Surgical Intervention with Adjunctive Cryotherapy for Canine Conjunctival Hemangiosarcomas versus Hemangiomas

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SURGICAL INTERVENTION WITH ADJUNCTIVE CRYOTHERAPY FOR CANINE CONJUNCTIVAL HEMANGIOSARCOMAS VERSUS HEMANGIOMAS

by

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B.S. BIOCHEMISTRY
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SURGICAL INTERVENTION WITH ADJUNCTIVE CRYOTHERAPY FOR CANINE CONJUNCTIVAL HEMANGIOSARCOMAS VERSUS HEMANGIOMAS

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ABSTRACT

Conjunctival hemangiomas and hemangiosarcomas are vascular tumors that affect the tissue in the eyes of canines. While relatively rare, these tumors can be costly to remove, and have a possibility of recurrence. Therefore, more understanding of the nature of these tumors and recurrence rates is of importance to the veterinary ophthalmologist. To date, there is limited data in the literature (2 retrospective studies) that examine the development, treatment, and recurrence of these vascular tumors; furthermore, the analyses do not examine the potential recurrence of tumors after treatment with therapies adjunctive to surgery (such as cryoablation, radiotherapy, and others). For this reason, additional information describing the characteristics and outcomes of patients diagnosed with these vascular neoplasms could help guide veterinary ophthalmologists to establish the best route of treatment for their patients. In this work, a retrospective analysis of canines treated at a specialty veterinary hospital in Albuquerque, New Mexico between 2011 to 2021 for conjunctival hemangiomas and hemangiosarcomas is presented. The recurrence rates between canines diagnosed histologically with conjunctival hemangioma versus hemangiosarcoma after surgical intervention with adjunctive cryotherapy were compared. In addition, analysis of the association between recurrence and location of the
neoplasms and recurrence and completeness of surgical excision was done. It was found that there was no statistically significant association between: recurrence and diagnosis, recurrence and location, and recurrence and completeness of excision. Further study is needed to advocate for the role of adjunctive cryotherapy in lessening the chance of recurrence when incomplete or narrow surgical margins are obtained. However, from this study it is recommended that monitoring of both hemangiosarcomas and the more benign hemangiomas is warranted after surgical intervention, as there is no statistical evidence that diagnosis of one or the other favors or lessens the chance of recurrence.
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Chapter 1

Introduction

1.1 Background and Significance

Canine conjunctival hemangiomas and hemangiosarcomas are vascular neoplasms affecting the conjunctival tissue in the eyes of canines. They are rare compared to other types of tumors affecting canines; conjunctival hemangiomas in canines have been reported to be around 2% of all canine ocular neoplasms, while conjunctival hemangiosarcomas have been reported to be around 1.14% of canine ocular neoplasms.\(^1\)

Grossly these tumors are exophytic, red masses, and when left untreated they have the potential to invade surrounding tissues, such as the cornea, impeding vision and causing discomfort.\(^1,2\) Surgical intervention can be curative, but recurrence of the tumors remains a possibility.\(^1,2\) Surgical intervention at a veterinary ophthalmologist’s office can cost upwards of $2000, depending on the extension, size, and location of the tumor.

Recurrence can increase the total cost of treating these neoplasms if surgical reintervention is required. Therefore, more understanding of the nature of these tumors and recurrence rates is of importance to the veterinary ophthalmologist.

There is little information describing the nature of conjunctival hemangiomas and hemangiosarcomas in the canine. While a decent amount of literature exists describing conjunctival vascular tumors in canines via case reports, there are only two retrospective studies analyzing the characteristics of these tumors, detailing surgical interventions, and describing recurrence of these tumors. However, these are limited by their retrospective nature. More specifically there exists no information regarding comparison of recurrence
of conjunctival vascular tumors when they are treated with the use of adjunctive therapies, including, but not limited to, cryoablation, radiotherapy, cautery, and photocoagulation. Continued retrospective analysis describing the characteristics and outcomes of patients diagnosed with conjunctival hemangioma or hemangiosarcoma would help elucidate the nature of these neoplasms and establish the best route for surgical intervention to achieve curative results with little to no recurrence.

1.1.1 Hemangioma and Hemangiosarcoma Origins and Risk Factors

Hemangiomas and hemangiosarcomas are vascular tumors, resulting from endothelial growth and proliferation.3 Hemangiosarcomas are the more malignant of the two tumor types, often invading into surrounding tissue, as well as peripheral tissue. Because hemangiomas and hemangiosarcomas are vascular in nature they can occur in any vascular tissue and are usually categorized as being visceral or cutaneous in location.3 Comparatively, the visceral tumors are more malignant in nature than the cutaneous tumors.3 Visceral tumors are typically found in the spleen, liver, lungs, and right atrium of the heart.4,5 Non-visceral hemangiomas and hemangiosarcomas are found in the dermis, subcutaneous, tissue, and conjunctiva.4 In the eye, hemangiomas and hemangiosarcomas occur in the well-vascularized conjunctival tissue. The conjunctiva is a mucous membrane that lines the inside of the eyelids and is continuous over the sclera of the eye and the third eyelid.6 Its functions include the prevention of corneal desiccation, increasing mobility of the globe and eyelids, providing a local immune response, and protection from pathogens and foreign bodies by providing a physical barrier to the inner contents of the globe.6 Locations of occurrence for conjunctival
hemangiomas and hemangiosarcomas have been reported to be found in the bulbar conjunctiva near the limbus, the conjunctival fornix, and the leading edge of the third eyelids.\textsuperscript{1,2} Specifically, there has been an association between hemangioma and hemangiosarcoma development in nonpigmented tissue.\textsuperscript{1,2} The protective role of pigmentation against conjunctival hemangiosarcoma will be discussed further.

\textbf{Figure 1.1}\textsuperscript{7} This figure models the location of the conjunctiva within the eye.

The ontogeny of visceral hemangiosarcomas has been suspected to lie in abnormal progenitor cells that migrate to various sites throughout the body.\textsuperscript{7} However, the development of these neoplasms in the conjunctiva has been suggested to be linked to ultraviolet (UV) radiation exposure.\textsuperscript{1,2} Factors that influence the amount of UV exposure include latitude, altitude, cloud coverage, and reflective surfaces.\textsuperscript{8} With latitudes approaching 0, which include areas approaching the earth’s equator, there is greater UV
exposure, as the sun is closer to the earth in these regions. For this same reason areas with higher altitudes also have more UV exposure. Thus, it may be more likely for conjunctival hemangiomas and hemangiosarcomas to develop in canines living in areas with higher exposure to UV radiation, such as in Albuquerque, New Mexico where the altitude is roughly 5300 feet, and the latitude is 35°N.

1.1.2 Species and Breed Predilection to Hemangiomas and Hemangiosarcomas

It has been suggested that there is a propensity for these conjunctival vascular tumors to occur largely in breeds that have significant outdoor activity.\textsuperscript{1,2} This is because these breeds tend to have more exposure to ultraviolet radiation, which has been hypothesized to be a risk factor.\textsuperscript{1,2} In one study by Pirie et al., an over-representation of Basset Hounds, English Springer Spaniels, Boxers, Labrador Retrievers, and English setters was noted with the development of hemangiomas within the Sporting group (as defined by the American Kennel Club) have the highest prevalence.\textsuperscript{1} For hemangiosarcomas the breeds most commonly represented were Beagles and Dalmatians with Australian Shepherds, Australian Cattle Dogs, Basset Hounds, and Great Danes being well represented, also.\textsuperscript{1} The Working group, according to the American Kennel Club, was highly represented in the hemangiosarcoma group in this study.\textsuperscript{1}
Table 1.1
Prevalence of Hemangiomas and Hemangiosarcomas in Overrepresented Breeds

<table>
<thead>
<tr>
<th>Dog Breed</th>
<th>Prevalence of Hemangioma</th>
<th>Prevalence of Hemangiosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basset Hound</td>
<td>10%</td>
<td>5.30%</td>
</tr>
<tr>
<td>English Springer Spaniel</td>
<td>8.60%</td>
<td>2.60%</td>
</tr>
<tr>
<td>Boxer</td>
<td>7.10%</td>
<td>5.30%</td>
</tr>
<tr>
<td>Labrador Retriever</td>
<td>7.10%</td>
<td>2.60%</td>
</tr>
<tr>
<td>English Setter</td>
<td>5.70%</td>
<td>0%</td>
</tr>
<tr>
<td>Beagles</td>
<td>4.30%</td>
<td>7.90%</td>
</tr>
<tr>
<td>Dalmatians</td>
<td>2.90%</td>
<td>7.90%</td>
</tr>
<tr>
<td>Australian Cattle Dog</td>
<td>1.40%</td>
<td>5.30%</td>
</tr>
<tr>
<td>Australian Shepherd</td>
<td>2.90%</td>
<td>5.30%</td>
</tr>
<tr>
<td>Great Dane</td>
<td>0%</td>
<td>5.30%</td>
</tr>
</tbody>
</table>

Table 1.1
This table represents data from a retrospective study analyzing 108 cases of conjunctival vascular tumors of canines. These breeds were determined to be the overrepresented breeds in the hemangioma and hemangiosarcoma groups in this study.

