing their correlation with postoperative parameters, particularly IOP.

Methods

Patient enrollment
All procedures and animal care were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-230901-2). Additionally, informed consent for clinical procedures and the use of related information for publication purposes was obtained from all animal owners involved in this study.

Patients diagnosed with primary angle-closure glaucoma and under follow-up after AGV surgery were enrolled in this prospective study, conducted from June 2021 to September 2023. All the AGV surgeries were conducted in the superior temporal quadrant of the globe and by a single board-certified veterinary ophthalmologist (SK).

Before the AGV surgery, patients were managed with individualized treatment plans based on the severity of glaucoma. These plans included medications, such as topical carbonic anhydrase inhibitors (2% dorzolamide) and prostaglandin analogs (0.005% latanoprost or 0.03% bimatoprost). Some patients were administered β-blockers as a sole agent (0.5% timolol) or a combination product (a fixed combination of 2% dorzolamide and 0.5% timolol). Additionally, both topical and systemic NSAIDs were administered to all patients immediately before the surgery.

The AGV (model FP8, New World Medical) surgery was performed using standard techniques; a 10-mm conjunctival incision was made 8 mm from the limbus, and the Tenon capsule was separated to create a pocket for AGV body placement. A 4 X 6-mm partial-thickness limbus-based scleral flap was made for the tube insertion. The pocket was treated with 0.5 mg/mL mitomycin C and subsequently rinsed with a balanced salt solution. The AGV body was then secured to the sclera 10 to 12 mm from the limbus using 8-0 nylon sutures. A 22-gauge needle track was created beneath the scleral flap, and the tube was carefully inserted into the anterior chamber. The surgical site was completed by suturing the edges of the scleral flap with 8-0 nylon, followed by a double-layer closure of the Tenon capsule and underlying conjunctiva, using 8-0 polyglactin 910 sutures in a continuous pattern.

The postoperative care comprised topical medications, including antibiotics, mydriatics, anti-inflammatory drugs, lubricants, and antiglaucoma medications as required based on the postoperative IOP. If postoperative complications, such as fibrin formation around or inside the tube, were observed, intraocular tissue plasminogen activator injections were administered (Supplementary Table S1).

All patients received routine ophthalmic examinations to assess surgical outcomes, including neuro-ophthalmic examinations, the Schirmer tear test (Schirmer Tear Test Strips; Merck Animal Health), IOP measurement using rebound tonometry (TonoVet; iCare), binocular indirect ophthalmoscopy (Vantage Indirect Ophthalmoscope; Keeler) with a 30-D condensing lens, slit-lamp biomicroscopy (SL-D7; Topcon), fluorescein staining (Flu-Glo; Akorn Pharmaceuticals), macroscopic examination of the bleb, and the Seidel test to check for bleb dehiscence. Patients presenting with an aqueous flare or complications related to the AGV implant, such as tube obstruction or compromised bleb stability, or those with other identified ophthalmic diseases were excluded from the study. Additionally, those who had simultaneous AGV and cataract surgeries, had the AGV implant removed, and underwent salvage procedures, such as intravitreal cidofovir or gentamicin injection, or lost vision for reasons other than increased IOP were excluded.

UBM examination of the bleb
UBM (50-MHz transducer; MD-320W; MEDA) examinations were conducted on patients who had undergone AGV surgery at least 6 weeks prior.
exhibited visibly observable blebs, and showed no complications related to the surgery. UBM scanning was consistently performed between 10 am and 12 pm in a dedicated, dimly lit examination room under fixed lighting conditions. The brightness, contrast, gain, and zoom settings were kept constant for all UBM scans. After topical anesthesia using 0.5% proparacaine eye drops, each dog was gently restrained in the most comfortable position, standing or sitting. The upper eyelid was raised from the side of the forehead to avoid globe or bleb compression. After confirming the dog’s stability, the probe was positioned at the margin of the bleb closest to the limbus. The scanning was then conducted perpendicularly to the limbus, capturing the sagittal section of the bleb along the full length of the AGV body. The scanning was performed as close to the median as possible while avoiding the simultaneous capture of the sclera and tube to prevent interference with scleral reflectivity by the tube (Figure 1). More than 5 images were captured for each bleb while monitoring real-time images, from which 3 images were selected for further analysis. All the UBM images and measurements were conducted by a single researcher (SL).

