Retrobulbar neurolytic ethanol injection for the treatment of end-stage canine glaucoma

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ABSTRACT

Background: Glaucoma is a chronic ocular disease of both dogs and humans that results in blindness and ocular discomfort. Most commonly, end-stage glaucomatous eyes in dogs are enucleated to provide comfort. This intervention requires significant financial investment, general anesthesia, and has a psychological impact on some owners. Retrobulbar neurolytic injections are used in humans to provide immediate and long-acting pain relief, while simultaneously preserving the globe.
Objectives: To determine the safety and efficacy of retrobulbar neurolytic ethanol injection in canine eyes with end-stage glaucoma.
Animals: 16 client-owned dogs (19 eyes) diagnosed with end-stage glaucoma.
Methods: All eyes underwent an ophthalmic examination, including Schirmer Tear Testing (STT), intraocular pressure (IOP) measurement, corneal touch threshold (CTT), anterior and posterior segment examination, and fluorescein staining. Subjects were sedated and administered a retrobulbar block with bupivacaine, followed by retrobulbar injection of ethanol or saline solution. At specified time points after the procedure, clients assessed their pet's comfort and side effects of the injections via survey. Subjects returned for enucleation. Owner perceived comfort after the enucleation was assessed at identical post-procedure time points and compared to that achieved with retrobulbar ethanol injection or control solution. Overall client satisfaction with each procedure, as well as the effects of retrobulbar ethanol injection on STT, IOP, CTT, and histological changes in retrobulbar tissues were investigated.
Results: Retrobulbar neurolytic ethanol injections did not signficiantly improve owner perceived comfort compared to control group treatment or provide more comfort than enucleation. Retrobulbar ethanol injections did not signficantly lower IOP, but did significantly elevate CTT. There was a trend towards lower STT in eyes receiving retrobulbar ethanol injections. Retrobulbar ethanol injections were safe, well tolerated, and no differences in client satisfaction with particpation in the study were noted in either injection group. Histologically, globes in the treatment group displayed significantly greater inflammation and fibrosis; retrobublar tissue samples were not significantly different between control and treatment groups with regard to inflammation or fibrosis.
Conclusions and Clinical Importance: Retrobulbar neurolytic ethanol injections were not determined to be an effective globe-sparing alternative treatment to provide analgesia for end-stage canine glaucoma. Enucleation remains an effective way to provide comfort to dogs with elevated IOP.

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GENERAL AUDIENCE ABSTRACT

The glaucomas represent a diverse group of blinding and painful diseases associated with elevated intraocular pressure (IOP). Despite advances in the medical and surgical treatment of glaucoma, the long-term prognosis in dogs remains dismal for IOP control, comfort, and globe retention. Blindness and pain are common long-term outcomes, necessitating surgical salvage procedures aimed at restoring patient comfort, including enucleation (eye removal), intrascleral prosthesis, or intravitreal chemical cyclodestruction. The most commonly performed, effective, and predictable of these options is enucleation, but this requires general anesthesia, a considerable financial investment, risks post-surgical complications, and has a negative psychological impact on some owners.

Retrobulbar neurolytic injections with absolute ethanol have been performed in humans with blind, painful eyes since the early 1900s. Immediate and long lasting pain relief can be achieved from 2 weeks to 2 years after a single injection. The purpose of this study is to determine the safety and efficacy of retrobulbar ethanol injections as a globe-sparing therapeutic option for end-stage glaucoma in dogs.

Nineteen dogs presenting to the VTH ophthalmology service with end-stage glaucoma were enrolled in a prospective, randomized, double-masked clinical trial. Subjects were sedated and administered a retrobulbar injection of ethanol (n=9) or control saline solution (n=10). At specified time points after the procedure, clients assessed their pet's comfort and side effects of the injections via survey. Three weeks later, subjects returned for enucleation and the level of comfort after the enucleation was assessed at identical post-procedure time points and compared to that achieved with retrobulbar ethanol injection or control solution.

Retrobulbar neurolytic ethanol injections did not signficiantly improve comfort compared to control group sham treatment or provide more comfort than enucleation. Retrobulbar ethanol injections did not signficantly lower IOP, but did significantly elevate corneal touch threshold in treated patients. There was a trend towards lower tear production in eyes receiving retrobulbar ethanol injections. Retrobulbar ethanol injections were safe, well tolerated, and no differences in client satisfaction with particpation in the study were noted in either injection group. Further investigation is warranted to determine the optimal volume of retrobulbar ethanol to provide analgesia for patients with end-stage glaucoma as well as to determine the duration of clinical effect of these injections.

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A. Glaucoma: Normal and Abnormal Intraocular Pressure

The canine glaucomas represent an array of neurodegenerative diseases with the commonality of elevated intraocular pressure resulting in progressive retinal ganglion cell death, optic nerve axon loss, and blindness. In dogs, the single greatest risk factor of the development of glaucoma is elevated intraocular pressure.[1]

Maintenance of normal intraocular pressure (IOP) is imperative for the health of intraocular structures, regulation of ocular perfusion pressure, preservation of ocular rigidity, and vision. Intraocular pressure is normally maintained within a physiologic range reported in dogs as 10.8+/-3.1mmHg (TonoVet), 18.7+/-5.5mmHg (TonoPenXL), 15.3+/-2.1mmHg (Perkins), 13.4+/-4.7mmHg (AccuPen), and 15.7+/-4.2mmHg (Mackay-Marg) by a balance of aqueous humor production and outflow.[1-4] Normal adult canine IOP values are established in the first weeks after birth.[5] IOP is not a stagnant value and diurnal variations exist, with normal fluctuations of 2-4mmHg reported in this species.[1] Glaucomatous animals also experience diurnal pressure fluctuations; however, they are more exaggerated with reported fluctuations of 10-15mmHg and the highest levels of IOP in the dog occurring in the morning and lowest in the afternoon.[1,6]

Aqueous humor production occurs in the non-pigmented epithelium (NPE) of the ciliary body by active secretion, ultrafiltration, and passive diffusion. Aqueous humor functions to provide structural rigidity, preserve a constant location of the refracting surfaces of the eye, refract light (refractive index 1.335) at the cornea/aqueous interface and at the aqueous/lens interface, and supply nutrients to the avascular structures of the

eye. The latter function explains why aqueous humor is an ultrafiltrate of plasma and therefore contains dissolved oxygen, electrolytes, cations, anions, and nutrients. Aqueous humor production by passive diffusion, ultrafiltration, and active secretion depends on vascular hydrostatic and oncotic pressure, as well as humoral and autonomic input to match outflow.[1,7] Diffusion of solutes across a concentration gradient occurs at the level of the capillary endothelial cell membrane and the double epithelial layer of the ciliary body and is the route most lipid-soluble substances enter the aqueous humor. Ultrafiltration is the enhanced movement of substances across a membrane due to hydrostatic force; this system relies on pressure differences between the vascular beds of the ciliary body capillaries and intraocular pressure. Active transport mechanisms contribute the majority of aqueous humor production and rely on ATP dependent pumps to move substances against a concentration gradient. Na^+, K^+-ATPase enzymes, concentrated on the lateral walls of the NPE, work in conjunction with carbonic anhydrase to actively secrete aqueous humor into the posterior chamber. Sodium is actively pumped into the posterior chamber in exchange for an unequal amount of potassium that is removed from the posterior chamber. The discordant movement of sodium cations into the posterior chamber subsequently creates an electrical gradient whereby anions (chloride, ascorbate, amino acids) move into the posterior chamber to maintain electroneutrality. Bicarbonate, created as an end product of the carbonic anhydrase reaction, is passively exchanged with a single chloride molecule as it moves into the aqueous humor. The increased osmolarity on the basolateral side of the NPE membrane facilitates of the movement of water through aquaporins into the posterior chamber. Movement of bicarbonate and sodium into the posterior chamber are codependent processes for the formation of aqueous humor.

Aqueous humor travels from the its site of production in the posterior chamber through the pupillary aperture into the anterior chamber where it exits the eye through the iridocorneal angle (ICA). Outflow pathways in the dog are divided into conventional or unconventional routes. The conventional route is also known as the corneoscleral trabecular meshwork or pressure dependent pathway, whereas the unconventional pathway is also called the uveoscleral pathway or pressure independent pathway. In healthy eyes, conventional outflow accounts for approximately 85% of aqueous humor drainage in the dog.[1] Aqueous humor enters the iridocorneal angle by traversing through the rarefactions of the pectinate ligament followed by the uveal trabecular meshwork, corneoscleral trabecular meshwork, and finally the juxtacanalicular zone, which is the site of greatest resistance to outflow. Once through the trabecular meshwork, the aqueous humor can conventionally drain to the angular aqueous plexus, episcleral venules, scleral venous plexus, and the vortex vein system to return to systemic venous circulation. Moreover, a smaller percentage of aqueous humor will dissect posteriorly through the ciliary body musculature to the suprachoroidal space.[8] A minute amount of aqueous humor is resorbed across the surface of the iris, ciliary body, and vitreous body.

Aqueous humor dynamics can be measured utilizing a multitude of invasive or non-invasive techniques including: manometry, constant rate/constant pressure perfusion, fluorophotometry, tonography, episcleral venule cannulation, and episcleral venomanometry.[1,7,9] These methods directly quantify aqueous humor formation, total aqueous humor outflow, and conventional outflow and allow for the estimation of unconventional outflow. Tonography, in combination with fluorophotometry, can reveal aqueous humor flow, conventional outflow, and therefore calculate unconventional

outflow. Mathematically, aqueous humor dynamics are represented by the modified Goldmann equation: IOP =$(F_{in}-F_u)/C_{trab}$ +EVP, where F_{in} is aqueous humor inflow, F_u is uveoscleral outflow, C_{trab} is the facility of trabecular meshwork outflow, and EVP is the episcleral venous pressure.[1,7] Total facility of outflow in the normal dog is reported as 0.24-0.30 uL/mmHg/min, as measured by tonography.[1] EVP in the normal dog is approximately 11 mmHg (range 10-12mmHg).[1,9] Aqueous humor formation in the dog is reported as 5.22 uL/min.[1]

B. Glaucoma: Pathogenesis

The single greatest risk factor for the development of glaucoma in dogs is elevated intraocular pressure. To date, the canine glaucomas all result from compromised outflow facility and hypersecretory, normotensive, and low-tension glaucomas have not been identified. Elevated IOP results in five consistent stages of disease regardless of the inciting etiology: (1) an initial event involving the aqueous humor outflow pathway, (2) physical changes causing aqueous humor outflow obstruction, (3) elevated IOP causing disruption of normal optic nerve axoplasmic and blood flow, (4) retinal ganglion cell dysfunction and death resulting in optic nerve degeneration and atrophy, and (5) visual field loss and blindness.

Various schemes exist to classify the glaucomas. Most commonly, the diseases are divided into congenital, primary, and secondary etiologies based upon the initial event leading to physical changes in the aqueous humor outflow pathway and subsequent obstruction. Congenital glaucoma is very rare and refers to extensive goniodysgenesis or

trabecular meshwork maldevelopment. Congenital glaucoma often coexists with other ocular malformations, particularly in the anterior segment. Primary glaucomas are heritable diseases that occur bilaterally and result in IOP elevation without concurrent ocular disease. The iridocorneal angle is characterized as open, closed, narrowed, or dysplastic (pectinate ligament dysplasia, goniodysgenesis). In primary glaucoma, the obstruction of the iridocorneal angle, as seen on gonioscopy, or of other outflow pathway structures deeper to open or dysplastic iridocorneal angles results in elevation of IOP.[10-12] Secondary glaucomas encompass diseases where concurrent ocular disease is present and explains the cause of elevated intraocular pressure. Numerous causes of secondary glaucoma exist including: uveitic (lens induced or other), post lensectomy (pseudophakic, aphakic), intraocular neoplasm, melanocytic, cystic, hyphema, lens displacement (anterior luxation, subluxation, posterior luxation), and pharmacologic (mydriatics, corticosteroids, non-steroidal anti-inflammatory agents).[1,7,12-17] The underlying mechanisms for pressure elevation have not been fully elucidated in all cases, but common lesions contributing to outflow obstruction include peripheral anterior synechiae, pupillary obstruction, iris bombe, formation of pre-iridal fibrovascular or cellular membranes, endothelialization or descemetization of the iridocorneal angle, infiltration of the iridocorneal angle with melanocytes, pigment, lens material, neoplastic cells, cysts (and their contents), and inflammatory cellular (red blood cells, white blood cells) or fibrinous debris.[1,12, 18-21]

Other causes of secondary glaucoma exist in the dog, though they are considered less common. This includes malignant glaucoma, which results in ciliovitreal or ciliolenticular block of the pupil due to aqueous humor misdirection from the posterior chamber through discontinuities in the anterior hyaloid face. Traumatic glaucoma from

blunt or penetrating trauma is very rare. Glaucoma secondary to silicone oil presentation in the anterior chamber after vitreoretinal surgery for the repair of rhegmatogenous retinal detachments can physically obstruct aqueous humor outflow pathways and necessitates manual removal.[22] Likewise, long standing rhegmatogenous retinal detachment can elevate IOP as normal rod and cone outer segments can physically obstruct the outflow pathways or formation of pre-iridal fibrovascular membranes across the iridocorneal angle are stimulated from vascular endothelial growth factor signaling from the hypoxic retina.[18,23] A form of phacomorphic glaucoma has also been identified in dogs with intumescent cataract causing forward displacement of the lens, iris base, and promoting pupillary block.[1]