In a separate retrospective study by Richardson et al. analyzing conjunctival hemangiomas and hemangiosarcomas Border Collies and Australian Cattle Dogs were highly represented among the groups that presented for conjunctival hemangiomas and hemangiosarcomas. In this study breeds with a likelihood to develop lower lid ectropion were represented in the group that developed tumors in the conjunctival fornix, which makes logical sense, as this anatomical location is more exposed to UV light when the lower eyelid conformation turns outward. In a study analyzing both visceral and non-visceral hemangiomas and hemangiosarcomas Whippets and Italian Greyhounds were highly represented. However, only 8 cases out of 76 cases on non-visceral hemangiosarcomas involved the eyes in this study. Thus, the studies analyzing conjunctival hemangiomas and hemangiosarcomas provide evidence to support the idea
that breeds with increased outdoor activity may have a propensity to develop these conjunctival vascular tumors.

Additionally, conjunctival hemangiomas and hemangiosarcomas appear to occur in middle-age to older dogs.\(^1,2\) In the most recent retrospective study analyzing canines with these conjunctival tumors the mean age for dogs that had developed conjunctival hemangiomas and hemangiosarcomas was roughly 8.69 years and the median age was 9 years of age.\(^2\) Similarly, another retrospective study analyzing conjunctival hemangiomas and hemangiosarcomas in dogs reported a mean age of roughly 8.4 years and 9.1 years of age at presentation for hemangiomas and hemangiosarcomas, respectively.\(^1\) The median ages for these two groups were 8 years for the hemangioma group and 9 years for the hemangiosarcoma group.\(^1\) Therefore, one can infer that these tumors are more likely to develop in middle-aged to older-aged dogs.

1.1.3 Diagnosis

The diagnosis of conjunctival vascular tumors is similar to diagnosing any other tumor of the body. Evaluation of the tumor location is easier to achieve in the case of conjunctival tumors in comparison to tumors hidden inside of body cavities, such as visceral hemangiosarcomas. Conjunctival hemangiomas and hemangiosarcomas can be diagnosed after ophthalmic examination based on gross appearance. Grossly these tumors are exophytic, red masses.\(^1\) In some cases, there can be corneal involvement of varying degrees, causing corneal vascularization.\(^1,2\) However, these tumors are definitively diagnosed after histological examination.
An ophthalmic examination for the initial diagnosis of conjunctival vascular tumors includes history and slit lamp biomicroscopy, although patients will typically receive examination beyond these two components of the ophthalmic exam. Key information in the history for the diagnosis of conjunctival hemangiomas and hemangiosarcomas include signalment, environment, onset, initial clinical signs, progression, duration, prior interventions, and nonophthalmic pre-existing disease. Prior to slit lamp biomicroscopy, the globe and adnexa can be examined, including the bulbar conjunctiva, the palpebral conjunctiva, and the third eyelid. Gross examination of the cornea and limbus can also be done prior to slit lamp biomicroscopy. Typically, corneas are clear and avascular.

However, in some cases of conjunctival hemangioma or hemangiosarcoma with corneal involvement, one will note the presence of vessels extending into the cornea or the presence of a mass lying on the cornea. Additionally, these lesions can have the appearance of an intrastromal hemorrhage. However, examination with a slit lamp allows for the observation of microscopic details and the diagnosis of conjunctival vascular tumors.

Conjunctival vascular tumors can also be examined histologically after surgical intervention. Hemangiomas and hemangiosarcomas are vascular tumors that contain blood, as opposed to other vascular tumors that contain fluids, such as lymph. Upon cytological examination conjunctival hemangioscarcomas also have cellular abnormalities, such as hyperchromasia, anisokaryosis, and they can have the presence of mitotic figures. It should be noted that the presence of mitotic figures, varying from low
to high in conjunctival hemangiosarcomas, does not dictate the tumors malignancy.\textsuperscript{12}

Other characteristics typical of hemangiosarcomas based on cytologic examination are spindle shaped, or fusiform, cells with a hemorrhagic appearance, vacuolated cytoplasm, and basophilic cytoplasm, meaning that they are likely to take up the basic hematoxylin stain.\textsuperscript{4} Thus, hemangiosarcoma can be defined by having these characteristics of malignancy.

Morphologically, hemangiomasarcomas have been reported to exist in 3 types: cavernous, capillary, and solid.\textsuperscript{3} Histologically, hemangiomas are described by having dilated vessels with thin walls with normal endothelial cell lining.\textsuperscript{12} Hemangiomas differ from telangiectasia, which is harmless in itself, in their lack of smooth muscle and pericytes surrounding the vessels.\textsuperscript{12} Typically, hemangiomas will have disrupted the surrounding tissue structure, as well.\textsuperscript{12} Much like any malignancy, hemangiosarcomas differ from hemangiomas in that they are more invasive in nature. Additionally, the endothelial cells forming these thin walled vessels are variable and exhibit the same polymorphisms described above in hemangiosarcomas compared to hemangiomas.\textsuperscript{12} Therefore, one can confirm a diagnosis of teleangiectasia versus hemangioma versus heamangiosarcoma histologically using these criteria.

Overall, a diagnosis of a conjunctival vascular tumor can be assumed after gross examination and a full ophthalmic examination. A definitive diagnosis can be obtained via histopathological examination after surgical intervention.
1.1.4 Surgical Intervention

Surgical intervention for conjunctival vascular tumors varies depending on the location of the tumor and involvement of the surrounding tissues, but they include excision via conjunctivectomy, third eyelid removal, and superficial keratectomy. These are microophthalmic procedures that require the use of magnification and microsurgery instruments. A microscope is required in some cases, such as limbal mass excision, as surgical microscopes are steady and offer 5.0x-40.0x magnification.

Gross excision can include a conjunctivectomy, conjunctivectomy and excision of Tenon’s capsule, superficial and deep lamellar sclerectomy, and a lamellar keratectomy or penetrating keratoplasty if indicated. Additionally, if the limbus and cornea are involved or the tumor has extensive involvement a penetrating sclerectomy, limbectomy, and keratectomy can be performed. A keratectomy includes removal of corneal tissue. With deeper involvement and more invasive excision, such as a penetrating sclerectomy whereby the interior portion of the globe is exposed, globe reconstruction may need to be done. Additionally, depending on the involvement and nature of the tumor, third eyelid excision or enucleation may be warranted. Thus, surgical intervention in most locations of the conjunctiva is limited to gross excision with more extensive surgical interventions being required based upon involvement of the tumor.

1.1.5 Adjunctive Cryoablation

Adjunctive therapies, including cryoablation, radiation, and photocoagulation, have been reported in a variety of cancers, including conjunctival vascular tumors. Cryoablation is a
technique whereby cells are destroyed both directly and indirectly via application of a metal probe that is in contact with the tissue and rapidly cooled.\textsuperscript{14} There are a variety of types of cryogens that are used for the cryoablation of tumors, including argon and nitrogen.\textsuperscript{15} In one type of cooling method, liquid nitrogen systems circulate liquid nitrogen through the probes to achieve rapid cooling of the probes, achieving temperatures similar to or lower than that which can be achieved using argon at around \(-195^\circ\text{C}\).\textsuperscript{15,16} Liquid nitrogen systems circulate the nitrogen, allowing for preservation of this resource.\textsuperscript{14} However, these systems have been claimed to be bulky and uneasily transported in a clinical setting.\textsuperscript{16} Another risk of using liquid nitrogen systems is the potential for the cryogen to experience vapor lock, which occurs when the heat from the tissues converts the liquid nitrogen into the gaseous phase, blocking the flow of liquid nitrogen through the probe.\textsuperscript{14} However, vapor lock is a preventable problem. Thus, cryoablation circulating liquid nitrogen can be utilized to kill tumor cells.

