LONG-TERM OUTCOME OF SURGICAL MANAGEMENT OF EPIBULBAR MELANOCYTOMAS BY COMPLETE EXCISION AND HOMOLOGOUS CORNEOSCLERAL GRAFTING IN 15 DOGS: A CASE SERIES

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TABLE OF CONTENTS

Abstract 3

Research goals 3

Introduction 4

Materials and methods 6

Results 8

Discussion 17

Conclusion 21

Acknowledgements 21

References 22

ABSTRACT

Objective to investigate the efficacy, durability, and the long-term outcome of utilizing frozen homologous corneoscleral grafting techniques as a treatment modality for extensive epibulbar melanocytomas affecting the eyes of canine patients.
Design Retrospective study.
Methods The medical records of dogs diagnosed with epibulbar melanocytomas and subsequent corneoscleral transplantation at the ophthalmology department of the 'Universiteitskliniek voor Gezelschapsdieren' (UKG) Utrecht from 2013-2023 were reviewed. Follow-up information was obtained by re-examination of patients or telecommunications with the owners.
Results The study population consisted of 9 females, 8 of which were neutered, and 6 males, 2 of which were neutered. The mean age at the time of surgery was 5 years and 2 months (range, 1 year 8 months – 7 years 5 months). Early complications included: graft edema (n=14), hypotony (n=12) consistent with anterior uveitis, hyperemic conjunctiva (n=10) and a small blood/fibrin clot in the anterior chamber (n=9). Long-term follow-up was conducted at a mean of 3 years, 5 months and 18 days post-surgery (range, 1 year, 1 month, 12 days – 6 years, 10 months, 17 days). 6 patients attended the long-term follow-up appointment, 2 owners were consulted via telephone. Long-term complications included graft edema (n=4), fibrosis (n=3), pigmentation (n=2) and lipid deposits (n=5). The corneal graft was successfully positioned in all patients with no long-term graft rejection. None of the patients experienced a recurrence of the tumor during the postoperative follow-up period. All patients maintained their vision following the surgical intervention and throughout the course of the study.
Conclusions Despite the technical challenges associated with this surgical technique, it represents a worthwhile approach for managing epibulbar melanocytomas. The benefits of stable graft placement, vision preservation, low tumor recurrence rates, and minimal long-term side effects make this procedure a valuable option for ophthalmologists and their patients.

Key words: Canine, epibulbar melanocytoma, sclerokeratoplasty, neoplasia

RESEARCH GOALS

Due to the nature of the study design, a retrospective case series, a research hypothesis could not be formulated. However, with the descriptive findings of our study we hope to generate hypotheses that can be tested more rigorously in a prospective study.
The aim of our study is to evaluate the efficacy, outcome, and complications following penetrating sclerokeratoplasty and frozen homologous corneoscleral grafting for the treatment of epibulbar melanocytomas in dogs. Also, to describe the clinical, ultrasonographic and histopathological findings of this tumor in the canine patients.

INTRODUCTION

Ocular melanocytic neoplasms are prevalent in various veterinary species. [1] In dogs, epibulbar melanocytomas are the most common ocular melanocytomas. [2] They account for 10-34% of all melanocytic ocular tumors. [3-7] A variety of locations have been described in dogs for melanocytic tumors such as the orbit, eyelid, sclera, cornea, anterior uvea, and posterior uvea. [1] Canine epibulbar melanocytomas, also known as limbal melanomas, are believed to originate from the melanocytes delineating the limbus at the junction of the corneal stroma and sclera. These tumors commonly arise from the dendritic melanocytic cells of the corneoscleral junction, typically occurring at the dorsal or dorso-lateral limbus. [8-10]

Epibulbar melanocytomas clinically present as a focal darkly pigmented, solitary, well-circumscribed, elevated mass located at the limbus of the eye commonly extending towards the scleral and the cornea. [11] Amelanotic epibulbar melanocytomas also occur but are much less frequently described in the literature. [10] Most dogs with an epibulbar melanocytoma present with minimal clinical symptoms (e.g. mild conjunctival hyperemia and/or epiphora). [12] It is also common for melanocytomas to be discovered incidentally. [13] In severe cases, there may be hyphema, uveitis, and vision loss. [10,14] Even without invasion into the eye, progressive expansion of the tumor into the cornea can lead to loss of vision for the patient. [10,14] Small melanocytomas can also cause color changes, alterations in the eye bulb shape and discomfort for the animal. [12,15] Epibulbar melanocytomas seem to mainly affect the cornea's dorsal and temporal quadrants. These quadrants are important for the patient's vision and are considered even more important than the ventral and nasal quadrants. Due to the position of the eyeball in the orbit, the long pars plana in the dorsal and temporal quadrants and the long nose of the dog, the ventral and nasal visual hemifields are affected. [16,17] It has been previously reported that 83% of epibulbar melanocytomas are localized in the dorsal arc from the dorsomedial to the ventrolateral limbus. [11] Canine epibulbar melanocytomas have been more commonly reported in heavily pigmented dogs, particularly in the German Shepherd, Labrador Retriever, and Golden Retriever. [10,18] There is evidence of a breed predisposition for these latter two breeds, with a suspected recessive mode of inheritance. [11] Several studies have suggested that epibulbar melanocytomas occur in two distinct age groups: a younger group of 2 to 4 years old and an older group of 8 to 11 years old. [10,19] It was previously believed that tumors in the younger group grew faster and were more aggressive than those in older animals, leading to a recommendation against immediate surgical treatment in older animals. [20] However, recent evidence has contradicted this, showing rapid progression of epibulbar melanocytomas in older dogs. Consequently, the advice for treatment has changed, and surgical treatment is now recommended regardless of age. [11,21] No predispositions have been found for gender, coat color or which eye is affected in dogs. [22]

Melanocytomas originate from the melanocytes in the deep limbal cornea, juxtaposed to the Descemet membrane, and infiltrate the corneal stroma and episclera, leading to a rainbow-like morphology. [20] Benign melanocytomas are distinguished from malignant melanocytomas by the mitotic index, nuclear pleomorphism, and the nuclear to cytoplasmic ratio. Melanocytomas are described as benign tumors, but they can be aggressive locally and even affect the anterior chamber. [10]