Elevated IOP initiates the neurodegeneration of the retina and optic nerve through various mechanisms that are still actively investigated. Ocular perfusion pressure is determined by subtracting IOP from the mean arterial pressure of the external ophthalmic artery and is decreased with elevated IOP or decreased systemic blood pressure.[24-25] The blood flow volume and velocity to the retina and optic nerve head are autoregulated, but with chronicity or high elevations in IOP this autoregulation is overwhelmed and ocular perfusion to these structures decreases. Evidence for retinal ischemic-reperfusion injury as well as decreases in short posterior ciliary artery number, lumen diameter, and optic nerve vascular velocity have recently been reported as contributory mechanisms for degeneration of these delicate structures in acutely glaucomatous dogs.[25-30] Large diameter and peripheral retinal ganglion cells are most sensitive to pressure elevations that disrupt axoplasmic flow and induce axon collapse, with subsequent atrophy and degeneration. The disruption of axoplasmic flow occurs most significantly at the scleral lamina cribrosa,

where extracellular matrix abnormalities contributing to architectural collapse in the face of elevated IOP can exacerbate this loss of flow. Disruption in axoplasmic flow deprives retinal ganglion cells of brain-derived neurotrophic factor promoting degeneration. The loss of retinal ganglion cells and their axons can cause a cascade of neural die back evident at the lateral geniculate nucleus.[31] Furthermore, local retinal and vitreal amino acid (glutamate) excitotoxicity injury perpetuate neural cell loss. The excess glutamate is postulated to come from dying retinal ganglion cells releasing intracellular stores, leakage from diseased retinal ganglion cell membranes, or disruption of glutamate removal systems by Muller cells.[1,25] Excessive extracellular glutamate stimulation induces intracellular calcium elevations, which disrupt normal mitochondrial function and promote the generation of radical oxygen species and apoptosis of inner and outer retinal segments and photoreceptors, resulting in visual field loss.

C. Glaucoma: Epidemiology

Glaucoma occurs with relative frequency in canine and human patients, with the literature citing a prevalence of 1-2% in the general canine population, including both primary and secondary causes.[1] Certain breeds have been noted to be at increased risk (incidence >2%) for primary glaucoma, including the American Cocker Spaniel, Basset Hound, Chow Chow, Shar Pei, and Boston Terrier.[1,23] The most recent epidemiologic study from Zurich reported an average age of onset for primary glaucoma of 7.3+/-3.6 years and breed predispositions for the Siberian Husky, Magyar Vizsla, and Newfoundland.[31] Interestingly, as in human patients, females are more often affected by primary glaucoma

than males, and in that study the gender ratio (F:M) was reported as 1.41:1 and ratios of greater than 2:1 have been noted within specific breeds, such as the Siberian Husky.[1] Gender differences including a significantly smaller iridocorneal angle opening distance and smaller anterior chamber depth and angle recess area are thought to contribute to the female propensity for this disease.[33]

The genetic risks for primary glaucoma have been identified in a few breeds afflicted with primary open angle glaucoma. Two mutations in the ADAMTS10 gene are responsible individually for primary open angle glaucoma in the Beagle and Norwegian Elkhound.[34] A mutation in the ADAMTS17 gene, which is responsible for primary lens instability, a known risk factor for secondary glaucoma, is responsible for primary open angle glaucoma in Petit Basset Griffon Vendeen.[34] These genes are inherited in an autosomal recessive fashion and require homozygosity to become phenotypically affected.

In the same epidemiologic study from Zurich, secondary glaucoma was reported to occur at an average age of 7.7+/-3.6 years and was most commonly diagnosed in Cairn Terriers due to ocular melanosis and Jack Russell Terriers secondary to lens displacement.[14] The most common causes of secondary glaucoma are anterior uveitis (23-44.9%), secondary to cataract surgery (5-15.8%), and due to lens displacement (15.2-22.6%).[14] Individual breeds appear to be at higher risk for secondary glaucoma as well. Boston Terriers are at significantly higher risk for blinding complications after cataract surgery (OR=290.44) with a greater than non-Boston Terrier incidence of glaucoma (38%).[35-36] Furthermore, the Labrador Retriever is reported to be at a significantly higher risk (35%) for the development of glaucoma post-phacoemulsification compared to non-Labradors.[37] In the Labrador Retriever, experiencing post-operative ocular hypertension

after phacoemulsification and increasing age were risk factors identified for the development of secondary glaucoma.[37] A histologic survey of eyes enucleated post-phacoemulsification for glaucoma highlighted the Cocker Spaniel, Shih Tzu, Bichon Frise, and Jack Russell Terrier as breeds considered at higher risk for pseudophakic or aphakic glaucoma.[36]

D. Glaucoma: Diagnosis and Clinical Signs

The diagnosis of glaucoma in veterinary medicine is typically made via tonometry, gonioscopy, and ophthalmoscopy. There are three types of tonometry: indentation, applanation, and rebound, with the latter two being the most commonly used. The TonoPen is an applanation tonometer that relies on the Imbert-Fick law, whereby pressure within a sphere is roughly equal to the external force needed to flatten a portion of the sphere. As such, applanation tonometers require corneal contact and therefore corneal anesthesia prior to use. Conflicting reports exist regarding the effect of topical anesthetics on IOP measurement. The TonoVet is a rebound tonometer that propels a magnetized probe and the rate of deceleration after rebounding off of the cornea generates an electrical signal that is translated to a measurement of pressure. The device must be held with the probe perpendicular to the central cornea and parallel with the floor, but topical anesthesia is not required. Both tonometers are subject to false readings due to technique, head and body position of the patient, periocular manipulation, extraocular muscle tone, tension on the neck, and temperament of the patient.[38-41] At higher IOP values, the TonoPen more often underestimates true IOP in comparison to the TonoVet.[1] Periodic screening of IOP is most

useful for cases of open angle glaucoma; however, the vast majority of veterinary patients have closed angle glaucoma, acute congestive events, or have angle closure occur concurrently with IOP elevation obviating the utility of periodic IOP measurement to "screen" for glaucoma. Once diagnosed, loss of IOP control, effectiveness of treatment, or determination of the need for additional therapy can be evaluated over the course of the day using an IOP curve that can highlight large diurnal fluctuations or loss of IOP control during non-waking hours. [42]

Gonioscopy permits visualization of the iridocorneal angle, pectinate ligament, and innermost part of the ciliary cleft. A variety of goniolenses exist that allow for direct (Koeppe, Lovac-Barkan, Wurst, Swan-Jacob, Richardson) or indirect (Goldmann, Posner, Sussman, Zeiss) visualization of the outflow pathway. Both systems require topical anesthesia and the use of a coupling gel to protect to the corneal surface and allow for 360-degree inspection of the angle. Direct and indirect viewing systems have been created that may or may not require rotation of the goniolens or movements of the observer to inspect all quadrants. Gonioscopy is useful to subjectively assess the state of the iridocorneal angle as open, narrowed, closed, or dysplastic (goniodysgenesis/pectinate ligament dysplasia). Given the subjectively of the diagnostic, no standardized and universally applied evaluation scheme exists, although many have been reported in various research scenarios.[11,43-45] Furthermore, when serial gonioscopic evaluations occur it's important that the user uses the same lens and assessment scheme to limit intrauser variability. Goniodysgenesis is an umbrella term that includes all forms of congenital iridocorneal angle malformation and, to that end, also includes pectinate ligament dysplasia. Serial gonioscopic evaluations of pectinate ligament dysplasia in the Welsh Spring Spaniel, Flat-

Coated Retriever, and Leonberger demonstrate this condition is more dynamic then previously reported and can progress overtime to complete angle closure and glaucoma; serial gonioscopic examinations are recommended in dogs of these breeds intended for breeding.[11,43-44]

Ophthalmoscopy, using both direct and indirect techniques, is employed for assessment of optic nerve neuroretinal rim thinning and optic cup enlargement consistent with glaucomatous damage. These tests are more useful in screening patients for signs of neurodegeneration prior to documented IOP elevation. In patients diagnosed with glaucoma, these tests enable the observer to monitor for disease progression despite the appearance of adequate control; for instance, in cases of primary closed angle glaucoma where a patient's IOP is within a normal range during a clinical appointment, progressive optic nerve head degeneration signals dysregulation outside of the IOP measurement periods.[46]

Glaucoma causes an array of clinical signs that vary along the timeline of acute or chronic elevation of IOP. Acute clinical signs include blepharospasm, ocular discharge, episcleral venous congestion, corneal edema, mydriasis, and vision loss. With chronic elevations in IOP, non-specific corneal neovascularization, iris degeneration, buphthalmos, lens instability or displacement, retinal degeneration, optic nerve cupping, blindness, and discomfort may be seen.

E. Glaucoma: Current Treatments

Regardless of etiology, elevated intraocular pressure must be addressed with hypotensive therapy until the patient's intraocular pressure normalizes. Both medical and surgical therapeutic options exist with the goal to normalize IOP, preserve vision, and reduce discomfort. In emergent settings, pressure can be precipitously dropped with oral glycerol, intravenous mannitol, or anterior chamber paracentesis.

Short term IOP control can be achieved with topical or orally administered medications multiple times a day with the intent to decrease aqueous humor production or enhance conventional or unconventional outflow. Miotic cholinergic drugs including direct acting parasympathomimetics (pilocarpine and carbachol) as well as indirect acting parasympathomimetics (demecarium bromide, and echothiophate iodide) can significantly lower IOP. The mechanism of action of these drugs in small animals has been studied with tonography and shown to increase conventional outflow in both normal and glaucomatous beagles; nevertheless, the local side effects, limited efficacy compared to alternative therapy, commercial unavailability, and effect on ciliary body musculature make the use of this class of drugs in clinical patients or patients on medications to enhance uveoscleral outflow infrequent.[47] Adrenergic agonists (epinephrine, dipivefrin, apraclonidine, brimonidine) and adrenergic antagonists (timolol, levobunolol, betaxolol, metipranolol, carteolol) function to lower aqueous humor production and may enhance outflow, however, their mechanisms of action are also not completely understood. Of these agents, the non-selective beta antagonist, 0.5% timolol, is most commonly used in glaucomatous dogs with contraindications in patients with cardiovascular or respiratory compromise as bradycardia and bronchospasm can be induced from topical use.

Carbonic anhydrase isoenzymes II, III, and IV are present in the canine ciliary epithelium and their contributions to aqueous humor production can be systemically (acetazolamide, dichlorphenamide, methazolamide) or topically (dorzolamide, brinzolamide) inhibited. Carbonic anhydrase is a ubiquitous enzyme, so topical administration is advantageous to limit systemic side effects. Topically these medications are effective, well tolerated, and commercially available and as such are commonly utilized in clinical cases to reduce aqueous humor production. Punctate keratitis and blepharitis have been reported as side effects of topical carbonic anhydrase inhibitors; however, these effects are self-limiting after cessation of use.[48]

Prostaglandin analogues are exceptionally potent topical hypotensive agents. Unosprostone, latanoprost, travoprost, bimatoprost, and tafluprost are commercially available prostaglandin F_2a analogues that induce rapid ocular hypotension and supraphysiologic miosis.[1,9,49-50] These agents are pro-inflammatory and are contraindicated in cases of uveitic glaucoma and anterior lens luxations, as they will worsen pupillary blockade in this latter scenario.