Cryoablation works in both direct and indirect methods to kill tissue.\textsuperscript{14,16} The success of cell killing is contingent upon the following factors: rate of cooling, final temperature, rate of thawing, and repetition of the freeze-thaw cycle.\textsuperscript{14,16} When cooling occurs above 0\(^\circ\text{C}\) cellular metabolism and the integrity of cellular proteins and lipids are affected.\textsuperscript{16} When cooling decreases below 0\(^\circ\text{C}\) ice crystal formation begins to occur extracellularly, which increases the extracellular tonicity, causing water to move from the intracellular space to the extracellular space.\textsuperscript{14,16} This leads to cellular dehydration, which can sometimes be sufficient to cause cell death.\textsuperscript{16} However, as the temperature drops to below \(-40^\circ\text{C}\), intracellular ice crystals begin to form, which could physically lead to damage to
intracellular structures, although the exact ways intracellular crystal formation leads to cellular damage are unknown. With rapid cooling the formation of intracellular ice crystals occurs quicker, leading to increased chances of cellular damage and death. However, the main cell killing is thought to lie in the rate of thawing. As the temperature begins to rise the crystals begin to attach to each other through recrystallization, which can lead to further cell damage and is fully optimized with slower thaw times. During thawing the extracellular space becomes hypotonic. Water then enters the intracellular space to compensate for this, leading to cellular swelling and rupturing of the plasma membrane. Lastly, repetition of the freeze-thaw cycle leads to further tissue damage and more successful chances of tumor death. With repetition there is more of a chance of killing cells that may have been damaged, but not killed in the previous cycles. Additionally, ice crystals grow larger upon repetition and the area of damage extends further to the periphery of the ice ball created by the probe, leading to larger areas of cell death. Thus, cryoablation works directly to kill cells through ice crystal formation using rapid cooling at temperatures below -40°C, slowly thawing the tissue, and repeating.

Indirectly, cryoablation leads to tissue death by affecting the vasculature of the surrounding tissue. During freezing the surrounding tissue undergoes ischemia and vessels constrict, leading to vascular stasis. During the thawing process these vessels dilate, and the damaged endothelium becomes leaky, leading to edema. Platelets begin to aggregate at the damaged endothelium and thrombus formation ensues. Additionally, neutrophils and macrophages rush to the site, which may be beneficial in
further tumor targeting.\textsuperscript{14,16} In the long-term over weeks to months inflammation and cytokine cascades occur at the site of cryoablation leading to further tissue necrosis.\textsuperscript{14} Therefore, cryoablation can be used in tumor killing via both indirect and direct methods.

1.2 Previous Studies
As mentioned above, there are only two main studies retrospectively comparing groups of canines with diagnosed conjunctival hemangiomas and hemangiosarcomas. This is likely because of the rare occurrence of these vascular neoplasms compared to other neoplasms.\textsuperscript{1} One of these studies analyzed the epidemiologic characteristics of canines with these diseases, and the other study analyzed recurrence rates between the two diseases after surgical intervention.

1.2.1 Retrospective Analysis of 108 Cases of Conjunctival Vascular Tumors
Pririe et al. examined 108 cases of conjunctival vascular tumors submitted to the comparative ocular pathology laboratory of Madison-Wisconsin between 1989-2004, comparing multiple characteristics between tumors diagnosed histologically as hemangiomas or hemangiosarcomas.\textsuperscript{1} They published their findings in 2006. This study focused on comparing hemangioma and hemangiosarcoma groups against control groups gathered from the comparative ocular pathology laboratory of Madison-Wisconsin and analyzing signalment, location of the tumor, pigmentation, size, duration from presentation to diagnosis, margins, adjunctive therapy, and geographic location of the patient and determining whether these factors significantly correlating with each group.\textsuperscript{1} However, due to the small levels of follow-up reports, these variables were not able to be
statistically analyzed to find correlation with recurrence in the hemangioma and hemangiosarcoma groups, presenting a limitation to this study.¹

The control group in this study served a variety of purposes, including minimizing geographic variation of submissions during the study and ensuring certain breeds were not over or underrepresented.¹ The authors used a logistic regression model to compare the response variable, which was the diagnosis of either hemangioma or hemangiosarcoma to the control group, to the following explanatory variables: age, gender, breed groups according to the American Kennel Club, and UV exposure.¹ It was found that the only significant difference between the hemangioma/hemangiosarcoma groups and the control groups was breed affected with Hound, Working, and Herding groups being at risk for developing a conjunctival vascular tumor.¹ It was indirectly thought that this breed representation supported the hypothesis that breeds with more outdoor exposure would be at risk for the development of these conjunctival tumors.¹ However, there was no statistically significant proof that there was a difference between cases with conjunctival vascular tumors and controls with exposure to UV light based on geographic location using the full logistic regression model.¹

The hemangioma group in this study included 70 tumors from canines.¹ Although no statistical analysis was performed to analyze possible associations between recurrence and diagnosis, 16 of 29 cases for which follow-up information was provided had no report of recurrence.¹ It should be noted that 2 of the cases that were classified as having recurrence were submissions after tumor regrowth had already been noted with no prior
histopathology report in file.\(^1\) In this group ancillary therapy was used in 14 cases with 12 of those cases utilizing cryoablation.\(^1\) 8 of the cryoablation cases had follow-up data available and none had recurrence.\(^1\)

The hemangiosarcoma group in this study included 38 of the 108 cases with only 20 of those cases having follow up information available.\(^1\) 11 of the 20 cases reported recurrence, but 2 additional cases were submitted after regrowth of the initial tumor, arguing that potentially a total of 13 out of 20 of these hemangiosarcomas experienced recurrence.\(^1\) In this group ancillary therapy was done noted for 8 of the 20 cases with 7 of these utilizing cryoablation, and 5 cases receiving this cryoablation experienced recurrence.\(^1\)

Limitations of this study included the inability to obtain follow-up information due to the retrospective nature of the study and the lack of statistical analysis describing recurrence rates and factors influencing them, such as diagnosis and adjunctive therapy. Only 49/108 (45.4\%\) cases had follow-up information, which would allow the researchers to describe recurrence. This led to a lack of statistical analysis that would describe the association of characteristics, such as recurrence and diagnosis. Additionally, this study did not show statistically significant correlation between recurrence and cryoablation, as this statistical analysis was not performed. However, it should be noted that this study did provide epidemiologic information describing the diseases, which was the main objective of this study.
1.2.2 Retrospective Analysis of 52 Cases of Conjunctival Hemangiomas and Hemangiosarcomas

Dr. Sarah Richardson and Dr. Anna Deykin published a retrospective study of 52 dogs diagnosed with either conjunctival hemangioma or hemangiosarcoma in 2021. This study analyzed the recurrence rates of canines in South East Queensland, Australia that had surgical excision of a conjunctival hemangioma or hemangiosarcoma between 2004 and 2020. Tumors occurred in the lateral bulbar conjunctiva, the palpebral surface of the third eyelid near the leading edge, and in the ventral conjunctival fornix. Grading of tumors involving the lateral bulbar conjunctiva was undertaken with higher grades being given to tumors with more involvement and invasion of local tissues. These grades allowed the researchers to assign a surgical dose for later statistical analysis, comparing both surgical dose versus diagnosis and surgical dose versus recurrence.

In this study statistical analysis included use of a Fisher’s exact test, a two-sample t-test, and a Kaplan-Meier time to event curve and a log-rank test of equality. The Fisher’s exact test was used to analyze the association between a variety of characteristics, such as location, corneal invasion, complete or incomplete excision, tumor location, surgical dose, histological diagnosis, and recurrence. The two-sample t test was utilized to compare the tumor volume with the probability of incompletely excising the tumor during surgical intervention and recurrence thereafter. Lastly, the Kaplan-Meier time to event curve and log-rank test of equality was used to assess the relationship between the time it took from patients presenting with a conjunctival vascular tumor to the time it
took to surgically intervene and the following: diagnosis, tumor volume, corneal invasion, and recurrence.²

The results in this study included a variety of information regarding the above characteristics. It was noted that the majority of tumors described in this study occurred in the lateral bulbar conjunctiva with hemangiosarcomas being more frequent in presence than hemangiomas in an insignificant way.² However, this study supported the observation that these tumors occur in nonpigmented tissue, as roughly 97% of the tumors analyzed occurred in nonpigmented ocular tissue.² There is a suspicion that UV light poses a risk factor for the development of conjunctival hemangiomas and hemangiosarcomas, and it has been demonstrated that pigment plays a protective role against the damaging effects of UV light.¹,²,¹⁷ Additionally, tumor volume was collected for 46 tumors with no statistical difference between the hemangioma and hemangiosarcomas being noted and having an average size of roughly 90mm³ and 155mm³ for hemangiomas and hemangiosarcomas, respectively.²

In the above mentioned study surgical intervention was the primary and only intervention used to treat the conjunctival vascular tumors save for one case where cryoablation was used as an adjunctive therapy.² About 73% of tumors assigned a grade prior to surgical intervention had a lamellar sclerectomy and a keratectomy, depending on corneal involvement of the tumor.² 53 tumors were defined as having complete excision based on their surgical margins. However, the location of tumors that had complete excision versus incomplete surgical excision had no statistical difference, and those that were not
completely excised were not shown to have a diagnosis of hemangiosarcoma over hemangioma in a statistically significant way.  