Multiple therapeutic treatment options have been described for melanocytic neoplasms. These include radiotherapy, laser therapy, cryotherapy, enucleation and surgical removal with different grafting techniques (e.g. homologous, third eyelid, xenogeneic, synthetic). [21-26] The best treatment for the tumor depends on the preference and capabilities of the clinician, the owner's financial status but also the size, location and rate of tumor growth. [27,28]

MATERIALS AND METHODS

Case selection

The medical records of dogs diagnosed with epibulbar melanocytomas and subsequent corneoscleral transplantation at the ophthalmology department of the 'Universiteitskliniek voor Gezelschapsdieren' (UKG) Utrecht from 2013-2023 were reviewed. Breed, age at onset, clinical history, ophthalmic examination results, surgical treatment, primary surgeon and assistant surgeon, complications, and surgical outcome were recorded.

Ophthalmic examination

All dogs were subjected to a complete ophthalmic examination of both eyes. Ophthalmic examinations were performed by a board-certified (ECVO) veterinary ophthalmologist and/or a resident in veterinary ophthalmology, and included neuro-ophthalmic testing, Schirmer tear test (STT, Schirmer Tear Test®, MSD Animal Health, Boxmeer, The Netherlands) hand-held slit-lamp biomicroscopy (SL-15/17, Kowa Optimed, Inc), tonometry (TonoVet, Icare® Finland Oy, TonoVet Plus, Icare® Finland Oy), fluorescein staining (BioGlo BIO FLUORO, BIOTECH) and indirect ophthalmoscopy (Video Omega 2C®,Heine Optotechnik, Herrsching, German). Affected eye, localization and size of the tumor were recorded. When performed, gonioscopy findings were reviewed.

Ocular ultrasonography

All ultrasonographic examinations were performed by a resident in veterinary diagnostic imaging and a board-certified (ECVDI) veterinary radiologist and included imaging of the eye and retrobulbar area. Both eyes were examined. Standard transcorneal ultrasonography of the canine globe was performed using a linear transducer in combination with a curvilinear transducer set between 8 and 12 MHz (PHILIPS C8-5, PHILIPS L12-3 PHILIPS L15-7io HD11 XE, and Epiq 5, Philips healthcare, Eindhoven, The Netherlands).

Donor material + harvesting protocol

All cases were treated via penetrating sclerokeratoplasty using frozen homologous corneoscleral grafting. The homologous donor material originated from otherwise healthy young laboratory Beagle dogs taking part in a terminal study not related to the current study. Prior to harvesting the eyes were quickly evaluated for normal corneal health. Directly (within 2 hours) after euthanasia both globes were enucleated. The globes were carefully rinsed with an antiseptic solution (1:10 diluted povidone iodine 5% solution) and copiously flushed with sterile saline solution. With a sharp scalpel blade (no. 11) a perforating scleral incision was made circa 1cm posterior from the limbus and was continued over the entire circumference. The uveal tissue was carefully removed from the corneoscleral button. The donor material was directly frozen after harvesting and kept at -18 degree Celsius in a Lacriforte drops solution.

Surgical procedure

Dogs were prescribed topical and oral anti-inflammatory drugs four days prior to surgery (gentamicin + dexamethasone eyedrops four times daily on the eye to be operated and SID 1mg/kg prednisolon orally). On the day of surgery, the dogs were anesthetized by employees of the Anesthesia department of the UKG.

The dog is positioned in dorsal recumbency. The affected eye is aseptically prepared for surgery. Topical tetracaine and adrenaline is administered. The exact size of the epibulbar melanocytoma is measured with callipers and noted. The conjunctiva surrounding the neoplasm is dissected and removed. Subsequently, a circular non-perforating incision around the neoplasm was made using a trephine blade ca. 1mm larger than the neoplastic lesion. A perforating stab incision is performed at the cornea, followed by intracameral adrenaline and subsequent filling of the anterior chamber with viscoelastic (date and batch number are noted). The perforating corneal incision is completed using left and right corneal section scissors. Using closed Stevens tenotomy scissors, blunt dissection is used to separate the scleral tissue from the underlying uveal tissue. Before removal of the corneoscleral button, the donor button is prepared. A circular incision, endothelial side up, is made in the cornea and sclera of the donor using a trephine blade 1mm larger than the trephine used at the recipient site. Subsequently, the limbal melanocytoma is removed using curved Stevens tenotomy scissors. After removal of the tumor, it was fixated in a 4% buffered formalin container and sent to the Veterinary Pathological Diagnostic Centre (VPDC). The donor button is secured in the surgical area with 4 cardinal sutures. The cornea and sclera are closed with resorbable sutures (vicryl 9-0 at the cornea and vicryl 8-0 at the sclera) in a single continuous suture pattern.

Histopathology

Histological analysis of the masses was performed to determine the tumor type, assess the completeness of tumor excision, evaluate the mitotic index and potential malignancy features. The excised neoplastic material was routinely processed for histopathology and stained with hematoxylin and eosin for evaluation. All samples were examined by a board-certified veterinary pathologist (GG). Due to the high melanin pigment content of the tumor samples were decolorized for better assessment.

Follow-up

After the surgery patients were routinely prescribed medication. At the first check-up appointment a decision was made if further appointments at the UKG were necessary. Clients were contacted by email and telephone to participate in the current study. Clients that agreed to participate filled out a consent form. Six out of fifteen clients agreed to a long-term check-up. At the consultation a complete routine ocular examination took place with special interest for the graft location. Photos and video material were captured if patient collaboration allowed it.

RESULTS

Signalment

The study population consisted of 15 dogs diagnosed with clinically and histopathologic confirmed epibulbar melanocytoma. All dogs were surgically treated with 'en bloc' excision of the neoplasia and a homologous corneoscleral graft was used to restore the conformation of the globe. Table 1 describes the description and age of the patients on the surgery date. The right eye (OD) was affected in 8 dogs and the left eye (OS) in 7 dogs. The study population consisted of 9 females, 8 of which were neutered, and 6 males, 2 of which were neutered. The affected population consisted of 5 Labrador Retrievers, 4 Golden Retrievers, 1 Border Terrier, 1 mixed breed, 1 German Shepherd, 1 Shiba Inu, 1 Norwegian Buhund and 1 Rhodesian Ridgeback. The mean age at the time of surgery was 5 years and 2 months (range, 1 year 8 months – 7 years 5 months). The Labrador Retriever was the most common breed followed by the Golden Retriever.