Alternative topical therapies including purified 2% and 0.248% bovine and porcine neurohypophysis and 2% delta-9-tetrahydrocannabinol ophthalmic solutions cause significant IOP reduction in normal and glaucomatous canines.[51-52]

In addition to IOP control, strategies to address neurotoxic and neurodegenerative processes are considered in the management of glaucoma. Glutamate induced intracellular hypercalcemia resulting in cellular excitotoxicity and apoptosis and ischemic-reperfusion injury are significant pathologic events resulting in neurodegeneration. The efficacy of oral

calcium channel blockers (amlodipine) has yet to be demonstrated as an effective retinal neuroprotectant; however, its use in acute canine glaucoma is common. In murine models of ocular hypertension, methylprednisolone, minocycline, and timolol have the potential to provide early or late benefit in retinal ischemia-reperfusion injury.[53]

Medical therapy for canine glaucoma will ultimately fail to control IOP in all cases. In cases of primary glaucoma, when only one eye is affected, conflicting results regarding prophylactic treatment of the fellow eye are reported. Miller prospectively demonstrated delay in pressure elevations in prophylactic treated eyes of dogs with primary closed angle glaucoma using 0.5% betaxolol alone or combination 0.25% demecarium bromide and betamethasone/gentamicin.[54] Likewise, Dees concluded using 0.125% demecarium, 0.005% latanoprost, 2% dorzolamide, and 0.25% demecarium in combination with adjunctive topical anti-inflammatory corticosteroids or topical nonsteroidal anti-inflammatories was beneficial.[55] Conversely, a retrospective study performed by Stavinohova showed no preventative effect of 1% brinzolamide, 2% dorzolamide or combination 2% dorzolamide/0.5% timolol and a study using anterior-segment optical coherence tomography by Tsai suggested limited utility of latanoprost as a prophylactic treatment for primary angle closure glaucoma.[56-57]

When medical therapy fails or progressive optic nerve damage is documented, surgical intervention to preserve vision, control IOP, and prevent pain is indicated. Cyclodestructive procedures as well as gonioshunt implantation function to decrease aqueous humor production and provide an alternative outflow pathway, respectively. Both types of procedures require financial investment, general anesthesia, carry the risk of complications, and do not control IOP in the long term. Cyclophotocoagulation causes

coagulation necrosis of the ciliary body with light energy and has been described with diode laser (810 nm; near infrared spectrum) energy as well as other laser systems (neodymium-yttrium aluminum garnet (Nd:YAG), argon, and krypton). Diode laser energy can be delivered to the ciliary body through transscleral or intraocular (endolaser) routes and is preferentially absorbed by melanin containing tissue.[58] No comparative studies exist comparing the efficacy of transscleral cyclophotocoagulation to endolaser cyclophotocoagulation, although the latter is proposed to provide the advantage of direct visualization of the ciliary processes, which may be in altered anatomic locations due to buphthalmos.[1] In addition to unpredictable long-term effectiveness, cyclodestructive procedures can induce significant ocular morbidity including pain, decreased vision, hypotony, corneal ulceration, phthisis bulbi, cataract, and retinal detachment. Gonioshunt implantation is most commonly performed with either Ahmed valved or Baerveldt non-valved implants.[59-60] Long term IOP control (median 326 days) has been reported as favorable for the majority of patients in recent case series following Ahmed gonioimplantation.[61] Complications from gonioshunt implants include shunt occlusion, hypotony, shunt extrusion, and shunt failure due to capsular fibrosis surrounding the drainage plate; the latter effect can be mitigated with the use of mitomycin C and possibly pirfenidone.[61-62] Clinically cyclophotocoagulation and gonioshunt implantation are often performed concurrently with long term IOP (IOP<20mmHg) control and vision maintained 12 months later in approximately 70% and 50% of cases respectively.[59]

Ultimately, canine patients become refractory to both medical and surgical therapies and blindness and pain ensue. Therapeutic options for these eyes is directed at pain control and are limited to enucleation, evisceration with intrascleral prosthesis, and

pharmacologic destruction of the ciliary body with intravitreal injection of gentamicin or cidofovir. Globe removal via enucleation is overall the most commonly performed and uniformly effective salvage procedure for end-stage glaucoma, with owners invariably reporting favorable outcomes and notably increased comfort in their pet following the procedure, as indicated by increased activity or playfulness, increased appetite, decreased guarding behavior, and other observed changes.[1,63-65] While complications rates are low, reported negative outcomes of enucleation include hemorrhage, edema, infection, contralateral blindness from damage to the optic chiasm, orbital epithelial cyst formation, orbital emphysema, intraorbital implant infection and extrusion, and dehiscence.[63] This procedure also requires general anesthesia, a considerable financial investment, and has a negative psychological impact on some owners. In certain cases, owners may elect for euthanasia rather than bilateral enucleation. Intrascleral prosthesis is a cosmetic alternative to enucleation, albeit with a somewhat higher complication rate.[63,66] It is costlier than enucleation and requires specialized training and equipment, rendering it an impractical option for general practitioners. Additionally, patients undergoing intrascleral prosthesis remain at risk for the development of adnexal and corneal disease postoperatively, including corneal ulceration, implant extrusion, and keratoconjunctivitis sicca ("dry eye"). Additionally, significant comorbidities such as systemic disease that would make general anesthesia a risk to patient survivability, neoplasia or infectious diseases carrying a poor long-term prognosis may render enucleation or evisceration and intrascleral prosthesis an impractical option. Intravitreal gentamicin injection can be performed under topical anesthesia, sedation, or general anesthesia and has a reported failure rate of 35%, with 50% of eyes that fail the first injection not responding to a second injection.[1] Whereas others

have reported first time injection success rates as high as 86.4%[67] Previously reported negative outcomes of ciliary body ablation include chronic intraocular inflammation, pain, corneal opacity, intraocular hemorrhage, and phthisis bulbi.[1,68] Recently, a suggested potential correlation between malignant iridociliary epithelial tumors and ciliary body chemical ablation was reported.[68] Intravitreal cidofovir injection, although effective, provides no clear advantage over intravitreal gentamicin injection and is generally cost prohibitive.[69]

F. Glaucoma: Prognosis

Though numerous strategies exist for the management of glaucoma in the dog, veterinary ophthalmologists are still commonly presented with patients that respond poorly to the therapies available. Due to disease heterogeneity, individual patient prognosis is impossible to predict, with the variables of patient age, breed, magnitude of pressure elevation, chronicity of IOP elevation, and response to medical therapy all potentially affecting long-term outcome. Additionally, impairment of axoplasmic flow and evidence of neurodegeneration have been demonstrated at pressures within what is considered the normal range, prior to angle closure and fulminant congestive events that would be noticed by owners. This highlights the difficulty of catching this disease in earlier stages when medical and surgical interventions might be most advantageous.

With sufficient time, all patients with primary glaucoma will develop the condition bilaterally. Equally, medical and surgical IOP control strategies will ultimately fail to control IOP. Development of additional therapies may aid in further reduction of these

poorly responding cases, thus improving our ability to manage this uncomfortable and blinding disease.

CHAPTER 2: NEUROLYTIC INJECTION LITERATURE REVIEW

A. Neurolytic Injection: Therapeutic Use

Neurolytic injections for neuromuscular pain, trigeminal neuralgia, sympathetic blockade, inhibition of muscular spasticity, and pain of malignancy have been used in human medicine since the early 1900s[70-74]. These injections not only eliminate sensation of pain, but decrease the need for parenteral or enteral pain medication dependency, and promote post-operative rehabilitation. Injections can be performed percutaneously with knowledge of relevant anatomy or the aid of radiography, fluoroscopy, peripheral nerve stimulators, computed tomography, or ultrasound guidance. Neurolytic injections are reported at most sensory ganglia or peripheral nerves including: the retrobulbar space, intercostal nerve, intervertebral space and ventral nerve roots, intrathecal, at the celiac plexus, superior cervical ganglion, oval foramen, hip adductors, hamstring muscles, and hip flexors.[75] Effectiveness of these blockades is variable, but most reports indicate long-term pain control.[70,72,74]

In veterinary medicine, neurolytic injections have been performed experimentally in dogs, rabbits, and rodent models and clinically in dogs and horses.[76-83] Reported locations include the sciatic nerve, retrobulbar space, intrathecally, acupuncture points, lumbar muscles, abaxial sesamoid nerve, tibial and fibular nerves, palmar digital nerve, and the tarsometatarsal, intertarsal, and proximal interphalangeal joints.[76-77,79-88] Despite prohibition from the American Quarter Horse Association, neurolytic injections of tail motor function are routinely performed to limit undesirable tail movement in

performance horses. Similar to human medicine these blocks are performed with substantial knowledge of relevant anatomy, peripheral nerve stimulators, and perineural ultrasound guidance.[87]

Numerous agents have been clinically reported to effectively induce neurolysis including: 5-7% phenol, 2% chlorocresol, chlorpromazine, formaldehyde, 8-32% lidocaine, 80-100% ethanol, phenol-ethanol combinations, resiniferatoxin, 5% phenol-glycerol combination, botulinum toxin type A, capsaicin, and n-Butyl-p Aminobenzoate.[82-92] A distillate of the pitcher plant is commercially available (Sarapin), but clinical studies have failed to prove its efficacy.[93] The mechanism of action is not completely understood for every agent, but common histologic lesions include hemorrhagic necrosis, nerve fiber dissolution, Wallerian degeneration, surrounding soft tissue necrosis, dispersion of nerve fiber debris into the endoneurium, as well as peripheral nerve regrowth over time.[76,88-92,94-96] Nerve fiber regrowth varies in timeline depending on the agent used, volume injected, and nerve ablated, but is usually evident after approximately 3 months.[76,94] Nevertheless, signs of nerve regeneration do not necessarily correlate to loss of clinical improvement or immediate functional return. Of these agents, phenol and ethanol are most commonly used. Reported side effects of neurolytic agents include pain at the injection site, paralysis, soft tissue (muscular, vasculature, skin) damage, increased mean arterial blood pressure and heart rate, lack of effectiveness, and transient neurologic symptoms.[75,96-98]

Retrobulbar neurolytic injections have been performed in humans as a treatment for blind, painful eyes since the 1900s.[89-92] Neurolytic substances used in this manner include absolute ethanol (80-100%), phenol, and chlorpromazine.[89-92] Retrobulbar ethanol injection may be preferred if the eye is cosmetically normal or the patient is medically or psychologically unsuitable for enucleation or evisceration.[89-90] Ethanol injection provides pain relief by coagulation of proteins and precipitation of lipids of sensory nerve fibers.[89,91] Immediate pain relief is achieved in the majority of cases. Success rates of up to 87% for pain control lasting 2 weeks to 2 years are reported after a single injection.[76,89-91,95,99-101] However, pain may return as peripheral sensory nerves regrow. The safety and effectiveness of retrobulbar injections performed in conscious humans and unconscious canines has been established using local anesthetics to provide internal and external ophthalmoplegia, analgesia, and decreased need for postoperative pain medications following orbital or intraocular surgery.[102-104] Risks of the retrobulbar injection procedure include retrobulbar hemorrhage, intravenous injection of drug, globe perforation, intrathecal drug administration, apnea, cardiac arrest, and brainstem anesthesia.[78,102,105] The incidence of scleral perforation, the most severe complication of retrobulbar injection, was reported as 0.007% in humans after reviewing 26,857 consective injections.[106] Likewise, in a recent study, dogs receiving retrobulbar bupivacaine injections utilizing the inferiotemporal technique did not experience any adverse events.[78]

Retrobulbar alcohol injections in humans and experimental rabbit and rodent models are associated with minimal and transient immediate side effects. Retrobulbar administration of bupivacaine prior to ethanol is advocated to mitigate pain associated with

ethanol injection and to ensure proper placement of the injection into the intraconal space as demonstrated by mydriasis, corneal anesthesia, and centralization of the visual axis.[78] Moreover, after correct placement of bupivacaine, the needle is left in place not only to limit patient discomfort, but to also ensure that subsequent intravenous or intrathecal ethanol administration is avoided. Chemosis, blepharoedema, ptosis, external ophthalmoplegia, and facial swelling are transient side effects of retrobulbar alcohol injection that resolve without medical intervention.[76,90,98-99,101] In humans, magnetic resonance imagining and histologic evaluation of orbital tissues after retrobulbar ethanol injection shows mild changes consistent with inflammation.[100] Histologically, orbital tissues display fibrotic changes in humans and rat orbits, which may increase the difficulty in subsequent removal of the eye, if necessary.[95,97,100] Interestingly, a marked lowering of IOP following retrobulbar ethanol injection has been noted in humans and rabbits.[76,91] The underlying mechanism for the IOP lowering effect is unknown, but is hypothesized to be directly related to provocation of active hyperemia in the orbit and indirectly due to neurogenic influence.[76,91] This IOP lowering effect may contribute to preservation of globe size throughout the duration of the neurolytic effect.

B. Retrobulbar Injection: Technique and Relevant Anatomy

Local anesthesia is employed in a variety of ophthalmic procedures including blepharoplasty and adnexal surgery, corneal diagnostic sampling, intraocular pressure measurement, standing corneal surgery, and intraocular surgery. Anesthetic agents can be injected along the route of superficial nerves that can be palpated along the orbit, applied as topical medications, or injected more invasively through peribulbar/Sub-Tenon's,

intracameral, or retrobulbar routes. Depending on the route of delivery and intended target, regional anesthesia facilitates anesthesia of the eyelids, globe, or intraocular structures, induces akinesia of the eyelids and extraocular muscles, achieves mydriasis, abolishes the vagal induced oculocardiac reflex, and can negate the need for systemic neuromuscular blockade and mechanical ventilation.