The main characteristic analyzed in this study was the rate of recurrence in these conjunctival vascular tumors. Thirty patients had follow-up for at least 6 months, and 6 of these patients exhibited recurrence, which was about 10% of the total tumors diagnosed and treated with surgical intervention. Statistical significance was not found for an association between the following: diagnosis and recurrence, location and recurrence, surgical grade and recurrence, complete versus incomplete excision and recurrence, and time from presentation to surgery and recurrence. However, it should be noted that 5 of the 6 recurrences were hemangiosarcomas. Overall, this study showed that surgical intervention alone produced results with only about 10% recurrence rates in the patients that had reported follow-up, which was only about 57% of the tumors that received intervention.

Limitations of this study included common limitations seen with retrospective studies, such as incomplete medical records or inability to systematically track follow-up changes. Additionally, only one case received adjunctive cryotherapy, limiting the ability to interpret the benefits of pursuing such a therapy.

1.3 Introduction to Current Study

This study is a retrospective analysis of canines with conjunctival hemangiomas and hemangiosarcomas treated at one specialty veterinary hospital in Albuquerque, New
Mexico between 2011 to 2021. This study aims to compare recurrence rates between canines diagnosed histologically with conjunctival hemangioma versus hemangiosarcoma after surgical intervention with adjunctive cryotherapy. Other factors, such as age, breed, sex, location of occurrence, will be analyzed for correlation to recurrence rates and to support previous epidemiological findings of the disease.
Chapter 2

Methods

2.1 Purpose of Study

The primary objective of this retrospective study was to compare the recurrence rates of conjunctival hemangioma versus conjunctival hemangiosarcoma in canines after surgical intervention with adjunctive cryotherapy. It was hypothesized that recurrence rates would be influenced by the diagnosis and be significantly lower for canines diagnosed with hemangiomas versus hemangiosarcomas.

Secondary objectives included obtaining additional information on the characteristics of these tumors including location and incomplete versus complete excision at the time of initial surgical intervention. These characteristics were analyzed against the recurrence rates to determine if an association existed.

2.2 Patient Selection

Patient cases were retrospectively selected and analyzed using clinical records from patients treated at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico from 2011 to 2021.

Inclusion criteria included canines diagnosed with hemangioma or hemangiosarcoma via histopathology that underwent surgical excision with adjunctive cryotherapy of the masses. Conjunctival hemangiomas and hemangiosarcomas have been described in felines, horses, and other species. However, these neoplasms, while rare in the canine,
are even more rare in other species.\textsuperscript{3,9} Additionally, focusing on canines narrows any possible variation that may be seen when analyzing recurrence among different species. Therefore, this study was limited to the analysis of canines. Secondly, patients included in this study were diagnosed with either hemangioma or hemangiosarcoma affecting the conjunctiva and extending into non-vascular tissue, such as the cornea, in some cases. These diagnoses were confirmed definitively with histopathology. Hemangiomas and hemangiosarcomas can occur in any vascular tissue, but this study focused on conjunctival neoplasms, as they behave differently and in a less malignant way than visceral hemangiomas and hemangiosarcomas.\textsuperscript{2} Additionally, surgical intervention can vary depending on the location and extension of the vascular tumor. However, in this study all surgical intervention methods were considered, including the following: conjunctivectomy, sclerectomy, limpectomy, keratectomy, partial third eyelid excision, whole third eyelid excision, and enucleation. Lastly, only patients treated with adjunctive cryotherapy were included in the retrospective analysis. This inclusion criterion was important in analyzing how recurrence rates might be affected with the use of this adjunctive therapy. However, this criterion did not leave any patients subject to exclusion, as all patients diagnosed with conjunctival hemangioma or hemangiosarcoma at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico from 2011 to 2021 were treated with adjunctive cryotherapy when surgical intervention was pursued. It should also be noted that no patients treated at this location in this time frame received any other type of adjunctive therapy, such as laser therapy or radiotherapy.
Exclusion criteria excluded patients that did not undergo an appropriate follow-up period. More specifically, patients excluded in this study did not have a follow-up period with a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center for at least a 30-day period. Due to this exclusion criterion, one patient was excluded because there was no follow-up, and another patient was excluded because the pet returned for only one recheck examination 1 week after surgery. It should be noted that in a previous retrospective study analyzing conjunctival hemangiomas and hemangiosarcomas that underwent surgical intervention, recurrence was noted anywhere from 5 weeks to 1 year after surgical intervention; in this study recurrence was defined as the presence of a tumor resembling a hemangioma or hemangiosarcoma occurring in the same location as the previous tumor.2

2.3 Surgical Intervention

Surgical intervention varied depending on the size of the tumor, the tissues affected by the tumor, assessment of vision, and existence of other ocular disease. Surgical intervention in this study included conjunctivectomy, sclerectomy, limpectomy, keratectomy, partial third eyelid resection, complete third eyelid excision, and, in one case, enucleation. All surgical intervention was performed by one of two board certified veterinary ophthalmologists at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico. The number of cases receiving each surgical intervention can be viewed in Table 2.1. This table represents the surgical intervention pursued after initial presentation to VCA Veterinary Care Animal Hospital and Referral Center.
Table 2.1 Surgical Intervention Pursued after Initial Presentation

<table>
<thead>
<tr>
<th>Case number</th>
<th>Surgical Intervention</th>
<th>Complete vs. Incomplete Margins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Keratectomy, Sclerectomy, Conjunctivectomy</td>
<td>Incomplete</td>
</tr>
<tr>
<td>2</td>
<td>Keratectomy, Sclerectomy, Conjunctivectomy</td>
<td>Incomplete</td>
</tr>
<tr>
<td>3</td>
<td>Gross Debulk Under Heavy Sedation</td>
<td>Incomplete</td>
</tr>
<tr>
<td>4</td>
<td>Unspecified Surgical Resection</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>Partial Third Eyelid Excision</td>
<td>Complete</td>
</tr>
<tr>
<td>6</td>
<td>Keratectomy, Sclerectomy, Conjunctivectomy</td>
<td>Complete</td>
</tr>
<tr>
<td>6</td>
<td>Conjunctivectomy</td>
<td>Incomplete</td>
</tr>
<tr>
<td>7</td>
<td>Partial Third Eyelid Excision</td>
<td>Complete</td>
</tr>
<tr>
<td>8</td>
<td>Keratectomy, Conjunctivectomy</td>
<td>Complete</td>
</tr>
<tr>
<td>9</td>
<td>Conjunctivectomy</td>
<td>Complete</td>
</tr>
<tr>
<td>10</td>
<td>Conjunctivectomy</td>
<td>Complete</td>
</tr>
<tr>
<td>11</td>
<td>Unspecified Surgical Resection</td>
<td>Incomplete</td>
</tr>
<tr>
<td>12</td>
<td>Unspecified Surgical Resection</td>
<td>Incomplete</td>
</tr>
</tbody>
</table>

Table 2.1 This table describes the type of surgical intervention pursued for each case upon initial presentation to VCA Veterinary Care Animal Hospital and Referral Center. The description of whether complete or incomplete margins were obtained is also described.

The most common surgical intervention included gross excision of the tumor using microsurgical techniques. In the case of a conjunctivectomy, the mass was grasped with Colibri forceps and excised using tenotomy scissors, which are blunt-tipped scissors used primarily for conjunctival tissue. Two to three millimeters of grossly normal tissue were obtained in two cases in which detailed surgical reports were recorded. However, in one of these cases, normal tissue obtained was less than one millimeter in the region adjacent to the limbus due the location of the tumor. With gross excision of tumors on of the third
eyelid, the third eyelid was clamped about two thirds of the way down with mosquito hemostats, the third eyelid was elevated, and the mass was excised with tenotomy scissors. Roughly five millimeters of grossly normal tissue was taken in both cases where gross excision of a third eyelid tumor was performed. In one case, the tumor affecting the bulbar conjunctiva was closely associated with the third eyelid, and the third eyelid was amputated. In this instance gross excision involved blunt dissection to the sclera using tenotomy scissors, obtaining five to eight millimeters of grossly normal tissue containing except on the side near the limbus where only one millimeter of grossly normal tissue was obtained.