Patient	Breed	Gender	Affected eye	Age on date of surgery
1	Border Terrier	VG	OD	7 years, 3 months
2	Labrador Retriever	M	OD	5 years, 4 months
3	Golden Retriever	VG	OS	7 years, 1 month
4	Labrador Retriever	M	OD	5 years, 5 months
5	Golden Retriever	M	OS	5 years, 3 months
6	Labrador Retriever	VG	OS	5 years, 0 months
7	Mix breed	VG	OS	3 years, 3 months
8	German Shepherd	VG	OS	5 years, 3 months
9	Labrador Retriever	MG	OD	7 years, 5 months
10	Shiba Inu	M	OS	2 years, 7 months
11	Norwegian Buhund	V	OD	3 years, 8 months
12	Labrador Retriever	VG	OD	1 year, 8 months
13	Golden Retriever	MG	OS	7 years, 4 months
14	Golden Retriever	VG	OD	4 years, 4 months
15	Rhodesian Ridgeback	VG	OD	6 years, 8 months

Table 1. Signalment and eye affected

Ophthalmic examination

The epibulbar melanocytoma was described as a dark brown to black pigmented raised structure of varying size in all patients (Fig. 1). The size of the masses ranged from 3 to 10 mm (Table 2). Different locations were affected by the tumor: dorsolateral (n=5), ventrolateral (n=4), dorsomedial (n=3), lateral (n=2) and dorsal (n=1) (Table 2). No gonioscopic data was available for 5 patients. Gonioscopy was not assessable in 2 patients due to cornea irregularity (case no. 10) and blepharospasm (case no. 2). No extension into the drainage angle was observed in all 8 remaining patients. Patient symptoms of pre- and post-operative appointments are described in table 3,

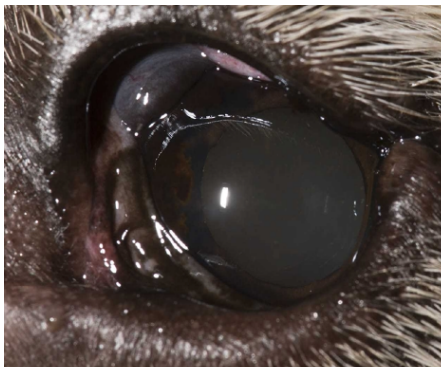

Fig. 1. Patient 7: melanocytoma OS dorsolateral

excluding the characteristic pigmented mass that was present in each patient preoperatively. Various preoperative signs were observed, including hypotony (n=5), mild (n=1) and moderate (n=2) hyperemic conjunctiva, nucleus sclerosis (n=2), decreased tear production (n=1), white crystalline corneal opacity ventrolateral to the pigmented mass (n=1), scarring from chorioretinitis OU, possibly associated with systemic infection (case no. 9, toxoplasmosis), minor episcleral vascular injection (n=1) and medial focally a small zone of lipidosis of the stroma (n=1). Furthermore, in patient 5 there were several preoperative symptoms, including a negative direct pupillary light reflex OS, negative consensual pupillary light reflex OD, fixed status of the pupil OS, peripherally epithelial pigmentation of the cornea OS, anterior uveitis OS and multifocal persistent pupillary membranes (PPM) on the anterior lens capsule. This dog had a history of trauma to OS of unknown cause resulting in dyscoria. In 7 patients, no abnormalities were noted besides the pigmented mass. In 1 case, preoperative data was not available (patient 7).

Ultrasonography

Ocular ultrasonography was performed by the UKG in all patients, apart from 2 cases. The ultrasound was conducted by the primary veterinarian in one case, but the data is unavailable (patient 7). In another dog, uncooperative behavior impeded a complete ultrasound. The owners deemed a repeated ultrasound under sedation to be unnecessary (patient 4). Among 10 patients, there were no indications of extension into deeper structures. In one patient, focal involvement of the iris could not be excluded (patient 3). In two patients, the mass appeared to be in direct contact with the ciliary body. In these patients the ciliary body could not be clearly differentiated from the mass but did not appear increased in thickness (patients 8, 9) (Table 2).

Preoperative medication

Patients were prescribed appropriate medication up until the day of the surgery. The pre-operative medication information was absent for patient 2. All 14 remaining patients started medication four days pre-operatively. All patients received systemic Prednoral® (prednisolone tablets, 1mg/kg, q 24h). Additionally, the patients received topical medication. Patient 1 was prescribed Maxitrol® (neomycin, polymyxin B, dexamethasone q 6h). Patients 3 through 10 (8/14) received topical Gentapolycort® (gentamycin/polymyxin B/dexamethasone, q 6h). Patients 11 through 14 (4/14) received Dexamytrex® (gentamycin, dexamethasone q 6h). Due to a high normal IOP (intraocular pressure) (24mm Hg), patient 11 was also prescribed dorzolamide/timolol 1 drop the evening pre-operatively and 1 drop in the morning of surgery. Patient 15 was prescribed Alfamydex® (gentamycin, dexamethasone q 6h).

Surgery

The surgeries were performed by different surgeons of the UKG: I.J.M. van Duijnhoven-Slenter (7/15), M.H. Boevé (7/15) and C. van Schaik-Verboven (1/15). Average surgery time was 118 minutes (range, 75-215 minutes). A canthotomy was necessary in 8/15 patients. Of these canthotomies, 7 were lateral and 1 was medial due to a particularly difficult location of the tumor near the nictating membrane. Canthotomies were closed with vicryl 6-0. Trephine blades were used for 14/15 surgeries, ranging from 7 to 11mm in size. For patient 14 Beaver blades 6400/6500 were used due to inadequate trephine blade size availability. Additional neuromuscular blocker cisatracurium was necessary in 4/15 patients (7, 8, 9, 14). Six patients received a tissue plasminogen activator (TPA) injection during the surgery (patient 5, 7, 8, 9,

10, 15). A surgical complication of hyphema occurred in patient 15. TPA was injected intracamerally during surgery, followed by a repeated injection 9 days postoperatively. This resulted in complete resolution of the hyphema. In patient 10, there were multiple intraoperative complications. There was extensive bleeding from the sclera and choroidal prolapse with residual prolapsing choroid in the anterior chamber postoperatively. This resulted in a prolonged surgery time of 215 minutes in total. During the dissection of the scleral flap, there were extensive hemorrhages that were difficult to control, potentially also due to hypertension during anesthesia.