Retrobulbar injections are performed commonly in clinical patients. Equine patients commonly suffer from ophthalmic disease. Retrobulbar injections delivered through the supraorbital fossa or in a Sub-Tenon's route enable clinicians to perform standing corneal and adnexal procedures limiting the need for general anesthesia in a species with greater anesthetic complications.[107] Four-point block and Peterson block techniques have been reported for use in bovine patients to facilitate standing enucleation or exenteration.[108] In canine and feline patients, these techniques are most often used in blinded eyes undergoing removal. The reported benefits of retrobulbar anesthesia prior to enucleation in small animals include lower inhalant anesthetic requirements, abolition of wind-up pain phenomenon, abolition of the oculocardiac reflex, and decreased need for post-operative rescue analgesia.[102-104] Achieving retrobulbar anesthesia can be technically challenging. Dissolvable orbital hemostatic sponges soaked in local anesthetic and orbital splash blocks have shown equal efficacy to retrobulbar local anesthetic injections; however, these techniques are employed after globe removal and do not provide the benefit of oculocardiac reflex or wind-up pain phenomenon abolition, which may increase perioperative anesthetic or analgesic requirements.[103-104]

Successful delivery of pharmacologic agents to the retrobulbar space requires a thorough understanding of the canine orbital anatomy, as well as consideration of the individual patient's axial globe length, superior-inferior globe diameter, skull morphology and therefore orbital depth, and relative position of the globe within the orbit (degree of enophthalmos or exophthalmos). Drug deliver can occur within the intraconal or extraconal space, with the intraconal space being the ideal target to affect the optic, oculomotor, trigeminal (ophthalmic and maxillary branch) and abducens nerves, as well as the ciliary ganglion. Compared to the perimandibular and combined superior-inferior peribulbar injection techniques, the inferior-temporal palpebral technique was determined to be the easiest to perform and most efficacious at drug delivery to the intraconal space in a latex injection cadaver model and contrast injection MRI study in dogs.[78] Using this technique, the inferior lid hair is clipped and the skin aseptically prepped with dilute Povidone iodine or Betadine™ solution and commercial eyewash solution. An approximate 20-degree angle (created by mechanical bending) of a 1.5-inch, 22-gauge spinal needle is positioned at the inferior orbital rim and inserted through the inferior lid at the junction of the middle and temporal thirds. Advancement proceeds until a slight popping sensation is detected to indicate the orbital fascia has been pierced. The needle is then directed slightly dorsally and nasally toward the apex of the orbit and advanced approximately 1-2 cm prior to aspiration and then injection of the desired substance. Consensus on the appropriate volume to inject is lacking, but given the diversity of orbital morphology in canine patients it stands to reason that these factors may influence the efficacy of a given injectate volume. Traditionally, drug volume is calculated based on patient body weight, ignoring the influence of skull morphology and body condition

score.[102-104] More recently this methodology has been called into question by Klaumann who reported a relationship between skull morphometrics from dolichocephalic, mesaticephalic, and brachycephalic breed cadavers and intraconal volume.[109] This study suggested a formula of 0.1mL/cm Lcr be used to calculate total intraconal anesthetic volume, whereby where Lcr is the distance in centimeters between the inion (center of the external occipital protuberance) and nasion (junction of the right and left nasofrontal sutures).[109] A calculated volume of contrast agent using the aforementioned formula was injected into cadaver heads and imaged with computed tomography to demonstrate the superiority of this calculation to volume calculation based on body weight.[109]

C. Assessment of Pain in Veterinary Medicine: Focus on Ocular Pain

The American Animal Hospital Association and American Association of Feline Practitioners identified ocular pain as a common source of overlooked pain in companion animals.[110] Although subjective, the accuracy of using a pain scoring system is enhanced by having a single observer assess all subjects.[111] Furthermore, it is now accepted that the most accurate method for evaluating pain in animals is not by physiologic parameters, but by observations of pain.[110] Various validated schemes for clinicians and owners to assess discomfort in companion animals exist; however, these schemes are generally reserved for patients within an intensive care unit setting or those who have undergone visceral or orthopedic surgery.[112] Few veterinary studies exist investigating ocular pain in companion animals. The majority of these studies are performed assessing post-operative pain control in end-stage diseased eyes undergoing enucleation.[102-104,113] Clinical assessment of post-operative ocular pain in salvageable eyes undergoing

phacoemulsification are reported using modifications of pain score assessment schemes created by the University of Melbourne and Sammarco.[114-115] These studies only included clinician assessment of discomfort while the patient was hospitalized, potentially ignoring decline or progression of post-operative pain after discharge.

Anecdotally, veterinary ophthalmologists agree that most owners are unaware of the impact of ocular pain on their glaucomatous pet, yet in hindsight after globe removal, owners commonly report improved social behavior, comfort, and activity. This assumption was recently reported as an abstract at the annual meeting of American College of Veterinary Ophthalmologists. The results of an owner survey to assess signs of ocular pain associated with end-stage glaucoma and satisfaction with enucleation revealed one-hundred percent of respondents would choose enucleation again over commonly discussed alternatives.[65] More specifically, 68% of respondents reported their dog slept less after enucleation, 63.6% noted an increase in play, 36.4% an increase in appetite.[65] Overall, 91% of owners though their pet's quality of life was better after enucleation.[65] In agreement with this study, overall client satisfaction was recently investigated for dogs undergoing bilateral enucleation (either simultaneously or over time) and was found to be high with 90% of clients reporting a positive effect on their dog's quality of life.[64] As veterinarians, we make medical recommendations with the best interest of our patients in mind, but understand that these recommendations must also conform to a client's individual goals for their pet. Removal of the globe has a negative psychological impact on owners, especially in dogs who may only have one eye

remaining and it is not uncommon for owners to refuse or substantially delay globe removal, despite strong medical recommendations, or clinician assessment of overt pain.

D. Conclusions and Research Justification

Unfortunately, in our canine patients, medical and surgical treatments for glaucoma almost universally fail at maintaining control of IOP over the long-term. Eyes that have become refractory to medical or IOP-lowering surgical treatment are quickly blinded and serve only as a source of discomfort for patients. The limited long-term success of both medical and surgical therapies means that salvage procedures to manage ocular pain and provide a cosmetically acceptable outcome are ultimately recommended. Equally, medical and surgical intervention taxes owners' financial resources and can strain relationships with their pets. Globe removal via enucleation is overall the most commonly performed and uniformly effective salvage procedure for end-stage canine glaucoma. However, this procedure risks minor complications, requires general anesthesia, a considerable financial investment, and has a negative psychological impact on some owners. In certain cases, owners may elect for euthanasia rather than unilateral or bilateral enucleation. Terminal life decisions can stem from psychosocial factors or culture differences, as has been documented in pediatric retinoblastoma patients where parental decision to allow or refuse potentially lifesaving enucleation has been studied.[116] Compounding factors were recently investigated at the Retinoblastoma Clinic of the Philippine General Hospital and beliefs that cancer is a fatal illness, the fear of unacceptable esthetic outcome of the surgery, and the cost of treatment were identified as common barriers to parental decision-making toward enucleation for their children.[116]

Additionally, significant comorbidities such as systemic disease that would make general anesthesia a risk to patient survivability, neoplasia or infectious diseases carrying a poor long-term prognosis may render enucleation an impractical option for owners of domestic species. There is a need for additional cosmetically acceptable, safe and cost-effective globe-sparing procedures for end-stage glaucoma that provide immediate, effective, and long-term pain relief.

The proposed study investigated the safety and efficacy of retrobulbar neurolytic ethanol injections in canine patients with end-stage glaucoma. We hypothesized that subjects treated with retrobulbar ethanol injection would have limited, if any, negative side effects associated with the injection, lower owner perceived pain scores than dogs receiving injection of control solution and that pain scores would be comparable to scores following enucleation. Additionally, we expected to see minimal inflammatory changes on histopathology in both groups and anticipated an IOP lowering effect and CTT elevating effect after retrobulbar alcohol injection, compared to retrobulbar control solution injection. If successful, we believed that this therapeutic option would be accepted as a globe-sparing, pain relieving treatment for refractory glaucoma patients that could be performed by general practitioners and ophthalmologists alike, with specific application to those patients with life-limiting comorbidities, those not well enough to undergo anesthesia, or owned by clients who would prefer to avoid globe removal for their pet.

CHAPTER 3: RETROBULBAR NEUROLYTIC ETHANOL INJECTION FOR THE TREATMENT OF END-STAGE CANINE GLAUCOMA

A. Introduction

Canine glaucoma is a common ocular disease and becomes refractory to medical therapy in nearly all cases, ultimately resulting in significant ocular discomfort and vision loss. The diagnosis of glaucoma in dogs is often made later in the disease process and the course of disease progression generally follows a more accelerated path than in humans. In human patients, tonometry, ophthalmoscopy, perimetry, gonioscopy, pachymetry, posterior segment optical coherence tomography, 3-dimensional imaging of the optic nerve head, and nerve fiber layer analysis are all important tests to diagnose glaucoma; whereas, in canine patients, tonometry, ophthalmoscopy, and gonioscopy are most often utilized in the clinical setting. Due to the delayed detection of glaucoma in dogs, considerable pathologic anatomical and physiologic changes have often occurred prior to the documentation of an elevated intraocular pressure. Current medical and surgical options for the treatment of glaucoma are designed to decrease aqueous humor formation and increase aqueous humor outflow in order to create a steady state of normal intraocular pressure and preserve vision. The long-term prognosis in dogs remains dismal for IOP control, comfort, vision, and globe retention. Blindness and pain, necessitating surgical salvage procedures to relieve discomfort are the most common long-term outcomes of this disease.

The most commonly performed, effective, and predictable option for patients with end stage glaucoma is enucleation. Success of this procedure and owner satisfaction post-operatively is very high; however, this procedure requires general anesthesia, a

considerable financial investment, risks post-surgical complications, and comorbidities that make general anesthesia a risk may render enucleation an impractical option. Furthermore, an owner's cultural and psychosocial factors, to which a clinician is not privy, may contribute to the decision to pursue euthanasia rather than unilateral or bilateral enucleation. The development of a safe, immediate, globe-sparing therapeutic option to provide long-term pain relief is ideal for canine patients with end-stage glaucoma and their owners.

Retrobulbar neurolytic injections with absolute ethanol have been performed in humans with blind, painful eyes since the early 1900s.[89-92] Immediate and long-lasting pain relief can be achieved from 2 weeks to 2 years after a single injection.[76,89-90,95,99-101] In veterinary species, the use of these injections has been limited to rabbit and rodent experimental models of normal eyes, and an evaluation of retrobulbar neurolytic injection has not been undertaken in the canine model or in any veterinary species suffering from end-stage glaucoma.[76] Promising *in-vivo* effects of retrobulbar neurolytic injections in humans, rodents, and rabbits as well as the safety of this minimally invasive procedure in dogs, serves as a promising potential therapeutic option for canine patients with end-stage glaucoma.[78,89-92]

In this study, we performed retrobulbar injections on canine patients with end-stage glaucoma to determine the effect on owner perception of patient comfort, quality of life, and potential side effects of injection. Ultimately enucleation was performed on all injected eyes and owners completed identical post-procedural surveys to elucidate if a superior pain-relieving effect was noted in the treatment group. Patients were clinically evaluated twice during the study period to determine long-term effects of the injections

on ocular health, IOP, STT, and CTT. All globes and a sample of the retrobulbar tissue were submitted for histopathologic evaluation. We hypothesized that retrobulbar injection with absolute (95%) ethanol is a globe-sparing therapeutic option that provides effective analgesia superior to retrobulbar injection with control solution and equal to standard enucleation for canines with end-stage glaucoma. Secondary hypotheses tested included: following retrobulbar ethanol injection owner perceived analgesia and quality of life improvement would be equal or superior to that following enucleation, minimal side effects would be noted, an IOP lowering and CTT elevating effect would occur, and histological changes of orbital tissue would be distinct between treatment and control groups.

B. Materials and Methods

Animals and study design

Canine patients presenting to the Virginia-Maryland College of Veterinary Medicine Teaching Hospital between July 2017 and January 2019 that had been evaluated by the Ophthalmology Service and diagnosed with glaucoma were potential candidates to be included in the study. Furthermore, patients that were non-visual, refractory to medical anti-glaucoma treatment, or where patient disposition did not allow for consistent medical therapy to control IOP in a range deemed appropriate to prevent pain (i.e. IOP >25 mmHg, despite maximum therapy) were eligible for enrollment. Dogs with patient-specific historical, systemic, or surgical complications preventing adherence to the study protocol, use of sedatives, or induction of general anesthesia were excluded. Patients chronically administered systemic analgesics or diagnosed with glaucoma secondary to systemic disease, orbital disease, or other painful ocular diseases (i.e. corneal ulceration) were

excluded from enrollment. Patients diagnosed with primary glaucoma or secondary glaucoma after lensectomy (pseudophakic or aphakic), rhegmatogenous retinal detachment, anterior chamber silicone oil presentation, lens instability, or chronic, inactive, and non-painful immune mediated panuveitis (i.e. uveodermatologic syndrome) were considered for enrollment. Historical data collected included signalment information, history of ocular disease, eye affected, current topical and systemic medication administration including dosage, frequency and route of administration. All patients received an ophthalmic and neuro-ophthalmic examination performed by 1 of 2 board certified ophthalmologists (IPH or JPP) or 1 resident in training (AME). In all cases, this examination included documentation of palpebral reflex, pupillary light reflex, oculocephalic and dazzle reflexes, menace response, Schirmer tear test I, intraocular pressure measurement via rebound tonometry (TonoVet), central corneal sensitivity (CTT) via Lunaeu Cochet-Bonnet esthesiometry, anterior segment examination via slit-lamp biomicroscopy, and indirect ophthalmoscopy. IOP measurements were performed in awake animals placed in sternal recumbency without pressure on the eyelids or jugular veins. CTT values were recorded as mm of filament length and values 0.5 mm that elicited a blink response were recorded as 0.5 mm and if no blink response was elicited at 0.5 mm then the value was recorded as 0 to indicate loss of corneal sensation. At the completion of the examination corneal fluorescein staining was performed.