When extension of the vascular tumors involved the limbus or cornea, a limbectomy and keratectomy was performed under general anesthesia. Due to the nature of hemangiomas and hemangiosarcomas being vascular tumors, in all cases where a keratectomy or limbectomy was required a conjunctivectomy was also required. This is because the cornea is an avascular tissue and extension of the lesion into the cornea comes from a tumor originating from the vascular conjunctiva.\textsuperscript{1,2} In the cases, requiring limbectomy or keratectomy as the surgical intervention, the patient was placed in dorsal recumbency and positioned under the operating light microscope for magnification. The incision was made in the cornea using a 6900 Beaver blade, extending into the cornea at an appropriate depth to ensure complete excision of the tumor. A linear incision was made in the cornea surrounding the following: the lesion, vessels that extended beyond the leading edge of the lesion into the cornea, and approximately 1.5-2 mm of normal cornea. This was done to ensure the tumor was completely excised as thoroughly as possible,
meaning there would be no evidence of cancer cells at the margins of the excision. The incision was continued into the conjunctiva and sclera in some cases with tenotomy scissors and/or a 6900 Beaver blade. The cornea was undermined with a Martinez corneal dissector to remove the tumor from the underlying layers as described above. The limbus and sclera were undermined with a 6900 Beaver blade, and the tumor was excised. When incisions were made in the cornea at a depth beyond the mid to posterior stroma an ACell® graft (ACell® Colombia, MD, US), consisting of decellularized porcine extracellular matrix derived from the urinary bladder, was placed. In the case where an ACell® graft (ACell® Colombia, MD, US) was required the graft was placed over the corneal excision site and sutured to the corneal and scleral borders. This took place after adjunctive cryotherapy was performed.

In one case, a third eyelid excision was performed due to a large bulbar conjunctival vascular tumor having a close association with the third eyelid. The third eyelid, also called the nictitating membrane, is found at the medial canthus of canines and its functions include protection of the cornea and production of a large portion of the aqueous tear film. Therefore, excision of the third eyelid requires close monitoring of tear production in the canine afterwards to address the possibility of corneal desiccation. In the case where amputation of the third eyelid was required, the base was first clamped with mosquito hemostats and tenotomy scissors were used to remove it.

In one case, an H-plasty of the lower eyelid was performed after a kissing lesion was identified approximately 5 months after removal of a hemangiosarcoma from the
palpebral surface of the third eyelid. This recurrent lesion was identified on the palpebral conjunctiva of the lower eyelid across from the location of the original lesion. H-plasty is a reconstructive blepharoplasty technique, allowing the surgeon to restore the eyelid when a lesion affects a large portion of the eyelid. In the case requiring a H-figure plasty, a Jaeger lid plate was placed below the mass and incisions using a #15 blade were made through the skin temporally, ventrally, and nasally to the lesion with tenotomy scissors being used to cut through the soft tissue. Two Burrow’s triangles were made near the bottom of the graft to permit sliding of the graft once the tissue containing the mass was excised. Grossly normal tissue was excised approximately 5.5mm temporal to the lesion, 8mm ventral to the lesion, and 4mm nasal to the lesion. The graft was slid dorsally into place and secured with sutures. It should be noted that in this case cryoablation was not performed due to the nature of the procedure. However, this intervention was pursued to treat a recurrent lesion, and cryotherapy was performed after excision of the initial tumor. Thus, this patient was included in this retrospective study.

2.4 Adjunctive Cryotherapy

Adjunctive cryotherapy (Frigitronics CE-2000 Cryosurgical System Trumbull, CT, US) was performed in all 13 surgical interventions to remove the vascular conjunctival tumors after patients presented to the ophthalmology department at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico. In one case treating recurrence that presented as a kissing lesion, requiring an H-plasty, the site was not treated with adjunctive cryotherapy due to the nature of the procedure. However, this patient had previously undergone adjunctive cryotherapy upon removal of the initial
conjunctival hemangiosarcoma. A nitrous oxide system with a supercool frigitonics probe was used for cryotherapy in all cases. Nine out of 13 cases (69.2%) had detailed descriptions of their cryoablation technique during the initial surgical intervention at VCA Veterinary Care Animal Hospital and Referral Center. Additionally, of the 6 instances where recurrence was noted, surgical intervention was only pursued in 5 of these instances and cryotherapy was only performed in 4 of them. In 2 of these 4 (50%) instances, the cryotherapy technique was described in detail.

In those cases with detailed descriptions of the adjunctive cryotherapy, at least two freeze-thaw cycles using a nitrous oxide system were accomplished at the surgical sites. In one case of recurrence, a triple freeze-thaw cycle was pursued at each surgical site. Cryotherapy was described to have extended into approximately 2-3 mm of surrounding normal tissue in 4 cases. Additionally, the ice ball was described in 2 cases as being approximately 6-7 mm in diameter at each freeze. In cases where cryotherapy was performed on the third eyelid and the technique was described, Styrofoam was placed behind the bulbar aspect of the third eyelid to protect the cornea during freezing.

2.5 Post-Operative Care
All patients were recovered after sedation or general anesthesia at VCA Veterinary Care Animal Hospital and Referral Center and sent home the same day. Patients were given a combination of topical antibiotics, topical anti-inflammatories, topical mydriatics, and oral pain medications depending on the type of surgical intervention they received. Antibiotics were given prophylactically to prevent bacterial infection post-operatively.
Anti-inflammatories, both oral and topical, were given for comfort, as were mydriatic agents.

2.6 Diagnosis

Diagnosing a conjunctival vascular tumor or a suspect hemangioma or hemangiosarcoma can be done after an ophthalmic examination based on appearance and observation. However, a definitive diagnosis is not obtained without histopathological confirmation. After surgical intervention, the masses were submitted for histopathological interpretation to one of the following institutions: University of Tennessee Veterinary Medical Center, University of Wisconsin-Madison Comparative Ocular Pathology Laboratory of Wisconsin, and Insight Veterinary Specialty Pathology. Based on histopathological findings, hemangioma or hemangiosarcoma was diagnosed definitively, and those patients not receiving a diagnosis of either of these were excluded from the study. Histopathology reports were examined for a definitive diagnosis of hemangioma or hemangiosarcoma and incomplete or complete excision.

2.7 Follow-Up

Follow-up periods were defined as the amount of time a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico examined the patient after the initial visit, diagnosing a conjunctival vascular tumor. A patient was excluded if they did not receive a follow-up period of at least 30 days after surgical intervention. At follow-up appointments a full ophthalmic examination was performed including, but not limited to, slit lamp biomicroscopy (Kowa SL-14 or SL-15
Slit Lamp, Kowa, Torrance, CA, USA) and indirect ophthalmoscopy (Keeler Vantage Plus Indirect Ophthalmoscope, Broomall, PA, USA). Additionally, when indicated, applanation tonometry (Tono-Pen XL, Mentor, OH, USA), Schirmer’s tear test measurements (Schering-Plough Animal Health, Union, NJ, USA), and evaluation of corneal fluorescein stain retention (Ful-Glo Strips, Akorn, Lake Forest, IL, USA) were performed.

2.8 Statistical Analysis

To compare the recurrence rates between the group of patients diagnosed with conjunctival hemangioma versus the group of patients diagnosed with conjunctival hemangiosarcoma, a Fisher’s exact test was performed. Additionally, a Fisher’s exact test was used to analyze recurrence and primary location of the tumor at initial examination, as well as recurrence and whether complete excision was obtained after the initial surgical intervention by a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center. This method of analysis was chosen for three main reasons. The first reason is that the data within this study aimed to compare two groups to assess if there was a statistical difference between them.\textsuperscript{19} Secondly, the data being compared between the conjunctival hemangioma group and the conjunctival hemangiosarcoma group was categorical in that the outcome was limited to a patient either experiencing recurrence within the follow-up time or not experiencing recurrence within the follow-up time.\textsuperscript{19} These criteria for analysis leave two analyses available: the Chi squared test and the Fisher’s exact test.\textsuperscript{19} Lastly, the Fisher’s exact test was chosen because the data being analyzed came from a small sample size. More specifically, more than 20% of the cells in
the contingency tables had expected frequencies of <5. In a Fisher’s exact test the null hypothesis is that of independence, meaning the categorical variables are not influenced by one another.