Postoperative medication

All 15 patients continued a tapering schedule of Prednoral® over the course of 2-3 weeks. Patients 1 and 2 were prescribed topical Maxitrol® (q 4h) up until the first control appointment. Patients 3 through 10 (8/15) received topical Gentapolycort® (q 4h for 4 days, then q 6h). Patients 11 through 14 (4/15) received Dexamytrex® (q 4h for 4 days, then q 6h). Patient 15 was given Alfamydex ® (q 4h for 4 days, then q 6h). All patients excluding patients 2, 4, 8 and 10 received Atropine. Patients 4 and 10 were instead given tropicamide. Brinzolamide was given to patient 2 and 4. Additionally, patient 10 was given Acetylcysteïne 10%. Patients 5 and 15 received Cyclosporine 2%. After the first check-up topical antibiotics were tapered over circa 3-6 weeks at ophthalmologists' discretion. Dexamethason 0,1% (q 6h) was started topically in a tapering schedule over the course of circa 12-15 weeks after ceasing of the topical antibiotics. Atropine was given for circa 5 weeks.

Histopathology

Histopathological findings confirmed melanocytoma in all cases (Table 2). Decolorization was applied to all samples. The results of decolorization were lacking in one patient (patient 7). Nine cases exhibited a low mitotic index. The mitotic index was not concisely reported for the remaining 5 cases, although no malignancy features were observed. Complete histological tumor removal was achieved in 4 cases (patients 6, 7, 10, 13), while uncertainty existed regarding complete excision in 8 cases. Data on the extent of tumor removal was lacking for 3 patients (patients 2, 5, 8).

Patient	Size tumor (diameter)	Localization	Histopathology	Ocular ultrasound
1	8 mm	Dorsolateral	Melanocytoma confirmed, Low mitosis index. Possibly not completely removed.	No obvious signs of extension towards deeper located structures.
2	9 mm	Dorsomedial	Melanocytoma confirmed, low mitosis index. Removal status indeterminable.	No obvious signs of extension towards deeper located structures.
3	7 mm	Dorsomedial	Melanocytoma confirmed. No malignant features. Possibly incompletely removed.	Focal involvement of the iris cannot be ruled out.
4	5 mm	Ventrolateral	Melanocytoma confirmed. No malignant features. Possibly incompletely removed.	No exam possible due to uncooperative behavior.
5	7 mm	Dorsolateral	Melanocytoma confirmed, low mitosis index. Removal status indeterminable.	No obvious signs of extension towards deeper located structures.

6	7 mm	Dorsolateral	Melanocytoma confirmed, low mitosis index. Completely removed.	No obvious signs of extension towards deeper located structures.
7	7 mm	Ventrolateral	Melanocytoma confirmed. Completely removed.	Examination external veterinarian, no data available.
8	5 mm	Ventrolateral	Melanocytoma confirmed. Low mitosis index. Removal status indeterminable.	Mild posterior convexity, direct contact with the ciliary body. No clear involvement of the ciliary body/cornea.
9	3 mm	Ventrolateral	Melanocytoma confirmed. No malignant features. Possibly not completely removed.	Mild posterior convexity with direct contact with ciliary body. Ciliary body cannot be clearly differentiated from mass.
10	10 mm	Lateral	Melanocytoma confirmed. No malignant features. Completely removed.	No obvious signs of extension towards deeper located structures.
11	5 mm	Dorsal	Melanocytoma confirmed. No malignant features. Possibly not completely removed.	No obvious signs of extension towards deeper located structures.
12	3 mm	Dorsomedial	Melanocytoma confirmed. Low mitosis index. Possibly not completely removed.	No obvious signs of extension towards deeper located structures.
13	8 mm	Dorsolateral	Melanocytoma confirmed. Low mitosis index. Completely removed.	No obvious signs of extension towards deeper located structures.
14	9 mm	Lateral	Melanocytoma confirmed. Low mitosis index. Possibly not completely removed.	No obvious signs of extension towards deeper located structures.
15	8 mm	Dorsolateral	Melanocytoma confirmed. Low mitosis index, possibly not completely removed.	No obvious signs of extension towards deeper located structures.

Table 2. Description mass including histopathology and ocular ultrasound

Follow-up

The first follow-up occurred on average 10.5 days (range, 1-19) after the surgery with a median of 11 days. In 10 patients, a second follow-up was necessary, which took place on average 33 days (range, 6-103) postoperatively with a median of 27.5 days. Four patients required a third consultation, on average 61.75 days (range, 42-103) postoperatively with a median of 51 days. Two patients (patients 1, 9) had a fourth consultation on 111 average (range, 110-112) days postoperatively with a median of 111 days.

Two patients visited the hospital's emergency department post-operatively. Patient 4 presented 2 days postoperatively with hematemesis, as well as lethargy. Subsequently, the decision was made to discontinue oral prednisolone. Additionally, metoclopramide was administered to address nausea, and omeprazole was given to protect the stomach. At the first follow-up at the ophthalmology department, postoperative symptoms were minimal. However, the patient exhibited hypotony and blepharospasm. Due to the uveitis stimulus, it was decided to restart oral prednisolone at half the previous dosage. At the second follow-up, the recovery was assessed as progressing favourably and as expected. There was some focal,

more extensive limbal pigmentation at the edge of the corneal transplant, which could represent a residual of an inflammatory reaction. This was to be monitored. As a result, the patient attended a third follow-up 103 days postoperatively. It was concluded that the right eye (OD) had fully recovered. The pigmentation had not increased. Patient 10 was admitted to the emergency department in the evening the day of the surgery for lethargy, mild hemorrhagic discharge and blepharospasm. The recommendation was made to admit the patient. If necessary, electrolyte levels were to be assessed, and fluid therapy was considered. However, the owners decided to take the dog home and wait until the next scheduled follow-up appointment.