**UBM image analyses**

For the scanned images, bleb wall thickness, bleb wall reflectivity, and scleral reflectivity were measured using image processing software (ImageJ, version 1.53; National Institutes of Health). The bleb wall thickness was defined as the distance from the first reflective signal of the bleb fluid cavity to the top of the conjunctiva. Given the variability in bleb wall thickness across the scan and depending on the measurement location, only the minimum and maximum distances were measured, and the mean value was obtained. For reflectivity, the pixel intensity was quantified by measuring 3 times within a 0.5 X 0.5-mm area with the least artifact interference in each of the 3 selected images. The mean value of these measurements was then calculated. The 2.0 X 2.0-mm area of the top right empty corner was designated as the background noise, which was measured using the aforementioned method and used as a correction value for calculation (Figure 2). From these normalized values, the relative reflectivity of each bleb wall, defined as its reflectivity compared to that of the sclera, was calculated as follows:

$$\text{Relative reflectivity} = \frac{\text{bleb reflectivity} - \text{background noise}}{\text{scleral reflectivity} - \text{background noise}}$$

**Histological examination**

An eye was enucleated at the client’s request 551 days after surgery due to uncontrolled IOP leading to blindness and subsequently underwent a histological examination (patient 7). The enucleated globe was preserved in 10% neutral buffered formalin for a week and was sagittally trimmed along the midline of the bleb and the AGV body. The tissue was processed for paraffin embedding and was sectioned...
to a thickness of 4 μm. H&E staining was employed to discern the general tissue structures, and picrosirius red staining was used to identify regions of collagen deposition. Immunostaining for α-smooth muscle actin (α-SMA) was performed based on established protocols to detect myofibroblasts; after deparaffinization and rehydration, these sections underwent a heat-mediated antigen retrieval using a citrate buffer (pH 6; Dako/Agilent). The sections were incubated with 0.3% hydrogen peroxide for 30 min to deplete endogenous peroxidase. These sections were then incubated with α-SMA mouse monoclonal antibody (1:200; Dako/Agilent) at 4°C overnight, followed by incubation with a horseradish peroxidase-conjugated secondary antibody at room temperature (20 to 25°C) for 1 hour. Visualization

**Figure 3**—Histological micrographs of the bleb surrounding the Ahmed glaucoma valve (AGV) body (patient 7). The inset boxes indicate areas of interest. A and B—Fibrous capsule surrounding the empty space where the AGV body was located, overlaid on the sclera. The outer wall of the fibrous capsule was composed of collagen fibers that were more mature and denser than those in the inner wall. H&E stain; scale bar = 2,000 μm (A) and 100 μm (B). C and D—Collagen deposition within the fibrous capsule showed that the dense collagen fibers of the outer wall were more prominent than those of the inner wall. Picrorasius red stain; scale bar = 2,000 μm (C) and 100 μm (D). E and F—Accumulation of myofibroblasts (brown), positively stained for α-smooth muscle actin, was observed throughout the fibrous capsule. α-Smooth muscle actin immunostain; scale bar = 2,000 μm (E) and 100 μm (F).
was achieved using 3,3’-diaminobenzidine, and Mayer hematoxylin was applied as a counterstain.

**Statistical analyses**

Breeds, age, sex, the operated eye, duration from AGV surgery to UBM imaging, and IOP at the time of UBM imaging were collected in all patients. Patient data and UBM measurements were expressed as the median and IQR. All statistical analyses were performed using open-source software (R statistical software, version 4.2.0; R Foundation for Statistical Computing). Pearson product-moment correlation was used to assess the relationships between the duration from AGV surgery to UBM imaging and either bleb wall thickness or relative reflectivity, as well as associations between IOP and these parameters. Additionally, a correlation between bleb wall thickness and its relative reflectivity was analyzed. Statistical significance was set at \( P < .05 \).