Following examination, if the dog was deemed a candidate for enrollment, clients were informed of appropriate options for continued management of their pet, which may include continued medical therapy, enucleation, evisceration/intrascleral prosthesis, and intravitreal gentamicin injection. For dogs that are candidates for enucleation, eligibility to

enroll in the retrobulbar ethanol injection trial, followed by enucleation and orbital biopsy was discussed. Informed owner consent was obtained prior to inclusion in the study.

After ophthalmic examination and owner consent obtained, enrollees were assigned an experimental ID number (1-20). A randomization table with 10 subject blocks was used to determine where the experimental ID number (1-20) fell within two treatment groups: 1 (ethanol, n = 10 dogs) or 2 (saline solution, n = 10 dogs). The list of treatment allocations was provided to the pharmacist who prepared a 2ml syringe of either saline (negative control) or ethanol. The syringe labeled with the experimental ID was provided to the investigator without any indication of the contents for injection. The owner was also blinded to the treatment or control group status of the patient.

Retrobulbar injection

Enrolled subjects were treated as outpatients. A standard sedative protocol was employed to include butorphanol 0.3 mg/kg intravenously and dexmedetomidine 10 mcg/kg intravenously administered simultaneously. While sedated, all subjects received flow by oxygen therapy and pulse oximetry was used to monitor vital parameters. Equal volume reversal agent, atipamezole, was administered intramuscularly at the termination of the procedure. Once sedated, the non-study eye was lubricated with topical ophthalmic lubricating ointment. The eye to receive retrobulbar injection was aseptically prepped with alternating washes of dilute Povidone iodine or Betadine™ solution and commercial eyewash solution.

Subjects were placed in sternal recumbency. The inferior lid hair was clipped and the skin aseptically prepped with dilute Povidone iodine or Betadine™ solution and

commercial eyewash solution. The inferiotemporal palpebral technique was employed to performed retrobulbar injections.[78] Briefly, an approximate 20-degree angle (created by mechanical bending) of a 1.5-inch, 22-gauge spinal needle was positioned at the inferior orbital rim and inserted through the inferior lid at the junction of the middle and temporal thirds. (Figure 1) Advancement proceeded until a slight popping sensation was detected, indicating that the orbital fascia has been pierced. The needle was then directed slightly dorsally and nasally toward the apex of the orbit and advanced approximately 1-2 cm. 0.5 ml of bupivacaine 0.5% for subjects weighing less than 5 kgs, 1 ml of bupivacaine 0.5% for subjects weighing 5-15 kgs and 2 mls for subjects over 15 kgs was injected into the retrobulbar space of all subjects. 5 minutes was allowed to elapse to determine if any complications from retrobulbar anesthesia were encountered. Dilation of the pupil, centralization of the visual axis, and CTT were assessed to indicate correct placement of the anesthetic injection. (Figure 2) The anesthetic injection spinal needle was left in place within the orbit and after 5 minutes, the treatment syringe prepared by the pharmacist (ethanol vs. saline) was administered. The subjective degree of exophthalmos was determined by the attending clinician and if it appeared severe or marked a single lateral tarsorrhaphy suture was placed. If a tarsorrhaphy was placed, then an Elizabethan collar was sent home with the patient to prevent suture dehiscence from self-trauma. The subject's sedation was then reversed.

Vital monitoring was continued until the subject regained consciousness and was able to support their weight in sternal recumbency, which indicated time 0. A repeat ophthalmic examination including central CTT, IOP, slit lamp biomicroscopy, and fluorescein staining was performed to determine any short-term complications 1.5 hours

post injection. Subjects were discharged 2 hours post injection. All subjects were discharged with 5 days of carprofen (2.2 mg/kg PO BID) and continued to receive their preexisting topical anti-glaucoma medication regimen.

Reevaluation and enucleation

Three weeks post retrobulbar injection, subjects were reevaluated by the Ophthalmology Service at the Virginia-Maryland College of Veterinary Medicine and hospitalized for enucleation. At the recheck examination, all dogs had a complete physical examination and preanesthetic bloodwork performed at the discretion of the attending clinician and anesthesiologist. Additionally, IOP, CTT, and repeat ophthalmic examination identical to the baseline evaluation was performed. Anesthetic premedication, induction, and maintenance protocols were determined at the discretion of the attending anesthesiologist. All patients received a retrobulbar block of 0.5% bupivacaine after anesthetic induction. Standard transconjunctival enucleation was performed by the same investigator (AME) and all globes and orbital soft tissue biopsies were submitted for histopathologic examination. Intraorbital silicone prosthesis was placed in some cases if owners requested it. All dogs received one dose of postoperative opioid analgesia with the drug type administered at the discretion of the attending anesthesiologists. Patients were discharged the following day with 5 days of carprofen (2.2 mg/kg PO BID) and an Elizabethan collar. Patients returned to their primary care veterinarians for suture removal 14 days postoperatively.

Assessment of pain relief, side effects, and outcome satisfaction

Clients filled out a baseline pain score survey (see appendix 1; survey 1) at the initial examination. Subsequent reevaluations were performed either via email or telephone with a single investigator (AME). The subsequent surveys were completed 2 days (48 hours), 1 week, 2 weeks, and 3 weeks post-treatment for both the injection procedure and enucleation procedure. A previously validated pain scoring survey (University of Melbourne Pain Scale) was modified to account for markers of ocular pain. Additionally, owner observations of ocular changes and side effects (see appendix 1; survey 2) following retrobulbar injection were recorded 2 days (48 hours), 1 week, 2 weeks, and 3 weeks post injection. If a temporary tarsorrhaphy was placed in the patient due to exophthalmos after retrobulbar injection, then owners were instructed to have this along with the Elizabethan collar removed by their primary care veterinarians 24 hours after injection prior to survey assessment. Finally, client satisfaction with outcomes of retrobulbar injection and enucleation was recorded for comparison between the procedures (see appendix 1; survey 3) three weeks post enucleation.

Histopathologic analysis

All globes and retrobulbar orbital biopsy samples were submitted for histopathologic evaluation. All tissues were placed in Davidson's fixative and stained with hematoxylin and eosin. A single board certified veterinary anatomic pathologist (KL), masked to the treatment group assignments, evaluated all samples. Tissues were divided into globe and retrobulbar tissue and a scoring system (Appendix 2: Histopathologic Scoring System) was created to objectively quantify the degree of inflammation and fibrosis/fibroplasia. Due to the surgical technique employed for enucleation and the

invasive nature of sample acquisition, hemorrhage was present in nearly all retrobulbar tissue samples and was thus not scored. The pathologist assigned suspected group assignments (control vs. treatment group) to the samples based on the nature and degree of histopathologic changes noted.

Statistical analysis

Normal probability plots showed that continuous data (IOP, STT, and CTT) were skewed. Subsequently continuous and ordinal data were summarized as medians (range). Categorical data were summarized as counts and percentages. Effects of treatment group, time, and the interaction between group and time on IOP, STT, and CTT were assessed using linear generalized estimating equations. Correlations between measurements within each dog were accounted for in the model using a compound symmetry correlation matrix. The interaction between group and time was further analyzed to compare groups at each time point and to compare time points within each group followed by Tukey's procedure for multiple comparisons.

For the injection portion of the study, scores (appearance, activity, interactions with family members, appetite, response to touch on the head, comfort, and discomfort) were compared between treatment groups at each time point using the Wilcoxon rank sum test. Scores were also compared between time points within group using Friedman's chi-square test followed by Bonferroni's procedure for multiple comparisons.

Occurrence of injection side effects (swelling, redness, squinting, third eyelid elevation, cloudiness, and discharge) was compared between groups at each time point using Fisher's exact test. Side effects were also compared between time points within

groups using the Mantel-Haenszel chi-square followed by Bonferron's procedure for multiple comparisons.

For the enucleation portion, scores (activity, interactions with family members, appetite, response to touch on the head, comfort, and discomfort) were compared between treatment groups at each time point using the Wilcoxon rank sum test. Scores were also compared between time points within group using Friedman's chi-square test followed by Bonferroni's procedure for multiple comparisons.

Scores from the client survey were compared between treatment groups using the Wilcoxon rank sum test. Histologic inflammation and fibrosis scores for globes and retrobulbar tissue were compared between treatment groups using the Wilcoxon rank sum test. Statistical significance was set to $p < 0.05$. All analyses were performed using SAS version 9.4 (Cary, NC, USA).

C. Results

A total of 19 eyes from 16 dogs were enrolled in the study. This included 6 spayed females and 10 neutered males. The mean ± SD age was 8.75 ± 2.32 years (range 3-13 years). Breeds included mixed (n=4), Cocker Spaniel (n=3), Boston Terrier (n=2), Border Collie (n=1), Great Dane (n=1), Miniature Poodle (n=1), Dachshund (n=1), Labrador Retriever (n=1), Basset Hound (n=1), and Greyhound (n=1). Nine dogs (9 eyes) were enrolled in the treatment group and 6 dogs (10 eyes) were enrolled in the control group. Patients with uncontrolled glaucoma in both eyes (n=1) were assigned the same treatment group based on random assignment of the first eye and treated simultaneously. This instance only occurred once during the study period and that dog was in the control

group. Two other patients had both eyes enrolled in the study, but they were enrolled weeks after initially completing the study in the first eye. Only 18 eyes from 15 dogs remained enrolled for the duration of the study. One eye was excluded from analysis two weeks after retrobulbar injection due to corneal perforation requiring premature rescue enucleation; this eye was enrolled in the treatment group.

Of the 19 eyes, primary glaucoma was diagnosed in 5 eyes. Secondary glaucoma was diagnosed in 14 eyes from a variety of causes: pseudophakia with rhegmatogenous retinal detachment (n=4), pseudophakia (n=3), aphakia (n=2), chronic rhegmatogenous retinal detachment (n=1), anterior lens luxation (n=1), silicone oil presentation in the anterior chamber (n=1), hypermature cataract and rhegmatogenous retinal detachment (n=1), chronic, inactive, immune-mediated panuveitis (n=1). All eyes had uncontrolled intraocular pressure (IOP > 25 mmHg) despite receiving a variety of topical antiglaucoma (2% dorzolamide, 0.5% timolol, 0.005% latanoprost) and anti-inflammatory medications (0.1% diclofenac, 0.5% ketorolac, 1% prednisolone acetate, 0.1% dexamethasone phosphate, Neomycin-polymyxin-dexamethasone, 0.03% flurbiprofen).

Intraocular Pressure Values

IOP was measured at baseline, prior to retrobulbar injection, and 3 weeks later when patients returned to the hospital for reevaluation prior to enucleation (second baseline). The median (range) IOP for the control and treatment groups at baseline was 55.5 (30-75) mmHg and 51.0 (26-84) mmHg, respectively. These values did not significantly differ. Within both the control and treatment groups a significant decrease in IOP was noted over the 3-week period after retrobulbar injection ($p=0.001$ and $p=0.03$),

respectively. At second baseline median (range) IOP for control and treatment groups was 24.5 (10-72) mmHg and 41.5 (7-69) mmHg, respectively. These values did not significantly differ between groups at second baseline.

Schirmer Tear Test I Values

STT I was measured at baseline, prior to retrobulbar injection, and 3 weeks later when patients returned to the hospital for reevaluation prior to enucleation (second baseline). The median (range) STT for the control and treatment groups at baseline was 15 (5-30) mm/min and 18 (9-34) mm/min, respectively. No significant differences were found between groups at baseline. At second baseline median (range) STT for control and treatment groups was 15.5 (10-26) mm/min and 13.5 (0-25) mm/min, respectively. Within the treatment group a significant decrease in STT was noted over the 3-week period after retrobulbar injection $p=0.003$; however, this decline did not differ significantly from the control group at second baseline.

Corneal Touch Threshold Values

CTT was measured (recorded in mm of filament length) at baseline, prior to retrobulbar injection, and 3 weeks later when patients returned to the hospital for reevaluation prior to enucleation (second baseline). The median (range) CTT for the control and treatment groups at baseline was 1.25 (0.5-4) mm and 1.0 (0-4) mm, respectively. No significant differences were found between groups at baseline. Within the control group no significant difference was noted over time. Within the treatment group a significant increase in CTT was noted over the 3-week period after retrobulbar injection $p=0.03$. At second baseline median (range) CTT for control and treatment

groups was 2 (0.5-3.5) mm and 0 (0-1.5) mm, respectively. A significant difference was noted between groups over time (baseline vs. second baseline) $p<0.0001$.

Client Pain Scale Surveys and Side Effect Surveys After Retrobulbar Injection

All owners completed the client pain scale survey at the predetermined timepoints for all eyes after retrobulbar injection, with the exception of dog 9, who was in the treatment group. Dog 9 was censored from the evaluation after completing a 48 hour and 1-week post injection survey, after which the eye perforated and was prematurely enucleated. No significant differences were noted between the treatment and control groups for any surveyed category at any time point (48 hour, 1, 2, 3 weeks post injection). Within the treatment group no significant changes in client pain scale scores were noted during the first three weeks after retrobulbar injection. Within the control group, a significant decrease in client pain scale survey scores was seen after retrobulbar injection with univariate analysis in the activity ($p=0.01$), family ($p=0.01$), and comfort ($p=0.01$) categories. These subcategories all lost significance in the multivariate analysis.