During the first consultation after surgery, all patients presented with early symptoms (Table 3): graft edema (n=14), hypotony (n=12) consistent with anterior uveitis, hyperemic conjunctiva (n=10) and a small blood/fibrin clot in the anterior chamber (n=9). Due to presence of fibrin in the anterior chamber of the eye and the potential for development of adhesions, patient 13 received a TPA injection at the first check-up. Less common side effects observed at the first examination included: blepharospasm (n=2), scleral vascular injection (n=2), a focal zone of hyper reflection at the tapetal fundus approximately the diameter of the optic nerve (n=2), a small amount of iris pigment on the anterior lens capsule (n=1), peripheral corneal epithelial pigmentation (n=1), mucopurulent exudate (n=1), moderately hyperemic sclera (n=1), posterior medial synechia (n=1), scarring from chorioretinitis OU (n=1), hemorrhagic discharge (n=1), and mild protrusion of the nictating membrane (n=1). The chorioretinitis is likely associated with a systemic toxoplasmosis infection (patient 9).

Five patients had symptoms consistent with the expected course after corneoscleral transplantation during the initial consultation and did not require a second follow-up. Ten patients returned for a second consultation. Among the previously mentioned common symptoms, hypotony (n=9) and graft edema (n=8) were still prevalent, while hyperemic conjunctiva (n=1) and a small blood/fibrin clot in the anterior chamber (n=2) had resolved in many cases. Other symptoms reported included blepharospasm (n=1), scleral vascular injection (n=2), stromal small bullae and iris pigment on the anterior lens capsule with fibrosis (n=1), peripheral corneal epithelial pigmentation (n=1), focal pigmentation on the anterior lens capsule (n=1), limbal pigmentation at the graft edge (n=1), mucopurulent/mucous discharge (n=2), moderately hyperemic and swollen sclera around the graft (n=1)(Fig. 2) , posterior medial synechia (n=1), scarring from chorioretinitis (n=1), a small, pigmented spot at the limbus – possibly translucent choroid (n=1), a lucent tunnel to the endothelium (n=1), posterior focal subcapsular cataract (n=1), decreased tear production (n=1), keratitis (n=1), diffuse mild clouding of the anterior lens capsule (n=1) and iris-colored deposits on the medial anterior lens capsule (n=1).

Fig 2. patient 14: mild diffuse graft edema. Sclera moderately hyperemic and swollen around graft.

Patient	Pre-operative symptoms	Symptoms first consultation	Symptoms second consultation	Symptoms third consultation
1	OD hypotony (13), Mild hyperemic conjunctiva.	OD hypotony (10), Hyperemic bulbar conjunctiva, diffuse graft edema, small blood clot present in the anterior chamber, small amount of iris pigment on anterior lens capsule.	OD hypotony (12), diffuse graft edema, stromal small bullae and iris pigment on anterior lens capsule with fibrosis.	OD hypotony (14), diffuse mild corneal edema, small string of fibrin from anterior lens capsule towards graft, small amount of iris pigment on anterior lens capsule.
2	OD Hypotony (9), STT 12, white crystalline corneal opacity ventrolateral to pigmented mass.	OD hypotony (6), Hyperemic conjunctiva, diffuse graft edema.	OD Hypotony (7), graft edema.	-
3	Hypotony OD (9) OS (6), Nucleus sclerosis OU.	Hypotony OD (12) OS (6), moderate hyperemic conjunctiva, diffuse graft edema, moderate amount of fibrin in the eye.	-	-
4	No abnormalities.	Hypotony OD (7), minimal focal graft edema.	Hypotony OD (6) OS (9), moderate graft edema, focal limbal pigmentation at graft edge.	Hypotony OD (9) OS (9), moderate graft edema, limbal pigmentation stable.
5	OS: Hypotony (11), Negative direct pupillary reflex, pupil in fixed status, moderate hyperemic conjunctiva, peripheral cornea epithelial pigmentation, uveitis anterior, anterior lens capsule multifocal PPM. OD: Consensual pupillary reflex negative.	Hypotony OS (8), graft edema, medioventral small fibrin cloth anterior chamber, cornea peripherally epithelial pigmentation OS.	Hypotony OD (10) OS (7), graft edema, cornea peripherally epithelial pigmentation OS.	-
6	No abnormalities.	OD: moderate hyperemic conjunctiva.	-	-
7	No records.	OS: hypotony (6), mild hyperemic conjunctiva, corneal graft edema, Mucopurulent exudate around eyelids.	-	-
8	OS: moderate hyperemic conjunctiva.	OS: Hypotony (7), moderate hyperemic conjunctiva, graft edema.	-	-
9	OU: hypotony (9), minor nuclear sclerosis. Scarring from chorioretinitis OU.	OD: hypotony (8), mild hyperemic conjunctiva. mild graft edema, thin sheet of fibrin in front of pupil, mild scleral vascular injection, clear discharge, medial synechia posterior. Scarring from chorioretinitis OU.	OD: Hypotony (7), dense graft edema, mucopurulent discharge, moderate scleral vascular injection, severely thickened corneal graft, in front of pupil two thin sheets of fibrin, medial synechia posterior, anterior lens capsule focal pigmentation. Posterior focal subcapsular cataract. Scarring from chorioretinitis OU.	OD: Moderate graft edema with slight pigmentation, reduced thickening of graft, synechia posterior, anterior lens capsule focal pigmentation. Posterior focal subcapsular cataract. Scarring from chorioretinitis OU.