**Results**

**Demographic details**

Sixteen eyes of 13 dogs that underwent AGV surgery were included in this study (Supplementary Table S1). The breeds comprised American Cocker Spaniel (7), Shiba Inu (2), Dachshund (1), Poodle (1), Coton de Tulear (1), and mixed breed (1). The median age was 9 years (IQR, 7.00 to 11.00). None of the eyes underwent any additional glaucoma surgeries, such as laser cyclophotocoagulation. Of 16 eyes, 4 were not receiving any antiglaucoma medications at the time of UBM imaging, whereas the remaining 12 eyes were receiving 1 to 3 different antiglaucoma medications. The median duration from AGV surgery to UBM imaging was 333 days (IQR, 92.25 to 737.00). The median IOP at the time of UBM imaging was 19 mm Hg (IQR, 15.75 to 33.00), irrespective of the use of antiglaucoma medications.

**Bleb UBM parameters**

Bleb wall reflectivity exhibited a broad range among the patients, with some cases showing significantly higher values by a median of 93.92 (IQR, 78.50 to 157.89). However, scleral reflectivity demonstrated less variability, with a median of 96.87 (IQR, 85.15 to 111.61). The relative reflectivity showed a median of 1.06 (IQR, 0.73 to 1.68). For bleb wall thickness, the median was measured at 1.02 mm (IQR, 0.78 to 1.23).

**Correlation analyses**

The analysis of correlations between the duration from AGV surgery to UBM imaging and bleb characteristics revealed no significant association with either relative reflectivity (Pearson \( r = −0.06 \); 95% CI, −0.54 to 0.45; \( P = .82 \)) or bleb wall thickness (Pearson \( r = −0.05 \); 95% CI, −0.53 to 0.46; \( P = .86 \)). When assessing the correlation between IOP at the time of UBM imaging and bleb characteristics, a significant correlation was observed with relative reflectivity (Pearson \( r = 0.60 \); 95% CI, 0.15 to 0.84; \( P = .01 \)), whereas no significant correlation was detected with bleb wall thickness (Pearson \( r = −0.40 \); 95% CI, −0.75 to 0.12; \( P = .13 \)). In assessing the correlation between bleb wall thickness and relative reflectivity, a significant negative correlation was observed (Pearson \( r = −0.72 \); 95% CI, −0.90 to −0.35; \( P = .002 \)).

**Histological findings**

In the histological examinations of the enucleated eye, the empty space formerly occupied by the AGV body was delineated by a wall of fibroblasts and connective tissue. A few macrophages and foreign-body multinucleated cells were present along the inner wall. Congestion and edema were present adjacent to the outer wall and within the Tenon capsule, accompanied by minimal infiltration of lymphocytes and neutrophils. Picrosirius red staining revealed distinctions between the Tenon loose collagen, dense sclera, and walls of the bleb cavity. The wall was brighter red than the scleral collagen, and the inner wall was more faintly stained. Immunostaining for \( α \)-SMA demonstrated positive staining in the fibroblast-like cells within the walls of the bleb cavity, suggesting the presence of myofibroblasts (Figure 3).

**Discussion**

Timely surgical intervention in glaucoma management is critical in controlling disease progression; however, the role of comprehensive postoperative care is equally vital, significantly enhancing both the longevity and effectiveness of surgical outcomes.\(^1\)\(^–\)\(^5\) The postoperative period is fraught with potential complications, with bleb fibrosis being a notable concern. The bleb fibrosis can lead to irreversible obstructions in the surgically created drainage pathway, which, in essence, can culminate in the failure of the surgery.\(^2\)\(^,\)\(^5\)\(^–\)\(^6\) Therefore, proactive efforts to prevent such irreversible changes and monitor the condition of the bleb are important for ensuring the long-term success of the surgery, emphasizing the necessity of understanding the variations in bleb characteristics and their impacts on postoperative outcomes. In this prospective study, the characteristics of blebs formed after AGV surgery in dogs were evaluated using UBM, with bleb wall thickness and reflectivity as the primary parameters. The relative bleb wall reflectivity, compared to that of the sclera, demonstrated a positive correlation with IOP, and a significant negative correlation was noted between bleb wall thickness and reflectivity.