Microsoft Excel with the XLSTAT (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France 2017) add in was utilized to perform Fisher’s exact tests to compare the following: recurrence versus diagnosis, recurrence versus location, and recurrence versus excision. Recurrence was defined as the appearance of a conjunctival vascular tumor in the same location as the previously excised tumor upon ophthalmic examination during a follow-up visit. Recurrence was definitively established after surgical intervention was pursued to remove the recurrent lesion, and it was submitted and interpreted via histopathology, confirming the presence of either a conjunctival hemangioma or hemangiosarcoma. Diagnosis was defined as being either hemangioma or hemangiosarcoma. Location was defined as being the primary location the neoplasm occurred and included the conjunctiva, limbus, and third eyelid. Excision was defined as being complete or incomplete after the first surgical intervention. This was based on whether the neoplasm extended to the surgical margins, as interpreted on histopathologic examination. It was not always known, however. In instances where complete versus incomplete excision was unknown or histopathological records were not available, these patients were not included in the statistical analysis.
Chapter 3

Results

3.1 Patient Characteristics

Patients included only canines. Patient characteristics for the canines diagnosed with either conjunctival hemangioma or conjunctival hemangiosarcoma between 2011 to 2021 at VCA Veterinary Care Animal Hospital and Referral Center in Albuquerque, New Mexico are shown in Table 3.1. The most common breeds represented were Border Collies and Australian Shepherds. The majority of the breeds represented in this sample could anecdotally be argued to be breeds that would be exposed to a significant amount of UV exposure due to outdoor activity, such as Coonhounds, Australian Shepherds, Queensland Heelers, Boxers, Pit Bull Terriers, Sheepdogs, Basset Hounds, and Border Collies. In the sample analyzed retrospectively, 4 of 12 patients were male (33.3%) and 8 of 12 patients were female (66.7%). The mean age of patients at the time of diagnosis by a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center was 9.83 years with a median of 10.5 years and a standard deviation of 2.33 years. The mean weight of these patients was 24.6 kilograms with a median weight of 24.3 kilograms and a standard deviation of 5.68 kilograms.
Table 3.1. Patient characteristics for canines with either hemangioma or hemangiosarcoma

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age at time of diagnosis (years)</th>
<th>Breed</th>
<th>Gender(^a)</th>
<th>Weight (kilograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>Coonhound Other</td>
<td>MI</td>
<td>36.1</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>Australian Shepherd</td>
<td>MN</td>
<td>23.3</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Australian Shepherd</td>
<td>FS</td>
<td>25.4</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>Queensland Heeler</td>
<td>FS</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Boxer Mix</td>
<td>FS</td>
<td>28.3</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Heeler Mix</td>
<td>FS</td>
<td>22.6</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Pit Bull Terrier</td>
<td>FS</td>
<td>22.4</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>Sheepdog Mix</td>
<td>MN</td>
<td>28.7</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>Basset Hound</td>
<td>FS</td>
<td>20.2</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>Pembroke Welsh Corgi</td>
<td>MN</td>
<td>12.8</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>Border Collie</td>
<td>FS</td>
<td>21.2</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>Border Collie</td>
<td>FS</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 3.1. Patient Characteristics.
\(^a\) M = male, F = female, N = neutered, S = spayed, I = intact

3.2 Diagnosis

Conjunctival vascular tumors occurred primarily in the limbus in 7 out of 13 cases, in the conjunctiva (either bulbar or palpebral) in 4 out of 13 cases, and in the third eyelid in 2 out of 13 cases. Nine out of 12 patients included in this retrospective study were diagnosed with conjunctival hemangiosarcoma after histopathological interpretation. Four out of 12 patients were diagnosed with conjunctival hemangioma after histopathological interpretation. It should be noted that one patient had two vascular neoplasms total with the left limbus being definitively diagnosed as a hemangiosarcoma and the right conjunctiva being definitively diagnosed as a hemangioma.
Six out of 9 patients diagnosed with hemangiosarcoma had involvement of the limbus. In the initial histopathology report of one case, four sections were examined, and a proliferation of spindle cells was noted to line blood-filled chambers. In this case, the neoplasm contacted the lateral and deep surgical margins, indicating incomplete excision, which was expected due to the large nature and extension of the mass. Minimal cellular abnormalities, including anisocytosis and anisokaryosis were noted. Recurrence occurred in this case. During a second histopathologic examination after surgical intervention to remove the recurrent neoplasm, irregularly shaped blood-filled space lined by plump endothelium was noted. However, complete excision was also noted, as the lesion did not extend to the margin in the examined section, indicating clean margins were obtained. In a second case, the sample was described as being infiltrative, containing dense amounts of spindle cells wrapped around collagen bundles, and having blood-filled vascular channels. The neoplasm extended to the margins, indicating incomplete excision. However, no recurrence was noted in this case. The third case included three separate reports after surgical intervention of the initial tumor and recurrence afterwards. After recurrence, the histopathology report examined three sections where mid-corneal neoplastic cells were noted and were arranged around vascular blood-filled channels. It was diagnosed as hemangiosarcoma at this point, but it was hypothesized to have progressed from a hemangioma after continued UV light stimulation. Clean margins were obtained, according to the report. In a fourth case, the histopathologic report described an irregular, densely cellular, and regionally necrotic mass containing solid sheets and irregular vascular channels. Anisocytosis and anisokaryosis were noted, as was solar elastosis. The neoplasm extended to the margins, indicating the existence of incomplete
margins. In the fifth case, the histopathologic report described a highly cellular infiltrative mass with endothelial cells forming blood-filled, vascular channels. Anisocytosis and anisokaryosis and karyomegalic cells were present with mitotic figures noted. Incomplete excision was determined in this case. Recurrence did occur, but a histopathologic report for this recurrence was not available. This globe was eventually enucleated due to uncontrolled primary glaucoma, which was unrelated to the hemangiosarcoma. In the sixth case, the histopathology report described variably sized vascular channels, mitotic figures, anisocytosis, and anisokaryosis. The neoplastic cells extended to the surgical margin, indicating incomplete excision.

Two out of the 9 cases diagnosed with hemangiosarcoma involved the third eyelid. In the first case, the histopathology report described this mass as exophytic, unencapsulated, well-demarcated with a dense cellular proliferation of endothelial cells forming blood-filled vascular channels. Anisocytosis and anisokaryosis were noted and mitoses were 13 per ten 400x fields. Complete excision was noted. This case had noted recurrence in the form of a kissing lesion on the palpebral conjunctiva of the lower eyelid. Recurrence was confirmed on the histopathologic report where the submitted tissue was described as an ulcerated, exophytic mass consisting of spindle cells forming small vascular channels. Complete excision was noted here. In the second case, the histopathologic report described an unencapsulated, exophytic neoplasm with numerous variable blood-filled vascular channels. Anisocytosis, anisokaryosis, and anisonucleoli were noted. Solar elastosis was also noted. Neoplastic cells were 3 mm from the surgical margins,
indicating that complete excision was obtained. This case did have recurrence, but surgical intervention was not pursued.

One out of the 9 cases diagnosed with hemangiosarcoma involved only the conjunctiva and/or underlying sclera. In this case, the histopathologic report described variably sized, blood-filled, vascular channels. Anisokaryosis and anisocytosis with 8 mitoses were noted. As neoplastic cells did not extend to the surgical margins, complete excision was obtained and noted.

Two out of the 4 patients diagnosed with hemangioma had the neoplasm involve the limbus. In the first case, where limbal involvement of the neoplasm was noted after ophthalmic diagnosis, the histopathology report described this neoplasm as a conjunctival hemangioma with incomplete excision. In this sample, well-differentiated endothelial cells formed vascular channels that extended to the margins. The incomplete excision could be explained by the location of the tumor and nature of the surgical intervention, which was a gross excision via conjunctivectomy. It is also interesting to note that solar elastosis was reported on this report. In the second case, the histopathologic report described a proliferation of endothelial cells forming blood-filled vascular channels. One mitotic figure was noted, and neoplastic cells extended to within 1 mm of the margin, indicating complete, but narrow, excision. There was no recurrence in this case.

Two out of the 4 patients diagnosed with hemangioma had the neoplasm extend only to the conjunctiva and/or underlying sclera. In the first case, the histopathology report noted
a discrete vascular mass composed of variable vascular channels. Suspected complete excision was noted in the report. No recurrence was noted in this patient. In the second case, the histopathologic report noted blood-filled vascular channels formed by a proliferation of endothelial cells with mitotic figures noted. Excision was noted to be likely complete, but could not be deemed as certainly complete, as the sample was small. This patient had no recurrence.