10	No abnormalities.	OS: Hypotony (13), STT OD 9 OS 11, moderate diffuse graft edema, thin layer fibrin with pigment in anterior chamber, blepharospasm, hemorrhagic discharge.	OS: Hypotony (12), STT OD 8, OS 12, mucous discharge, blepharospasm.	-
11	No abnormalities.	OD: graft edema with severe swelling, mild amount of fibrinous material on anterior lens capsule.	OD: mild graft edema, keratitis. Diffuse mild clouding of the anterior lens capsule; iris-colored deposits on the medial anterior lens capsule.	-
12	No abnormalities.	OD: Conjunctiva moderately hyperemic, graft edema. Dorsolateral a focal zone of hyperreflection, approximately the diameter of the optic nerve.	-	-
13	No abnormalities.	OS: Hypotony (9), moderate hyperemic conjunctiva, graft edema, blood cloth dorsolateral in anterior chamber with fibrin, moderate blepharospasm, dorsomedial focal zone of hyperreflection approximately the diameter of the optic nerve, photophobia, mild protrusion nictating membrane.	OS: hypotony (11), in sclera a few mm from the medial and lateral transition to the limbus a small, pigmented spot – possible translucent choroid. In the corneal transplant there appears to be a lucent tunnel to the endothelium, but no additional swelling.	-
14	OD: minor vascular injection episclera, medial focally a small zone of lipidosis of the stroma.	OD: Hypotony (12), mild hyperemic conjunctiva, graft edema, focal blood clot with fibrin dorsal at the pupillary margin with fibrin sclera moderately hyperemic.	OD: hypotony (12), mild diffuse graft edema, moderate hyperemic sclera and swollen around graft, mild hyperemic conjunctiva, Focal blood clot with fibrin dorsal at the pupillary margin.	OD: hypotony (14), graft edema.
15	No abnormalities.	OD: hypotony (13), Diffuse graft edema. thick blood clot ventrolateral anterior chamber, scleral vascular injection.	OD: hypotony (13), graft edema. mild scleral vascular injection.	-

Table 3. Short term symptoms after surgery

Four patients returned for a third follow-up. For the other 6 patients, a third follow-up was not indicated. Patient 1 presented at the 3rd follow-up with OD hypotony (14), diffuse mild corneal edema, a small string of fibrin from the anterior lens capsule towards the graft and a small amount of iris pigment on the anterior lens capsule. Since many symptoms persisted, this patient also returned for a 4th and final follow-up. At this point, only graft edema, iris pigment on the anterior lens capsule with a small string (possibly PPM and not fibrin) were observed. Patient 4 also had a third follow-up, showing persistent hypotony (9) and moderate graft edema. However, limbal pigmentation was stable, leading to no further follow-up

needed. Patient 9 returned for both a 3rd and 4th follow-up. During the process, this patient was tested for toxoplasmosis, with a positive result. At the 3rd follow-up some symptoms were still present OD: moderate graft edema with slight pigmentation, reduced graft thickening, posterior synechia, focal pigmentation on the anterior lens capsule, posterior focal subcapsular cataract, and scarring from chorioretinitis OU. A fourth consultation was conducted due to the dog experiencing blepharospasm and scleral vascular injection. Findings included hypotony (5), mild serous discharge, mild photophobia, moderate uveitis, synechia posterior, focal pigmentation on the anterior lens capsule, posterior focal subcapsular cataract, and scarring from chorioretinitis OU. Lastly, patient 14 had a 3rd follow-up, showing only hypotony (14) and graft edema before being discharged. In none of the cases there was evidence of tumor regrowth in the short term. Despite the removal of a relatively large portion of the cornea and sclera with the mass, there were no major short-term complications regarding the homologous graft. All patients retained vision in the affected eyes.

Patient	Time after surgery	Edema	Fibrosis	Pigmentation	Lipid deposits
6	6 years, 10 months, 17 days				X
11	3 years, 5 months, 19 days	X		X	X
12	3 years, 5 months, 29 days	X		X	
13	3 years, 4 months, 5 days	X	X		X
14	2 years, 24 days	X	X		X
15	1 year, 1 month 12 days		X		X

Table 4. Common long-term graft descriptions

To assess long-term complications, the patients were approached for a follow-up appointment. Of these, 6 patients attended the long-term follow-up appointment, 2 owners were consulted via telephone. Unfortunately, 2 patients had already passed away and for 5 patients the current contact information was unknown. The long-term complications of the patients that attended the long-term follow-up are detailed in table 4. Long-term follow-up was conducted at a mean of 3 years, 5 months and 18 days post-surgery (range, 1 year, 1 month, 12 days – 6 years, 10 months, 17 days). All grafts remained in situ at the long-term follow-up. All patients retained vision. No recurrence of the tumor was observed in all cases.

The graft of patient 6 was observed to be remarkably clear. At the transition from graft to cornea, silver-white lipid deposits were noted. Parallel to and ventromedial to the graft a narrow, crescent-shaped 'halo' of less dense stromal lipids were visible. Other ophthalmologic findings were nucleus sclerosis OU, capsular fibrosis (OS>OD) and slight dyscoria.

At the level of the graft of patient 11, the sclera was slightly thicker than the surrounding sclera. The cornea of the graft is moderately edematous and thickened with focal

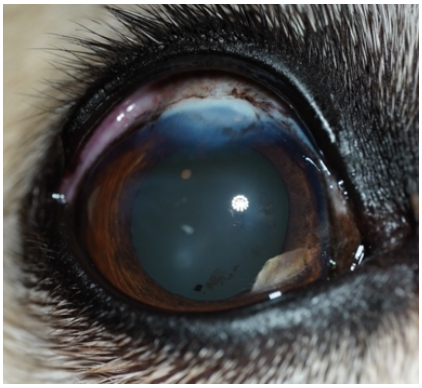

Fig 3. Patient 11: dorsal moderate graft edema with focal endothelial deposits.

endothelial deposits (fig 3). Centrally 2 small foci of endothelial deposits were observed. At the lens focal deposits of uveal pigments were visible on the anterior lens capsule. The sclera of patient 12 appeared to be slightly raised to the surrounding sclera. The corneal part of the graft was moderately thickened and edematous but otherwise clear. A moderate amount of pigmental deposition was seen at the cornea endothelial side of the graft (fig 4). Patient 13 showed cholesterol deposits in the superficial stroma at the edge and centre of the graft and endothelial deposits at the centre of the graft. Moderate graft edema with focal thickening. Posterior lens capsule shows focal fibrosis. The lens showed focal posterior cortical cataract and perinuclear cataract. The previously observed focal zone with some multifocal hyperreflective zones had now decreased in diameter from the entire diameter to approximately 1/2 the diameter of the optic nerve. For patient 14 the corneal part of the graft had diffuse moderate swelling with mild edema and focal fibrosis. Lipid deposits were observed along the edge of the cornea-graft transition. The corneal part of the graft was slightly raised for patient 15. The endothelial side showed mild fibrosis. A slight amount of lipid deposits was visible along the donor button's edge. The remains of PPM were seen, similar to previous consultations. The current eye status of patient 7 and 9 was inquired over the telephone. Both dogs were doing very well. Vision was intact and there appeared to be no signs of tumor regrowth according to the owners.