In human studies\(^18\)\(^–\)\(^20\),\(^27\)\(^–\)\(^28\) from shunt device surgery to nondevice filtration surgery, including trabeculectomy, various methods have been introduced to assess the prognosis of surgery by evaluating blebs. Yamamoto et al\(^18\) and Pavlin et al\(^19\) used UBM to examine the intrableb structure after trabeculectomy and established a classification system for the filtering blebs. They compared bleb wall reflectivity against scleral reflectivity and classified them as low, medium (almost identical between bleb and scleral reflectivity values), and high. These studies revealed that the groups with low or medium bleb wall reflectivity had controlled IOP without medication.
Salhy et al\textsuperscript{20} and Özen et al\textsuperscript{28} similarly evaluated bleb wall reflectivity as in previous studies\textsuperscript{28,29} and confirmed that successful blebs had lower reflectivity than unsuccessful blebs. Similar to these findings, this study has shown a positive correlation between bleb wall reflectivity and IOP. It is imperative to consider that this study compared IOP and reflectivity regardless of the use of antiglaucoma medications. A considerable portion of the eyes in this study were prescribed antiglaucoma medications (12 out of 16 eyes), potentially maintaining controlled IOP levels regardless of the bleb condition. Due to many eyes requiring antiglaucoma medications postoperatively and the limited number of eyes investigated, drawing a significant comparison between eyes maintaining stable IOP without these medications and those requiring them was challenging. Nevertheless, the average relative reflectivity in eyes not requiring antiglaucoma medications was lower by more than half compared to those requiring the medications (0.63 vs 1.32), suggesting that eyes necessitating antiglaucoma medications may exhibit higher bleb wall reflectivity. It was noted that all patients included in this study had closed iridocorneal angles on gonioscopy and collapsed ciliary clefts on UBM before surgery. Given that the majority of the outflow pathways were obstructed in these cases, it can be hypothesized that the characteristics of the bleb may be a major factor influencing IOP in such scenarios.

Tissue trauma inflicted by filtering surgery may lead to a surge in fibroblastic activity. Various cytokines and growth factors can stimulate the accumulation of the extracellular matrix and strengthen the scaffold.\textsuperscript{6,26} As the stimuli persist, the transformation of fibroblasts into myofibroblasts evokes contractile activity associated with bleb scarring, which subsequently inhibits aqueous humor filtration, leading to surgical failure.\textsuperscript{6,29,30} The increase in bleb wall reflectivity observed in various studies\textsuperscript{26,28,31} is presumed to be a manifestation of increased fibrosis, as in the current study. It is known that tissue reflectivity in UBM images primarily represents the density of collagen fibers.\textsuperscript{32,33} From a histological point of view, the more severe the fibrosis, the higher the collagen fiber density.\textsuperscript{34} In previous histological studies\textsuperscript{35–37} on filtering blebs, the collagen in the connective tissue of functional blebs was widely distributed and revealed loosely formed myxoid appearances, whereas the failed blebs comprised dense collagen tissue. In this study, histological examination of the enucleated eye, which failed to maintain controlled IOP after AGV surgery, identified a bleb composed of collagen fibers and fibroblasts with a density similar to that of the sclera. Increased fibrosis, as evidenced by denser collagen, might have contributed to the failure of the bleb. Additionally, the detection of myofibroblasts through α-SMA immunostaining supported the hypothesis that fibroblastic transformation is critically involved in bleb scarring and the impairment of aqueous humor drainage.\textsuperscript{6,29,30}

There are various opinions regarding bleb wall thickness in human patients. Leung et al\textsuperscript{31} demonstrated that nonfunctional encapsulated blebs exhibited thicker bleb walls than functional blebs through anterior segment optical coherence tomography (AS-OCT) analysis. In addition, using AS-OCT, Jung et al\textsuperscript{30} revealed that bleb wall thicknesses were greater in the failed group than in the successful group. They hypothesized that the thinner the wall, the higher the permeability and, consequently, a better controlled IOP.\textsuperscript{30} However, Ciancaglini et al\textsuperscript{25} and Shin et al\textsuperscript{35} measured bleb wall thickness using AS-OCT; no significant association was observed between bleb functionality and thickness. The latter study suggested that aqueous humor flows through the conjunctiva in a bleb with a thin wall, whereas it flows through subconjunctival absorption in a thickwalled bleb.\textsuperscript{39} However, Narita et al\textsuperscript{40} confirmed that the walls of successful blebs were thinner than those of failed blebs (844 ± 289 vs 667 ± 239 μm) using AS-OCT. Waibel et al\textsuperscript{41} also demonstrated similar results (670 ± 51 vs 414 ± 71 μm). This study observed a significant correlation between IOP and relative reflectivity; nevertheless, no such correlation was observed with bleb wall thickness. However, the negative correlation identified between relative reflectivity and wall thickness suggested that an increase in bleb wall reflectivity, potentially indicative of fibrosis, was associated with a tendency for the wall to become thinner (Figure 4).