Histopathology reports were not available for 2 cases: 1 report after initial surgical intervention and 1 report after surgical intervention excising a recurrent lesion. The definitive diagnosis for these cases was reported in the patients’ medical records, but details of histopathological findings, including complete vs. incomplete excision was not available.
Table 3.2. Location, diagnosis, affected eye, recurrence, and excision status of sample patients

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Location</th>
<th>Hemangioma or Hemangiosarcoma</th>
<th>Eye</th>
<th>Recurrence</th>
<th>Excision</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limbus</td>
<td>HSA</td>
<td>OS</td>
<td>Yes</td>
<td>Incomplete</td>
<td>8mm</td>
</tr>
<tr>
<td>2</td>
<td>Limbus</td>
<td>HSA</td>
<td>OS</td>
<td>No</td>
<td>Incomplete</td>
<td>7mm x 4mm</td>
</tr>
<tr>
<td>3</td>
<td>Limbus</td>
<td>HA</td>
<td>OD</td>
<td>No</td>
<td>Incomplete</td>
<td>4mm</td>
</tr>
<tr>
<td>4</td>
<td>Conjunctiva</td>
<td>HA</td>
<td>OS</td>
<td>Yes</td>
<td>Unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>5</td>
<td>Third Eyelid</td>
<td>HSA</td>
<td>OD</td>
<td>Yes</td>
<td>Complete</td>
<td>6mm x 5mm x 4mm</td>
</tr>
<tr>
<td>6</td>
<td>Conjunctiva</td>
<td>HA</td>
<td>OD</td>
<td>No</td>
<td>Complete</td>
<td>1mm</td>
</tr>
<tr>
<td>7</td>
<td>Limbus</td>
<td>HSA</td>
<td>OS</td>
<td>No</td>
<td>Incomplete</td>
<td>3-4mm</td>
</tr>
<tr>
<td>8</td>
<td>Limbus</td>
<td>HA</td>
<td>OD</td>
<td>No</td>
<td>Complete</td>
<td>4mm</td>
</tr>
<tr>
<td>9</td>
<td>Conjunctiva</td>
<td>HSA</td>
<td>OD</td>
<td>No</td>
<td>Complete</td>
<td>unknown</td>
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<tr>
<td>10</td>
<td>Conjunctiva</td>
<td>HA</td>
<td>OD</td>
<td>No</td>
<td>Complete</td>
<td>5mm</td>
</tr>
<tr>
<td>11</td>
<td>Limbus</td>
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<td>OD</td>
<td>Yes</td>
<td>Unknown</td>
<td>15mm x 6mm x 4mm</td>
</tr>
<tr>
<td>12</td>
<td>Limbus</td>
<td>HSA</td>
<td>OS</td>
<td>Yes</td>
<td>Incomplete</td>
<td>6-7mm</td>
</tr>
</tbody>
</table>

Table 3.2. This table describes the primary location, initial diagnosis, affected eye, whether excision obtained complete or incomplete margins, and the size of the conjunctival masses upon initial presentation.
a. HSA = hemangiosarcoma, HA = hemangioma

3.3 Follow-Up

Total follow-up times for patients after receiving surgical intervention was between 5 weeks and 5 years. The mean total follow-up time after the first surgical intervention was 56.3 weeks. The median total follow-up time after the first surgical intervention was 31.5 weeks. However, the standard deviation was large at +/- 71.5 weeks, implying there was a great deal of variation amongst follow-up time with patients. Recurrence was noted between 2 months and 6 months after surgical intervention. Recurrence was noted in 6 out of 13 cases (46.2%), which was around 4.5 times greater than recurrence rates previously reported in one study. However, this was slightly less than recurrence rates for hemangiosarcomas reported in a study retrospectively analyzing conjunctival hemangiomas and hemangiosarcomas where recurrence occurred in 11/20 cases (55%)
where follow-up information was available.\(^1\) Of the recurrent cases in this study, 5/6 (83.3\%) were diagnosed as hemangiosarcomas after the initial surgical intervention and 1/6 (16.7\%) was diagnosed as hemangioma initially. It should be noted that this hemangioma was then reclassified as a hemangiosarcoma after surgical intervention to remove the recurrent lesion, and histopathologic interpretation suggested that it evolved from the previous hemangioma.

### 3.4 Recurrence Rates

Three separate Fisher’s exact tests were performed to evaluate the following relationships: recurrence versus diagnosis, recurrence versus excision, and recurrence versus location. For the relationship between recurrence and diagnosis, the relationship between a patient presenting with recurrence after the first surgical intervention by a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center was analyzed against the patient receiving a definitive diagnosis of hemangioma and hemangiosarcoma. In this analysis the null hypothesis was that these two categorical variables were independent of one another. The p-value reported was 0.266, which was not less than 0.05. Therefore, the null hypothesis could not be rejected. In other words, recurrence was not influenced by diagnosis and these variables could be interpreted to be independent of each other in this retrospective analysis.

Secondly, the relationship between recurrence and excision after the first surgical intervention by a veterinary ophthalmologist at VCA Veterinary Care Animal Hospital and Referral Center was analyzed. In this analysis the null hypothesis stated that
recurrence and whether the excision was complete or incomplete, as determined by the neoplasm contacting the surgical margins, were independent of one another. The p-value was 1 in this case, which was not less than 0.05. Therefore, the null hypothesis could not be rejected. Overall, recurrence was not influenced by whether complete excision was obtained after the first surgical intervention by a veterinary ophthalmologist at the site in this retrospective study.

Lastly, the relationship between recurrence and primary location of the tumors, as determined after ophthalmic examination and histopathological interpretation, was analyzed. In this analysis, the null hypothesis was defined as recurrence and primary location being independent of each other. The p-value was calculated to be 0.372, which was not less than 0.05. Therefore, the null hypothesis could not be rejected. In other words, location of the tumor could not be stated to have had an influence on recurrence in this retrospective study.

Overall, the primary hypothesis that the diagnosis of hemangioma versus hemangiosarcoma influencing the chance of recurrence amongst canines with conjunctival vascular tumors was rejected in this retrospective study. Additionally, the location or completeness of surgical excision did not influence recurrence rate in this sample either.
Chapter 4

Discussion

4.1 Background

Canine conjunctival hemangiomas and hemangiosarcomas are rare neoplasms that, if left untreated, can infiltrate into the surrounding tissue.\textsuperscript{1-3} While they have been observationally noted to have little to no chance of metastasis, they can become intrusive and uncomfortable to the untreated patient. Previous studies analyzing the characteristics of these neoplasms and their epidemiology are limited. This retrospective case analysis represents the third known retrospective analysis to demonstrate recurrence rates among canines with either conjunctival hemangioma or hemangiosarcoma after receiving surgical intervention. However, this analysis represents a group where adjunctive cryotherapy was performed in every case.

4.2 Conclusions

Overall, this retrospective study demonstrated no association between recurrence and diagnosis, location of the neoplasm, or completeness of excision after surgical intervention. While adjunctive cryotherapy may help reduce recurrence of neoplasms that may have more likelihood of recurrence with surgical intervention alone, the data from this study alone was not enough to prove this. Future studies should be conducted to further determine the benefits of adjunctive cryoablation.
4.3 Recurrence versus Diagnosis

4.3.1 Hypothesis

The primary objective of this study was to evaluate the rate of recurrence between canines diagnosed with conjunctival hemangioma versus conjunctival hemangiosarcoma after surgical intervention and adjunctive cryotherapy. It was hypothesized that recurrence rates would be significantly higher in those patients diagnosed with hemangiosarcoma versus hemangioma.

4.3.2 Previous Studies

In a large retrospective analysis of 108 cases of conjunctival hemangiomas and hemangiosarcomas between 1989 and 2004, it was observed that hemangiosarcomas were more likely to recur than hemangiomas. However, this was not proven to be statistically significant, as the amount of cases where follow-up information was available was deemed to be too small for statistical analysis, according to the authors. Another large, retrospective study of 52 canines diagnosed with either conjunctival hemangioma, conjunctival hemangiosarcoma, or both between 2004 and 2020 found no statistical significance for the likelihood of recurrence based on diagnosis. The patients in this study were not treated with adjunctive cryotherapy except for in 2 cases. Future analysis comparing the outcomes of groups in this study to these prior studies would provide information on whether the use of adjunctive cryotherapy is beneficial. In this the aforementioned study, recurrence was noted between 5 weeks and 1 year, which was similar to the this current study, where in which recurrence was noted between 2 months and 6 months.
4.3.3 Current Study

This retrospective analysis of 13 cases, where a case was defined as an initial presentation of hemangioma or hemangiosarcoma where surgical intervention was pursued, did not confirm statistical significance between diagnosis and likelihood of recurrence after surgical intervention with adjunctive cryoablation. Statistical analysis catering to small sample sizes was applied via the use of a Fischer’s exact test.19 However, by observation 5/8 (62.5%) patients with hemangiosarcoma had recurrence, whereas only 1/5 (20%) of patients with hemangiomas had recurrence.

4.3.4 Conclusion

Overall, it may be stated that, while observationally conjunctival hemangiosarcomas are more likely to recur than conjunctival hemangiomas, these differences were not statistically significant. It cannot be concluded that hemangiosarcomas have a higher likelihood of recurrence than their benign counterparts.