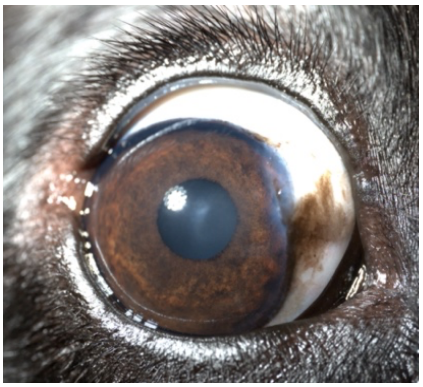

Fig 4. Patient 12: dorsomedial graft thickened and edematous.

Fig 5. Patient 13: moderate graft edema with lipid deposits.

DISCUSSION

Signalment

In the present study 9 out of 15 dogs are either Labrador Retriever or Golden Retriever. The sample size of the study is not big enough for reliable statistics. However, the high percentage does support the breed predisposition theory. Previous studies suggested two distinct age groups (2-4 years, 8-11 years) for the occurrence of epibulbar melanocytomas. [10,18] In the current study the young age group is represented with 4/15 dogs. The older group is noticeably absent. It can be theorized that this is due to owner unwillingness to have their elderly dog operated and does not indicate the absence of the occurrence of the tumor in this age group. The middle-aged group (4-8) is considerable with 11/15 dogs. The size of this group is remarkable and does not support the previously suggested distinct age groups. The distribution of affected eyes (8 OD, 7 OS) does not seem to indicate an affected eye predisposition. There is a slight discrepancy in the gender of the patients (9 females, 6 males), but due to the small sample size no conclusion can be drawn from this.

Ophthalmic examination

Epibulbar melanocytomas typically present with minimal clinical symptoms, consisting of mild conjunctival irritation and/or epiphora. In 7 patients, no clinical symptoms were observed besides the pigmented mass. In the remaining patients, various preoperative symptoms were reported (table 3). A commonly described preoperative symptom was hypotony (n=5). The exact cause of low IOP is unknown. It is likely that growth of the neoplasm caused irritation, resulting in the development of secondary uveitis. This phenomenon, often described in intraocular tumors, is also referred to as pseudo-uveitis. [29,30] Nuclear sclerosis was also reported in 2 patients in both eyes, explainable as a normal aging process of the lens in geriatric patients. Mild to moderate conjunctival hyperemia was also commonly reported, consistent with previous reported clinical symptoms for epibulbar melanocytomas in the literature. [21] Patient 5 had multiple pre-operative symptoms (table 3). However, this patient had an unknown trauma to the left eye 3 years pre-operatively, which may explain several symptoms such as negative direct pupillary light reflex, fixed pupil, and peripheral corneal epithelial pigmentation. Additionally, patient 9 was observed to have bilateral chorioretinitis and anterior uveitis, and this dog tested positive for toxoplasmosis during the process. Studies on toxoplasmosis in dogs have shown that these symptoms are common associated with toxoplasmosis. [31] The tumor was localized in the dorsal arc from dorsomedial to ventrolateral limbus in 83% of cases in previous studies. [11] In this study, the tumor was observed at various locations, with the dorsolateral region being the most common location (n=5). All localizations were consistent with the previously reported 83% and were located in the area of the dorsal arc from dorsomedial to ventrolateral. [11]

Ultrasound

The purpose of the ocular ultrasound was to rule out any extension into deeper structures. Ocular ultrasound data was available for 10 patients with epibulbar melanocytomas. Previous studies have shown that epibulbar melanocytomas can grow locally in an aggressive manner and affect the anterior chamber of the eye. [10,12,19] It was previously reported that intraocular invasion occurred in 16% of cases. [9] In the current study, in 2 patients there was direct contact between the melanocytoma and the ciliary body. The ciliary could not be clearly differentiated from the mass but did not appear increased in thickness. In 1 patient, focal involvement of the

iris could not be excluded. However, no intraocular invasion was observed in any of the 10 patients. These findings suggest that while epibulbar melanocytomas can invade local structures, intraocular invasion may be less common than previously reported. [9] The difficulty in differentiating the melanocytoma from the ciliary body in some cases highlights the importance of careful imaging and evaluation when assessing the extent of these tumors.

Surgery

In the present study, the surgeries used frozen homologous graft. This technique provides several benefits for the veterinary field. It is generally inexpensive and provides the opportunity to store the tissue over a long period of time with preservation of the collagen structure. As the amount of suitable donor eyes offered is limited and inconsistent over time, cryopreservation is a necessity at the moment for most veterinary clinics. However, the preservation technique is not flawless. Complete corneal clarity is difficult to achieve, as the endothelial cells are unlikely to survive the freezing process. The endothelium is the main corneal layer responsible for corneal transparency, as it is able to maintain stromal dehydration. [32,33] The Corneal endothelium has limited regeneration capacity. Consequently, the vast loss of corneal endothelium due to prolonged freezing leads to a blurred cornea. There is a paucity in studies conducted on the maximum duration of cryopreservation and can therefore not be reliably determined. In Costa et al [33], it is suggested that corneal and scleral tissue can be cryopreserved with low bacterial contamination, high organization and collagen preservation at -20° Celsius for up to 8 years.