No significant differences pertaining to side effects of retrobulbar injection were found between or within groups throughout the duration of the study.

Enucleation Surveys

All owners completed the client pain scale survey at the predetermined timepoints for all eyes after enucleation, with the exception of dog 9, who was in the treatment group. Dog 9 was censored from the evaluation due to complications encountered during

the retrobulbar injection evaluation portion of the study. No significant differences were noted between the treatment and control groups for any surveyed category at any time point after second baseline (48 hour, 1, 2, 3 weeks post enucleation). Within the control group a significant decrease in client pain scale survey scores was seen after enucleation with univariate analysis in the activity (p=0.005), family (p=0.02), comfort (p=0.002), and discomfort (p=0.03) categories. Within the treatment group a significant decrease in client pain scale survey scores was seen after enucleation with univariate analysis in the activity (p=0.002), family (p=0.009), touch (p=0.02), comfort (p=0.008), and discomfort (p=0.007) categories. These subcategories all lost significance in the multivariate analysis for both the control and treatment groups.

A comparison of all patient scores prior to intervention (baseline) to all time points after enucleation was performed to detect the overall effect of enucleation on the control and treatment groups. Within the control group a significant decrease in client pain scale survey scores was seen after enucleation with univariate analysis in the activity (p=0.002), family (p=0.02), comfort (p=0.0003), and discomfort (p=0.0004). Within the treatment group a significant decrease in client pain scale survey scores was seen after enucleation with univariate analysis in the activity (p=0.001), family (p=0.008), touch (p=0.03), comfort (p=0.0001), and discomfort (p=0.001). Multivariate analysis of these variables in the control group revealed persistent significance in comfort beginning two- and three-weeks post enucleation (p=0.01, p=0.01 respectively), and discomfort beginning one week after enucleation through the end of the study (p=0.03, p=0.01, p=0.01). Multivariate analysis of these variables in the treatment group revealed

persistent significance in comfort beginning one week after enucleation through the end of the study (p=0.03, p=0.01, p=0.01).

Client Satisfaction Survey

No significant differences were found between groups for any variable pertaining to the client satisfaction survey.

Histopathology

The primary changes were similar between globe and retrobulbar tissue and consisted of mild to moderate inflammation and mild to moderate fibroplasia. The inflammation was primarily mononuclear consisting of macrophages and lymphocytes. The inflammation and fibroplasia tended to colocalize in the samples and in the sections. (Figures 3-5) Globes from the treatment group displayed significantly more inflammation and fibrosis (p=0.01, p=0.03, respectively). No significant difference between treatment groups was found for retrobulbar tissue inflammation or fibrosis scores. The pathologist blindly assigned treatment group or control group labels to samples to see if a clinical impression of histopathologic samples was seen. The pathologist correctly assigned control group labels to 7/10 (70%) of samples that belonged to the control group. The pathologist correctly assigned treatment group labels to 6/10 (60%) of samples that belonged to the treatment group.

D. Discussion

This study is the first of its kind investigating the effects of neurolytic retrobulbar injections using absolute ethanol in dogs with end-stage canine glaucoma. Overall, no significant therapeutic benefit for analgesia was determined for dogs receiving retrobulbar neurolytic ethanol injection and major side effects were noted in 3 dogs. Despite this, these injections were easy to perform and were perceived by owners to be well tolerated in patients.

All patients in this study received retrobulbar injections under the same sedation protocol using 10 mcg/kg of dexmedetomidine and 0.3 mg/kg of butorphanol administered intravenously. All retrobulbar injections were performed using the aforementioned inferiotemporal technique without complication.[78] All retrobulbar injection techniques have the potential for extraconal drug delivery due to improper needle placement or egress of fluid from the intraconal space to the extraconal space. This may result in the clinical appearance of correct intraconal neural blockade, however, the inferiotemporal technique has been proven to be the most accurate for proper intraconal placement.[78] Correct anatomic location of injectate within the intraconal space was assumed in our patients objectively and subjectively by visualizing centralization of the visual axis, mydriasis, and loss of corneal sensitivity, as has been previously reported.[78,117] The most consistent of these changes was centralization of the visual axis and complete loss of corneal sensitivity (CTT filament length <0.5 cm), which occurred in all eyes after placement of bupivacaine. The effect of dexmedetomidine and butorphanol on CTT in dogs has not been studied. When administered intramuscularly at 0.2 mg/kg, butorphanol was shown to have no significant effect on cornea blink reflex when the effects were studied for 45 minutes post administration.[115] The CTT of the

untreated eye was not measured, but presumed to be retained to some degree (CTT filament length >0.5 cm) given that ophthalmic procedures performed on sedated animals such as superficial keratectomy, require the use of topical anesthetics as sedation alone is ineffective to provide corneal analgesia. Confirmation of corneal anesthesia after retrobulbar bupivacaine injection confirmed correct needle position prior to retrobulbar ethanol or saline injection. Mydriasis was the least consistent finding seen in patients after retrobulbar bupivacaine injection and was only seen in 2 patients. The inutility of mydriasis as a sign of proper retrobulbar anesthetic placement in this study, as compared to others, is due to confounding factors of ocular disease and ocular pharmacologic agents in our study population as compared to normal dogs used in other studies. These included fixed miosis due to prior latanoprost administration (n=5), preexisting mydriasis (n=4), inability to visualize the pupil due to corneal or anterior chamber disease (n=3), synechiae (n=3), or lens dislocation occluding the pupillary aperture or entrapping the iris (n=2).

Retrobulbar volumes of bupivacaine and ethanol or saline were arbitrarily assigned to patients based on body weight as has previously been reported.[102-104] In this study dogs weighing less than 5 kgs were administered 0.5 ml of bupivacaine and 1 ml of study solution (saline vs. ethanol). Dogs weighing less than 15 kgs, but greater than 5 kgs were administered 1 ml of bupivacaine and 2 ml of study solution. Dogs weighing more than 15 kgs were administered 2 mls of bupivacaine and 2 mls of study solution. The orbital dimensions of healthy dogs are significantly variable across breeds, skull types, and body condition score; furthermore, the effect of prolonged intraocular pressure elevations and subsequent varying levels of buphthalmos influence the residual volume

of the orbit, which may have affected injectate distribution or efficacy. Immediate post-injection side effects were noted in 12 dogs (n=6 control, n=6 treatment) including conjunctival chemosis (mild n=2, moderate n=2, severe n=1), conjunctival hyperemia (n=1), and exophthalmos (n=6). Exophthalmos causing lagophthalmos necessitated the placement of a single lateral temporary tarsorrhaphy in 5 dogs that was removed by their primary care veterinarians one day following retrobulbar injection. Exophthalmos was attributed to the volume of bupivacaine and study solution placed in the orbit and further influenced by the degree of buphthalmos present in some globes as well as the significant breed related skull morphology variation seen in our subjects. Determination of the most appropriate orbital volume for retrobulbar injection has recently been studied by Klaumann, et al. who reported a relationship between skull morphometrics from dolichocephalic, mesaticephalic, and brachycephalic breed cadavers and intraconal volume.[109] This study suggested a formula of 0.1mL/cm L_{cr} be used to calculate total intraconal anesthetic volume, where L_{cr} is the distance in centimeters between the inion and nasion.[109] A calculated volume of contrast agent using the aforementioned formula was injected into cadaver heads and imaged with computed tomography to demonstrate the superiority of this calculation to volume calculation based on body weight.[109] This study does not completely pertain to the present investigation given that we injected two different solutions into the retrobulbar space and it is unknown at what ratio retrobulbar anesthetic should be combined with retrobulbar neurolytic injection to ensure proper needle placement and prevent pain associated with ethanol injection in dogs.

The presence of chemosis may be related to local hypersensitivity to the drugs administered, extraconal placement of retrobulbar solution, or egress of intraconal

solution to the extraconal space due to high injection volume. Extraconal placement of neurolytic solution may have contributed to adverse effects seen after retrobulbar injection. Schirmer tear test I measurements showed significant decreases over-time within the treatment group only; although these changes were not significantly different from the control group at the 3-week recheck after retrobulbar injection. The underlying mechanism for this is unclear, but it is known that glaucomatous eyes have decreased corneal sensitivity compared to normal eyes and therefore may have less basal and reflex tearing, yet it would be expected this factor would influence both groups equally.[119] Despite the lack of statistically significant differences between groups, a clinician should consider the possible sequela of decreased tear production prior to neurolytic retrobulbar injection as severe neurogenic keratoconjunctivitis sicca (KCS) may occur with lacrimal nerve or lacrimal gland chemical ablation. Two dogs in our study developed absolute KCS (STT = 0 mm/min) following retrobulbar ethanol injection and one of these cases displayed concurrent signs of xeromycteria. Optimizing orbital drug volume and unequivocally confirming intraconal placement of neurolytic substances may decrease the risk of extraconal spread of drug and resultant lacrimal nerve or gland damage. Drug delivery through peribulbar routes, ultrasound guided intraconal needle placement, or computed tomography guided intraconal needle placement have been performed, but have not shown a clear superior advantage over conventional techniques.[120-122]

Corneal touch threshold increased significantly over time within the treatment group and was significantly higher than the control group at 3 weeks following retrobulbar ethanol injection. Corneal anesthesia is not a complete measure of ocular analgesia, but does signify inhibited transmission of noxious stimuli via the ophthalmic

branch of the trigeminal nerve. Topically applied anesthetics prevent corneal pain by anesthetizing distal nerve fibers of the trigeminal nerve. As such, proximal destruction of the nerve with neurolytic ethanol injection should decrease or ablate corneal sensitivity. The duration of this effect in dogs in unknown and the benefit of decreased corneal sensation may come with risks of neurotrophic keratitis and corneal ulceration. Corneal sensitivity was significantly decreased within the treatment group over time and this decrease was significantly different from the control group 3 weeks after neurolytic injection. As such, neurolytic retrobulbar ethanol injections were effective at decreasing corneal sensitivity over time and theoretically should improve ocular pain. One patient in the treatment group, experienced a corneal perforation 1.5 weeks after retrobulbar injection. At the time of evaluation, an axial corneal perforation sealed with fibrin was documented and STT values were within normal limits. It is possible this patient developed a severe corneal ulceration secondary to neurotrophic keratitis, self-trauma due to irritation from retrobulbar ethanol, or egress of ethanol from the retrobulbar space through the conjunctival entry point and causing corneal ulceration . Given the size and location of the perforation, CTT was not performed at presentation for perforation, as all other measurements were performed on the axial cornea and corneal sensitivity varies by region.[123] Due to the severity of this ocular condition the eye was enucleated and the patient dropped from the study analysis for all subsequent time points.

Despite the short and long-term negative effects associated with retrobulbar ethanol injection, no statistically significant differences were noted either between or within groups over the three-week injection portion of the study. This would implicate the overall safety of retrobulbar neurolytic ethanol injections; however, it is important to

recall three dogs did develop significant clinical side effects including absolute keratoconjunctivits sicca (n=2) and corneal perforation (n=1). Furthermore, no statistically significant difference of owner perceived side effects between treatment or control groups was noted over the study indicating that owner's perception of retrobulbar injection was satisfactory. The lack of significant negative side effect perception within the treatment group as well as between treatment and control groups may relate to the common notion that owners are often unaware of glaucoma associated ocular pain in dogs or to lack of specificity in our questionnaire to raise concerns about post injection side effects.

Increased intraocular pressure outside of a physiologic range causes discomfort. Retrobulbar neurolytic ethanol injections have been demonstrated to induce a marked lowering of IOP in humans and rabbits.[76,91] The underlying mechanism for the IOP lowering effect is unknown, but is hypothesized to be directly related to provocation of active hyperemia in the orbit and indirectly due to neurogenic influence.[76,91] This hypothesis is not explained in the literature but may stem from the observation that episcleral vasculature drains aqueous humor from the conventional outflow route via anastomosis with the aqueous collector channels of the episcleral venous system. As such, IOP is dependent on EVP as a factor for resistance to outflow and if EVP increases, IOP increases and vice-versa.[9] Orbital hyperemia and vasodilation from inflammation secondary to retrobulbar ethanol injection may stimulate vasodilation of the episcleral veins, lowering resistance to outflow and EVP and therefore lowering IOP. Under normal physiologic circumstances, intraorbital pressure establishes a positive pressure gradient between the suprachoroidal and intraorbital spaces.[124] This allows fluid, solutes, and large

protein molecules to easily exit the eye by passing through the spaces surrounding the neural or vascular scleral emissaria or through the scleral substance itself and is a significant factor in establishing IOP and aqueous humor outflow through the uveoscleral pathway.[124] Theoretically glaucomatous dogs with elevated IOP and lower intraorbital pressure, which may occur after orbital hyperemia induced retrobulbar ethanol injection, would have an even greater pressure gradient across these structures, enhancing outflow and contributing to a lower IOP. This IOP lowering phenomenon seen in humans and rabbits is believed to help preserve the globe size throughout the duration of the neurolytic effect, which would be especially beneficial in dogs, as canine scleral rigidity is less than that of humans and more prone to stretching.[124] However, our study failed to confirm that retrobulbar neurolytic ethanol injections significantly lowered IOP in patients compared to those receiving sham therapy. A significant IOP lowering effect was seen over time within both treatment and control groups, but the difference between groups failed to reach significance after three weeks. This trend towards lower IOP was of greater magnitude in the control group with the median IOP reaching high-normal levels (IOP = 24.5 mmHg) at second evaluation 3 weeks after retrobulbar injection, whereas, the median IOP in the treatment group remained elevated (IOP = 41.5 mmHg).