The decrease in bleb wall thickness may have implications for aqueous humor dynamics within the bleb, influencing its overall performance, which could be significant in relation to various elements that influence bleb morphology and might affect surgical outcomes.\textsuperscript{20,22} Aqueous humor shunted from the anterior chamber to the subconjunctival space is absorbed primarily by a transvenous route and secondarily by transconjunctival egress.\textsuperscript{37,42} The conjunctiva and Tenon capsule must have a high

![Figure 4](image-url)
degree of hydraulic conductivity for these processes to work optimally. However, proximity to the collagen-rich bleb, that is, the encapsulated bleb, lowers the ability of tissue for hydraulic conductivity. In addition, excessive fluid pressure applied to the bleb can cause ischemia, owing to blood vessel collapse in the tissue, and provoke a vicious cycle of chronic inflammation and fibrosis. As the bleb becomes fibrotic via the aforementioned process, IOP gradually increases because of the unabsorbed aqueous humor; therefore, along with the increased internal pressure load within the bleb, compression of the collagen fibers in the connective tissue of the bleb wall may occur, which may be the cause of the bleb wall thickness decrease. The histological examination in this study revealed mild inflammation and high-density collagen fibers in the fibrous encapsulation tissue, consistent with this phenomenon.

This study had some limitations, primarily attributed to the relatively small sample size, which constrains the generalizability of the findings. The patients evaluated were a heterogeneous group with numerous uncontrolled variables present. Future research is warranted to undertake a large-scale study that examines the relationship between various factors that may influence bleb characteristics, such as age, breed, the application of perioperative antiglaucoma medications, owner compliance in applying the medications, whether the medications contained preservatives known to induce fibrosis or not, other surgeries, size of the globe, area of the bleb, and volume of the bleb. Additionally, each patient in this study was subject to UBM examination only once and measured only in one direction, which limited the ability to assess dynamic changes in bleb characteristics over time and observe the bleb’s overall structural features. Nonetheless, even though it does not reflect the complex 3-D aspect of the bleb, this report on the characteristics of the bleb obtained from 3 images might contribute to improved management of canine patients who have undergone AGV surgery. Therefore, further studies incorporating multiple UBM assessments from various imaging directions are needed to comprehensively understand the progressive nature and multidimensional aspects of bleb transitions.

In conclusion, this study evaluated postoperative bleb characteristics following AGV surgery in dogs using UBM, revealing that the increased relative reflectivity significantly correlates with higher IOP. Additionally, a negative correlation was observed between bleb wall thickness and the relative reflectivity, suggesting a potential association with bleb functionality, particularly in fibrosis-related changes. Therefore, the use of UBM to assess blebs may be considered in cases where it is feasible, potentially aiding in the planning of subsequent treatments and contributing to early intervention strategies.

Acknowledgments

None reported.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

Funding

This study was supported by the BK21 FOUR Future Veterinary Medicine Leading Education and Research Center, the Research Institute for Veterinary Science, and the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2021R1I1A1A01058695).

References


22. Lee S, Kim J, Jeong S. Modified trabeculectomy using Ologen® collagen matrix with or without a cyclodestruc


27. Özen B, Yüce B, Öztürk H. The significance of ultra


29. Välimäki J, Usitalo H. Immunohistochemical analy


32. d’Anjou MA, Pennick D. Practical physical concepts and arte


40. Amar N, Labbé A, Hamard P, Dupas B, Baudouin C. Filtering blebs and aqueous pathway an immunocytologi


42. Freedman J. Tube-shunt bleb pathophysiology, the cyto


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org