4.4 Recurrence versus Excision

4.4.1 Hypothesis

Secondary objectives included obtaining additional information on the characteristics of these tumors. It was hypothesized that incomplete versus complete excision would not influence the rate of recurrence when surgical intervention was followed by adjunctive cryotherapy.
4.4.2 Previous Studies

In a previous study analyzing 108 cases of conjunctival vascular tumors retrospectively, no statistical analysis analyzing the relationship between recurrence and completeness of excision was achieved due to the low number of follow-up data.\(^1\) Another retrospective study analyzing 52 canines diagnosed with conjunctival hemangioma or hemangiosarcoma retrospectively, found that complete versus incomplete margins did not affect recurrence after surgical intervention in a statistically significant manner.\(^2\)

4.4.3 Current Study

In this study, analysis of whether there existed a likelihood of recurrence based on incomplete versus complete margins was performed with a Fischer’s exact test. Complete excision, defined as clean margins noted in the histopathology reports, was achieved in 6/12 cases with 2/6 (33.3%) of those having recurrence and 4/6 (66.7%) having no recurrence. Incomplete excision was noted in 6/12 cases with 3/6 (50.0%) having recurrence and 3/6 (50.0%) having no recurrence. The relationship between cleanliness of the margins and recurrence was not found to be statistically significant.

4.4.4 Conclusion

Adjunctive cryoablation is used to target and kill rapidly growing cells, such as malignant proliferating hemangiosarcoma cells. Utilization of adjunctive cryoablation during surgical intervention of conjunctival vascular tumors around the excision site, including normal conjunctival tissue, would, theoretically, eliminate any unexcised neoplastic cells. Many veterinary ophthalmologists utilize this and other adjunctive therapies for this
purpose, as hemangiomas and hemangiosarcomas are microscopic in nature, making it difficult to ensure that all the cells have been removed with surgical excision alone. Additionally, the location of these neoplasms makes it difficult to take wide surgical margins, making adjunctive cryotherapy a useful tool for the veterinary ophthalmologist.

Overall, it was shown that complete versus incomplete excision did not influence recurrence in a statistically significant way. However, it is difficult to conclude that adjunctive cryotherapy lessened the chance of recurrence from this data alone.

4.5 Recurrence versus Location

4.5.1 Hypothesis

Again, secondary objectives included obtaining additional information on the characteristics of these tumors, such as how the location of the tumor might influence recurrence rates. It was hypothesized that surgical excision with adjunctive cryotherapy would lead to an independent relationship between location of the neoplasm and likelihood of recurrence.

4.5.2 Previous Studies

One previous retrospective study, analyzing 108 cases of conjunctival vascular tumors in canines, noted on observation that hemangiosarcomas were more likely to occur on the bulbar conjunctiva, but this was not proven in a statistical way. However, in this study, analysis to find an association between location and recurrence was not described. In the another previous retrospective study, analyzing 52 patients, there was no statistically
significant association between location of the neoplasm and recurrence.\textsuperscript{2} Tumors in this study were located on the bulbar conjunctiva, adjacent to or on the limbus, and on the palpebral surface of the third eyelid.\textsuperscript{2}

### 4.5.3 Current Study

In this study, the association between recurrence and location of the neoplasm was analyzed. Excess UV light exposure is thought to increase the risk of developing a conjunctival hemangioma or hemangiosarcoma.\textsuperscript{1-3} Thus, it would make sense that these vascular tumors would be found in nonpigmented, vascular tissue. In this retrospective study, the neoplasms were all described in nonpigmented conjunctiva and at least 3 histopathology reports noted solar elastosis. The primary tumor locations upon presentation were: the bulbar conjunctiva, third eyelid, and limbus. Seven out of 13 (53.8\%) of the tumors were located on the limbus with 3 recurring and 4 having no recurrence. Four of 13 (30.8\%) of the tumors were located on the bulbar conjunctiva with 1 recurring and 3 having no recurrence. Two of 13 (15.4\%) of the tumors were located on the third eyelid, both of which had recurrence. Tumors involving the limbus often extend into the conjunctiva or require more technical surgical intervention to excise the mass and obtain clean margins. Thus, it would be thought that limbal involvement would lead to higher rates of recurrence. However, location and recurrence were not associated in a statistically significant way.
4.5.4 Conclusion

Adjunctive cryotherapy could eliminate the possibility of recurrence in those areas that are difficult to achieve wide surgical margins, such as those extending to or approaching the limbus and cornea. However, this data alone did not support the idea that adjunctive cryotherapy was the reason there was no association between location and recurrence, particularly because this was an observance from previous studies where this therapy was not utilized.

4.6 Other Limitations

The limitations in this study were mainly due to its retrospective nature due to missing information or existence of follow-up examinations, surgical reports, and histopathology reports. Ideally, follow-up information in the form of ophthalmic examination by a veterinary ophthalmologist would occur for intervals spanning at least 1 year post-operatively. However, because this study was retrospective, follow-up for this time period was not always achieved and information was, at times, limited to 1-2 follow-up examinations of at least 30 days after intervention. Additionally, although the majority of surgical reports provided information on the type of surgical intervention pursued, size of the mass, and size of the margins obtained, not all surgical reports in this study provided that level of detail. Lastly, histopathology reports were not always obtainable due to the retrospective nature of this study. However, it should be noted that these were very minor limitations. The mean and median follow-up times for the group analyzed was 56.3 and 31.5 weeks, respectively, 10/12 patients had detailed surgical reports, and histopathology was only missing for 1 case.
Another limitation in this retrospective study arose from the small sample size. It is difficult to obtain large samples of canines with conjunctival hemangiomas and hemangiosarcomas, as these are rare neoplasms. However, utilizing statistical analysis specific for small sample sizes was beneficial.

4.7 Future Directions

4.7.1 Meta-analysis

Future directions should consider the performance of meta-analysis using data from the previous two retrospective studies along with this study to address the significance of cryoablation with recurrence along with confirming that there is no statistically significant association between recurrence, completeness of excision, and location of the neoplasm. This meta-analytic approach would widen the sample population, allowing for statistical analysis that could either confirm previous reports or elucidate associations within the group. Specifically, analysis of the following should be pursued: diagnosis and recurrence, location and recurrence, location and diagnosis, recurrence and complete versus incomplete margins, and adjunctive cryotherapy and recurrence. If the sample size increased in such a way that would allow for a Chi squared test, stronger evidence could be made to either support or disprove the results from these three independent studies. Quantitative analysis could also be utilized to compare the recurrence rates between patients receiving adjunctive cryotherapy to those that did not. This could provide support for or against the use of adjunctive cryotherapy as a necessary curative asset in
the treatment of conjunctival hemangiomas and hemangiosarcomas via surgical intervention.

4.7.2 Randomized Clinical Trial

Additionally, a prospective randomized clinical study could be beneficial in directly analyzing the potential benefit of utilizing adjunctive therapies, such as cryoablation, radiotherapy, and laser therapy, in the intervention of conjunctival hemangiomas and hemangiosarcomas. The primary limitation of pursuing such a study would include the low rate of occurrence of these types of vascular tumors. A study of this nature could take several years to enroll enough patients for convincing statistical analysis.

The design of this study would require assigning the aforementioned adjunctive therapies to patients separated in various groups via a randomization method. Due to the nature of the adjunctive therapies, blinding would not be possible. After obtaining a pre-determined number of enrolled patients, follow-up data would be collected primarily to analyze recurrence rates among the different groups treated with the various adjunctive therapies. However, secondary information could be gathered to report characteristics of these tumors, such as location, breed, age, completeness of excision, etc. This study would allow for more control not only in assignment of adjunctive therapies, but also follow-up procedures, as a follow-up protocol could be instated. This is a key feature that is limiting in retrospective studies.
4.7.3 *In vitro* and *In vivo* Studies

Lastly, future studies to evaluate the cause of conjunctival neoplasms, such as hemangiomas and hemangiosarcomas, could be beneficial. As of now, the hypothesis is that these neoplasms are UV induced. Studies confirming this in the conjunctiva would be beneficial. Growing these canine conjunctival hemangioma or hemangiosarcoma cells *in vitro* in controlled environments and exposing them to specific amounts of UV light (e.g., varying wavelengths for varying amounts of time) and observing, measuring, and analyzing tumor growth could help determine the influence of UV light in tumor progression/development. The control in this study would be cells grown without UV light exposure. *In vitro* studies should be pursued before moving to animal models.

However, *in vivo* studies could include taking a mice strain and injecting hemangioma or hemangiosarcoma cell lines from canines to analyze whether UV light increased the likelihood and size of the mass would help support the hypothesis that these neoplasms are induced by increased exposure to UV light. It must be determined whether mice can support canine strains of conjunctival hemangiomas and hemangiosarcomas. If this is determined, exposing certain groups of mice to UV light after ensuring they have grown canine conjunctival hemangiomas or hemangiosarcomas, and observing their tumor progression could help elucidate the role of UV light in tumor progression. However, further research after this would still be required to definitively confirm that UV light exposure induces hemangioma or hemangiosarcoma formation in the conjunctiva.
References