In addition to CTT assessment, the analgesic effect of retrobulbar neurolytic ethanol injections was further characterized by subjective measures, as has been previously reported.[78] Clients were surveyed on the appearance of their dog's eye, activity level, interactions with family members, appetite, response to touch around the eye, comfort level, and discomfort level with lower scores associated with more positive assessments. No significant differences in improvement or decline were noted between

the treatment and control groups for any surveyed category at any time point (48 hour, 1, 2, 3 weeks post injection). Within the control group, a significant decrease in client pain scale survey scores (positive improvement) was seen after retrobulbar injection in the activity, family interaction, and comfort categories. However, these subcategories all lost significance in the multivariate analysis. Unfortunately, we were unable to prove a clinical improvement in dogs receiving retrobulbar neurolytic ethanol injections for end-stage glaucoma. It is unclear why the control group appeared improved after receiving sham treatment. Owners were masked to the treatment group assignments limiting the likelihood for placebo effect in this group alone. Alternatively, both groups may have been afflicted with placebo effect given the desire to see improvement in their dog's comfort level or a substantial improvement in dogs in the treatment group may have been confounded by mild discomfort from retrobulbar ethanol such that no statistically discernible improvement was identified. The lack of significant improvement in the treatment group may be related to low numbers of animals in this group and confounded by the loss of one patient in this group during the study. Furthermore, the heterogeneity of clinical signs with glaucoma vary based on etiology and acute or chronic nature of the disease so it is possible we had too few subjects with similar enough diseases to elucidate a treatment effect. The duration of the neurolytic effect may not have been fully appreciated during the three-week period after injection given the brevity of the study. This effect is considered less likely given the almost immediate effect noted in people after neurolytic injection, but it remains unclear if this is true for dogs. Lastly, many owners are unaware of the discomfort glaucoma causes their pets. Stoic patients or lack

of recognition of ocular signs of pain, for example, squinting, which most owners do not associate with pain, may have decreased their perception of improvement.

Regarding enucleation, our study like others before it, showed that clients perceived notable clinical improvement in their pets after enucleation.[64-65] Within the control and treatment groups a significant decrease in client pain scale survey scores (improvement) was seen after enucleation with univariate analysis in the activity, family, comfort, and discomfort categories. In addition, within the treatment group a significant decrease in client pain scale survey scores was also seen in the touching around the face category. These changes did not maintain significance after multivariate analysis likely due to the small sample size. Interestingly, changes in appetite does not appear to be an effective way to assess ocular pain in dogs as evaluation of this factor never reach statistical significance in our study at any time point and a recent study on owner perception of quality of life post-enucleation for glaucoma indicated approximately one-third of owners noticed an improvement in appetite after enucleation.[65]

A comparison of all patients prior to intervention (baseline) to all time points after enucleation was performed to detect the overall effect of enucleation on the control and treatment groups prior to retrobulbar injection. Within the control group, a significant improvement in comfort beginning two- and three-weeks post enucleation and resolution of discomfort beginning one week after enucleation through the end of the study was documented. Likewise, in the treatment group a significant increase in comfort beginning one week after enucleation through the end of the study was noted. The quicker difference in improvement of comfort in the treatment group may have been related to discomfort associated with retrobulbar ethanol or due to discomfort associated with lower

tear production and adnexal disease after retrobulbar ethanol injection that was alleviated with enucleation. Alternatively, early improvement could be seen because of the effectiveness of retrobulbar ethanol at providing earlier postoperative comfort from the ablation of sensory nerves within the orbit. Overall, this data continues to support the notion that glaucoma is painful to dogs and enucleation is an effective treatment option that quickly relieves pain.[64-65] Based upon our findings, retrobulbar neurolytic ethanol injections are not recommended as a globe-sparing salvage treatment for end-stage glaucoma in dogs.

At the time of enucleation, retrobulbar samples were submitted for histopathologic evaluation. Clinically, as noted in human patients who have received retrobulbar ethanol injections, significant orbital fibrosis can make subsequent enucleation more difficult.[95,97,100] This was noted in 2 patients within our study at the time of enucleation who were later revealed to have received retrobulbar ethanol injections. The globes remained intact during removal, but were much more firmly adhered to the intraorbital contents, which resulted in decreased ability to manipulate tissues for exposure. The primary changes were similar between globe and retrobulbar tissue and consist of mild to moderate inflammation and mild to moderate fibroplasia. The inflammation was primarily mononuclear consisting of macrophages and lymphocytes. The inflammation and fibroplasia tended to colocalize in the samples and in the sections. Globes in the treatment group displayed significant increases in inflammation and fibrosis; however, this relationship was not true for retrobulbar tissues. Histologically these fibrotic changes were not noted in the extraocular muscles that were submitted with the globes. Extraocular muscles are known to be recalcitrant to injury and,

in humans and primates, respond to denervation and injection of local anesthetic bupivacaine with myofiber hypertrophy rather than degeneration.[125] This effect has not been specifically studied in dogs, but myofiber degeneration was not a common finding in our study population. It's possible more consistent histologic changes or focal areas of change secondary to ethanol injection were not submitted for evaluation due to variability of orbital mass contents and the amount and type of retrobulbar tissue submitted in each case. The lack of continuity with retrobulbar tissue samples as compared to globes, which are easier to ensure a similar sample is submitted each time, likely explains the lack of increased inflammation and fibrosis seen in retrobulbar samples.

Several study limitations must be addressed. The study design lasted a total of six weeks and an inherent shortcoming of all studies is trying to gather the most data with patients treated on an outpatient basis knowing that the number of rechecks must be a balance between study and owner practicality. As such, objective measures of IOP, STT, and CTT were only performed twice. Daily and weekly diurnal fluctuations in IOP and STT have been reported and it has been recommended that at least for IOP consistent measures with a single patient should be performed with the same instrumentation at the same time of day.[126] Our IOP readings were obtained with the same instrumentation, but at random times of patient rechecks and may have influenced the overall lack of significant change in IOP seen in this study between groups at second baseline. Within each group IOP significantly declined over the three weeks and is likely related to wide diurnal fluctuations of IOP in glaucomatous eyes as dogs returned to the VTH at variable times during the day and at varying time periods after last receiving topical antiglaucoma therapy. The limited duration of retrobulbar ethanol injection assessed (3 weeks) before

enucleation may have also masked a delayed IOP lowering effect of retrobulbar ethanol injection that could have presented itself earlier or later. In humans the timeframe for this phenomenon has not been reported and in rabbits, within 1 day a significantly lower IOP was documented, that returned to baseline values within 14-21 days.[76] Hospitalizing patients for observation during the course of the study, standardizing a time for appointments for patients to be evaluated, or performing and IOP curve while patients were in the hospital prior to enucleation may have revealed an IOP lowering effect that was not documented. The volume of injection was arbitrarily assigned to patients based on body weight as previously described. A more recent study describing a mathematical determination of retrobulbar injectate volume and the superiority of peribulbar anesthetic delivery to the intraconal space over retrobulbar injection was not utilized in this study given the publication of that data after the commencement of the present study.[109,127] We believe it would have negated some of our data to alter the injection protocol after some dogs had already been injected. Moreover, our original goal was to enroll 20 eyes in the study and ultimately only 19 eyes were enrolled due to difficulty finding cases that met our inclusion criteria. Thus, forgoing data for dogs previously injected to amend our injection protocol was not considered. The smaller number of dogs enrolled in our study likely contributed to the lack of statistical significance seen in those variables that lost significance between univariable and multivariable analysis. Originally our inclusion criteria was even more strict to only include dogs with primary, pseudophakic, and aphakic glaucoma. We expanded our criteria to include dogs with secondary glaucoma not associated with systemic or life-threating ocular disease (chronic retinal detachment, chronic inactive immune mediated uveitis) as these conditions do not cause non-IOP

related ocular pain and these eyes would also be candidates for globe-sparing gentamicin injection and did not meet criteria most clinicians would use to solely recommend enucleation (e.g. intraocular neoplasia). Using owner surveys has previously been validated as an accurate subjective measure of discomfort over objective measures and that it is enhanced when a singular observer performs this assessment.[111] Although the same owner performed each survey throughout the duration of the study, each dog was evaluated by its owner meaning that the surveys were truly completed by 16 observers. This added variability may have accounted for the lack of statistically significant findings in patient pain scores. Our owner survey to assess patient comfort was modified to assess ocular pain from previous studies that assessed postoperative comfort after phacoemulsification and post-enucleation in dogs while hospitalized.[102-104,111,114-115] None of these previous studies consider patient comfort after returning home and no standard survey for the assessment of ocular comfort exists. Serious painful complications (absolute keratoconjunctivitis sicca, corneal perforation) developed in three of our patients in the treatment group (3/9) and despite this no statistical difference within or between groups regarding owner perception of side effects. Furthermore, owners did not report noted increased discomfort in their pets as both dogs with absolute keratoconjunctivitis sicca completed the study and were not prematurely enucleated and the dog with a corneal perforation presented to the hospital prior to scheduled recheck due to the eye appearing deflated to the owner rather than the dog being more uncomfortable. This means either our survey was ineffective at capturing the discomfort in these patients or their owners were unaware of signs of ocular discomfort, which calls into question the accuracy of the assessments they completed. This mirrors the findings

of a recent survey of owner assessment of ocular comfort after enucleation for glaucoma that captured just two-thirds of owners noted improved activity levels and less sleep in their pets, despite 91% of owners believing their dogs quality of life had improved. Improvements in survey accuracy could have been attempted by having the investigator perform the assessment through the use of livestream video chatting software the investigator would be able to at least assess the appearance of the eye, side effects, and patient comfort.

CHAPTER 4: CONCLUSIONS AND FURTHER RESEARCH

Retrobulbar ethanol injections were well tolerated and easy to perform without complication. However, they do not effectively relieve the discomfort associated with end-stage canine glaucoma compared to enucleation, despite significantly lowering corneal sensitivity. Additionally, they do not appear to provide the IOP lowering effect seen in other species and may raise concerns for decreased tear production. Despite our results, evidence of efficacy in other species suggests that further investigation is warranted for this globe sparing procedure in dogs. More targeted delivery of neurolytic substances at appropriate intraconal volumes would potentially reveal a positive clinical effect in glaucomatous canine patients. Refinement of delivery technique with sub-Tenon's or peribulbar anesthetic approaches and/or the concomitant use of imaging modalities to ensure proper needle placement may also enhance the desired clinical effect while minimizing extraconal spread of solution. Once the technique is mastered, it would be important to assess the duration of the neurolytic injection effect. Based upon our survey, patient activity, family interaction, comfort, touch around the head, and improved discomfort are better assessments of ocular pain relief than changes in appetite or objective measures like CTT. Glaucoma is a painful disease that affects a large portion of our canine patients and continued investigations into pain-relieving and globe-sparing therapies are warranted.

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APPENDIX 1: OWNER SURVEYS

Survey 1: Client Pain Scale Survey

For each question that follows***, select only one answer*** that best describes your dog over the period since you last filled out this survey. For the questions that indicate changes in behavior, relate this assessment to the behavior your dog displayed prior to developing glaucoma.	
Which best describes the appearance of your dog's affected eye looks? *Note: after your pet's eye has been removed, please skip this question.*	Eyelids are completely open, in normal position, third eyelid is not more apparent than normal (0) Eyelids are partially closed so that the eye appears 25% smaller than the other eye, or the third eyelid is more apparent than normal (1) Eyelids are partially closed so that the eye appears 50% smaller than the other eye, mild tearing, or the third eyelid is partially covering the eye (2) Eyelids are partially closed so that the eye is barely visible, moderate tearing, or the third eyelid is covering the eye completely (3) Eyelids are completely closed, marked tearing (4)
Which best describes your dog's general activity level?	Normal general activity level (0) Minor changes, mildly decreased general activity level (1) Moderately decreased general activity level (2) Markedly decreased general activity level (3)
Which best describes your dog's interactions with family members or other pets in the household?	Normal (0) Mild decrease in playfulness/interactions (1) Moderate decrease in playfulness/interactions (2) Marked decrease in playfulness/interactions (3)
Which best describes your dog's appetite?	Normal (0) Mildly decreased (1) Moderately decreased (2) Markedly decreased (3)
Which best describes your dog's response to touching around the head or face?	Normal (0) Pulls away when you touch around the eye (1) Vocalizes when eye is touched, reluctant to move but will if coaxed (2) Violent reaction to touching of the eye, snapping, growling when approached, will not move when coaxed (3)
What is your overall impression of your dog's comfort level?	Substantially improved (0) Slightly to moderately improved (1) Unchanged (2) Slightly to moderately worse (3) Substantially worse (4)
Do you believe your dog is experiencing discomfort related to its eye condition?	No (0) Uncertain (1) Yes (2)

Survey 2: Injection Side Effects Survey

Since the injection procedure was performed, please indicate which of the following changes have been noted.	
Increased swelling around eye	No (0) Yes (1)
Increased redness of eye	No (0) Yes (1)
Increased squinting/holding eye shut or partially shut	No (0) Yes (1)
Increased protrusion of third eyelid	No (0) Yes (1)
Increased cloudiness of eye	No (0) Yes (1)
Increased discharge from eye	No (0) Yes (1)

Survey 3: Procedure Satisfaction Survey

For each question that follows, ***select only one answer*** that best describes your dog over the study period	
How would you assess the changes in your dog's behavior following the injection?	Negative (0) Unchanged (1) Positive (2)
How would you assess the changes in your dog's behavior following the removal of the eye?	Negative (0) Unchanged (1) Positive (2)
What was your overall satisfaction with your pet's behavior following the injection procedure?	Poor (0) Good (1) Very good (2) Excellent (3)
What was your overall satisfaction with your pet's behavior following removal of the eye?	Poor (0) Good (1) Very good (2) Excellent (3)
Would you choose to have the injection performed on your pet again?	No (0) Yes (1)

APPENDIX 2: HISTOPATHOLOGIC SCORING SYSTEM

	Globe	Retrobulbar Tissue
Inflammation	0 Normal 1 Mild inflammation of any type 2 Moderate inflammation of any type	0 Normal 1 Mild inflammation of any type 2 Moderate inflammation of any type
Fibroplasia	0 normal 1 Mild fibroplasia 0-10% of tissue 2 Moderate fibroplasia >10% of tissue	0 normal 1 Mild fibroplasia 0-10% of tissue 2 Moderate fibroplasia >10% of tissue

APPENDIX 3: FIGURES

Figure 1. Inferior-temporal technique for retrobulbar neurolytic injection.

Figure 2. Mydriasis and centralization of the visual axis of the right eye after retrobulbar bupivacaine and ethanol injection.

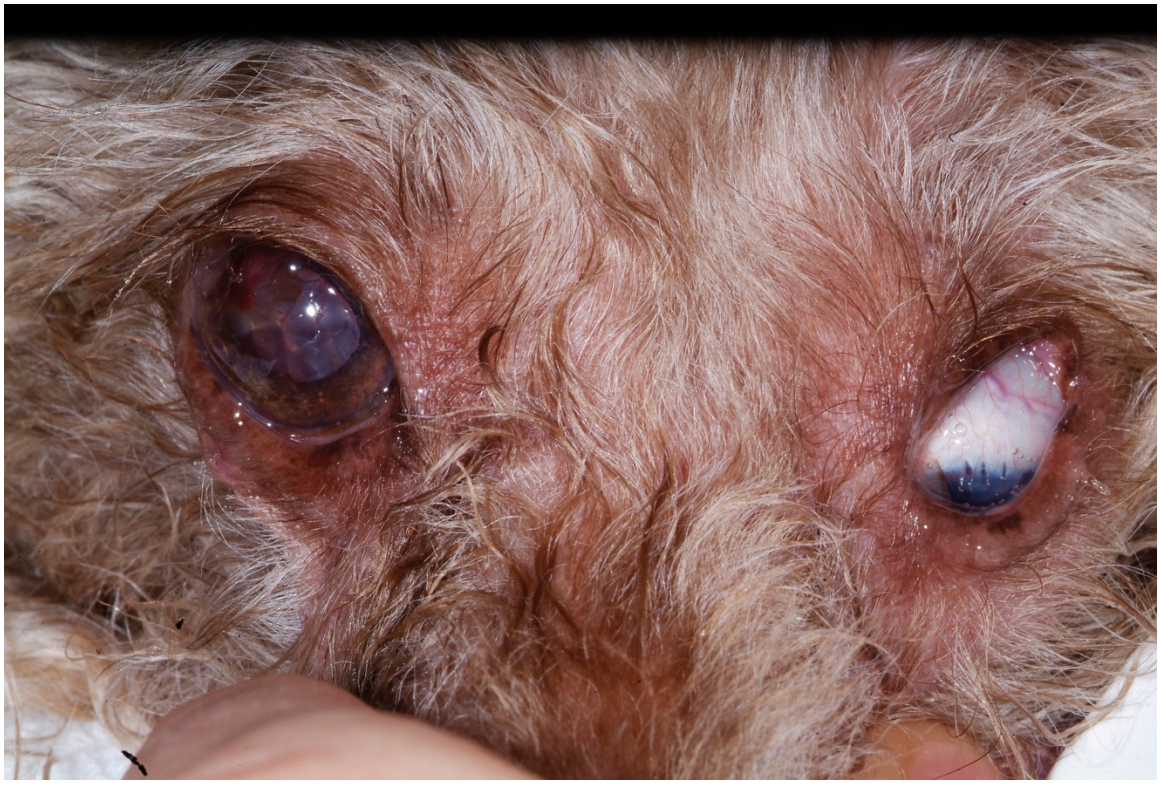

Figure 3. Clusters of lymphocytes surround blood vessels and infiltrate retrobulbar adipose tissue. Bar equals 250 μm

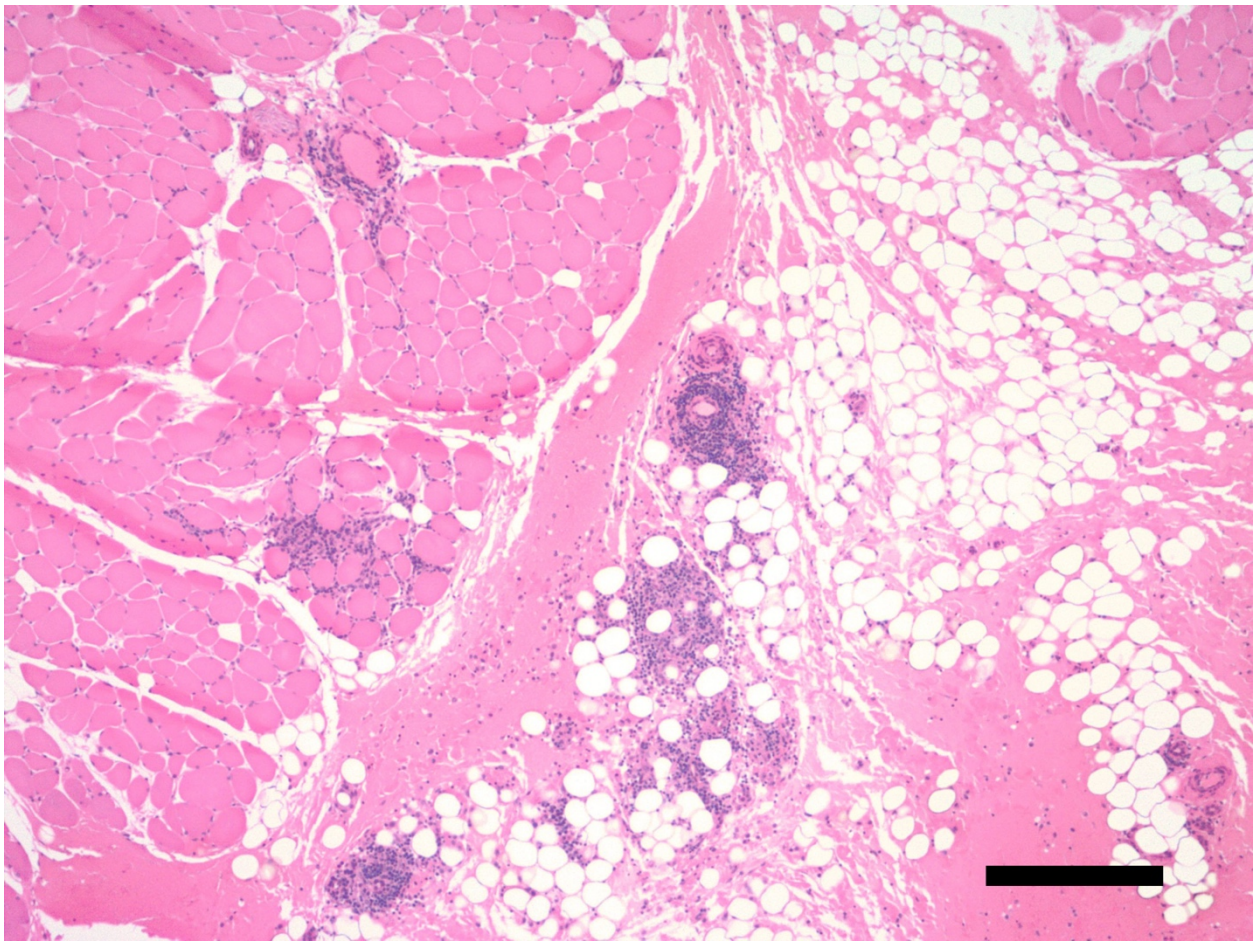

Figure 4. Infiltrates of lymphocytes and macrophages in the retrobulbar adipose tissue with some early fibroplasia. Bar equals 100 μm

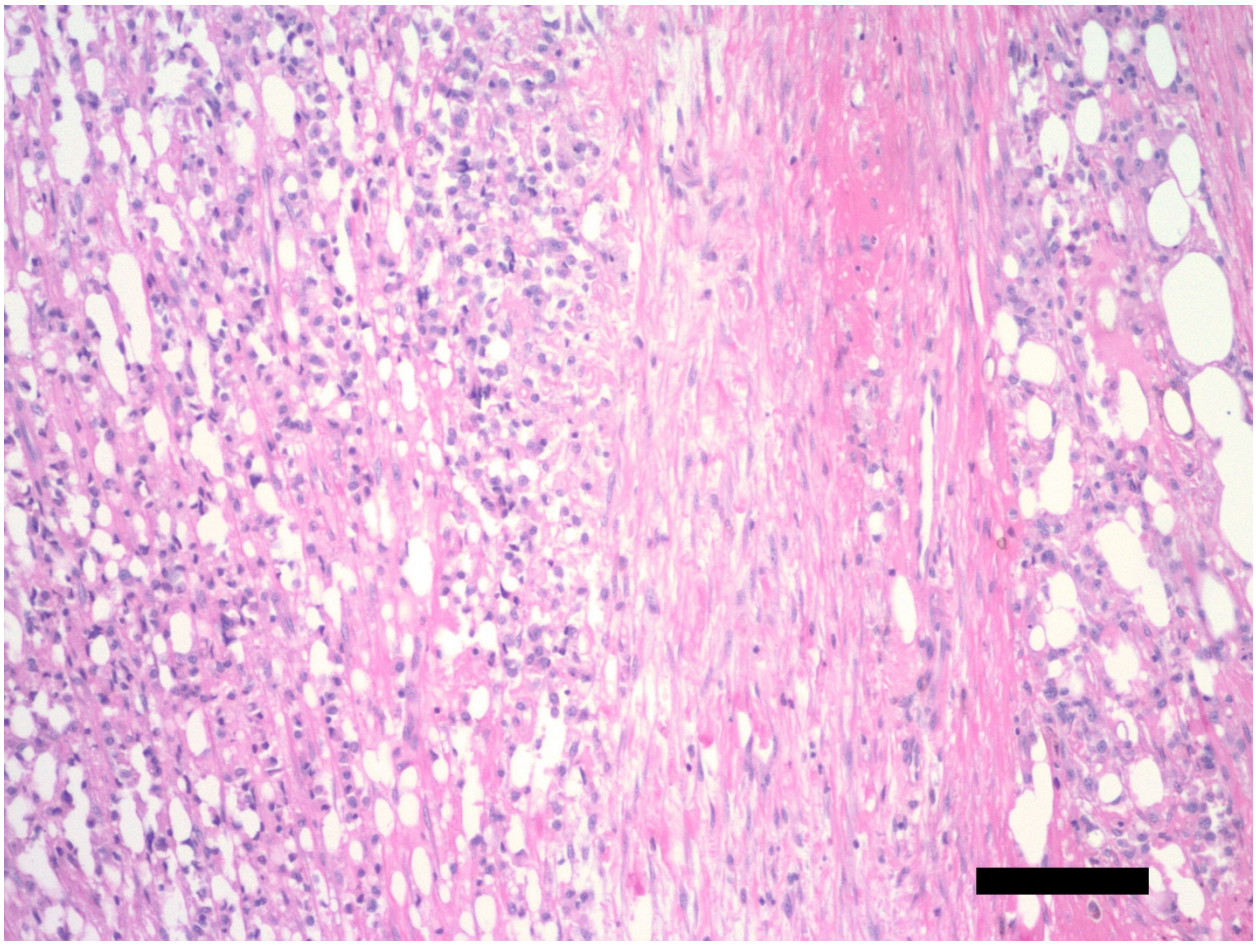

Figure 5. Disorganization and myocyte degeneration in retrobulbar skeletal muscle. Bar equals 100 μm