In the study by Mironovich et al [34], the risk factors for corneal conjunctival grafting failure were described. It was concluded that surgery on the opposite eye relative to surgeon handedness was significantly associated with an increased risk of graft failure. However, in the present study the handedness of the surgeons was not specified. Although there was no graft failure, it is advisable to take the study of Mironovich et al, into account if possible. Furthermore, it was described that the use of 7-0 and 8-0 sutures versus 9-0 sutures and the use of a combined simple continuous and interrupted suture pattern were significantly associated with an increased risk of graft failure. In this study, the corneal graft was closed using 9-0 vicryl and the sclera was closed using 8-0 vicryl. Additionally, the donor button was first secured with 4 cardinal sutures before the cornea and sclera were closed with a single continuous suture pattern. The use of a single continuous suture pattern and the chosen suture material is therefore advantageous, as it significantly reduces the risk of graft failure. The treatment of epibulbar melanocytomas in dogs has been a topic of discussion, with various treatment modalities being explored. In a comparative study [22], radiotherapy was found to have more severe long-term symptoms, including focal scleromalacia, lipid keratopathy, sectoral cortical cataract, localized bullous keratopathy, and globe perforation, although these effects occurred in only 20% of cases. Interestingly, the recurrence rate in this study was low (1/30). Laser therapy, on the other hand, has been reported to cause corneal fibrosis and pigmentation as long-term effects. The advantages of this technique include its minimally invasive nature and the lack of a need for donor tissue. [35] However, a major disadvantage is the relatively high chance of recurrence. [25] Another treatment option is lamellar resection followed by cryotherapy. Long-term effects include mild or marked corneal lipidosis, with marked lipidosis appearing to occur more quickly in cases of extensive limbal melanoma. It is important to note that there was no recurrence with this procedure. However, the inflammation following the procedure can be significant and acute, necessitating careful

follow-up evaluations. [26,35] Enucleation is only recommended when there is rapid tumor growth with extension into intraocular structures. [36] A previous study described the treatment of epibulbar melanocytomas in dogs using full-thickness resection and homologous corneoscleral grafting. In this study, corneal fibrosis and/or pigmentation were observed in all patients as long-term effects. Additionally, lipid keratopathy and mild anterior cortical cataracts were noted. [21] Another treatment option is a full-thickness resection and a synthetic graft. A study describing this procedure reported graft failure, with the graft remaining in the desired location for 7 weeks before being surgically removed. However, healthy new tissue formed beneath the graft. [24] A less commonly described treatment option is full-thickness resection with xenogeneic submucosa. The short-term postoperative symptoms include corneal clouding, granulation throughout the graft, neovascularization, and hyperemia. A previous case report described this method as useful, with the notable finding that the area of corneal repair becomes completely transparent after the procedure. [23] The treatment of epibulbar melanocytomas in dogs requires careful consideration of the potential risks and benefits of each treatment modality. While radiotherapy and laser therapy have been used, they are associated with long-term complications and a higher risk of recurrence. Lamellar resection followed by cryotherapy and full-thickness resection with homologous corneoscleral grafting or synthetic grafts have shown promising results, but careful follow-up is necessary. Enucleation remains an option for rapidly growing tumors with intraocular extension. Further research is needed to optimize treatment strategies and minimize long-term complications.

Histology

In previous studies, it was established that the distinction between benign and malignant tumors can be determined based on the mitotic index, nuclear pleomorphism, and nuclear-to-cytoplasmic ratio. [8,11,37] Earlier studies have reported malignant features in 5 out of 30 dogs. [11] In the current study population however, no malignant features were observed. A low mitotic index was reported in 9 out of 15 patients. For the remaining patients the mitotic index was not discussed. A previous report [11] has suggested that 18% of the study population exhibited histological tumor necrosis, but in the current study no tumor necrosis was observed on histological examination. Similar to the findings reported by Maggio et al [21], the current study did not identify any malignant characteristics on histopathological examination, and all tumors were characterized as benign. Over the years, different pathologists were involved in the histological analysis of the patients, which led to a lack of consistency in the reporting. Going forward, it would be ideal for pathologists to establish a consistent approach in reporting either malignant features or the mitotic index, and evaluating nuclear pleomorphism and the nuclear-to-cytoplasmic ratio could provide additional insights. It is difficult for pathologists to determine the real removal status of the tumor. The pathologists can only assess the cut surfaces, not the surface on the other side of the cornea or sclera. In the histopathological examination, it was reported that in 4 patients the tumor was completely removed, while in the remaining cases the tumor was not completely removed (n=1), there was doubt about the complete removal (n=7), or no data was available (n=3).

Follow-up short term

Postoperative examination revealed several complications, including neovascularization (n=2), corneal pigmentation (n=1), and corneal edema (n=14). Previous studies have documented these complications following penetrating keratoplasty, where they were described as clinically insignificant. [21] In patients 12 and 13, a focal zone of hyper-reflection

approximately the diameter of the optic nerve was observed during the first postoperative examination, which was not detected preoperatively. Despite the precautions taken (low light source setting, turning off the light source when no procedures were performed), focal phototoxicity of the retina may have occurred due to the operating microscope light and the duration of the procedure. In patient 13, at the long-term follow-up 3 years and 7 months post-operation, fundoscopy revealed the hyperreflective zone, which had now reduced to half the diameter of the optic nerve. During penetrating keratoplasty, intraoperative complications such as uveal contusions and intraocular hemorrhages may occur. Other potential lesions include conjunctival hyperemia, fibrin and cellular debris in the anterior chamber, and hypotony. These findings are consistent with the commonly observed symptoms at the first postoperative examination, including hypotony (n=12), hyperemic conjunctiva (n=10), graft edema (n=14), and a small blood/fibrin clot in the anterior chamber (n=9). These symptoms are to be expected with intraocular surgery and with the use of frozen homologous grafts.

During the second follow-up visit patient 11 exhibited more clinical signs compared to the initial assessment. The dog owner reported that the dog had not received any medication for 2-3 weeks prior to the second visit due to personal circumstances. This lapse in treatment may explain the worsening of the clinical signs observed. Patient 1 was a patient who required more frequent follow-up examinations before being discharged. In this patient, the initial examination revealed diffuse graft edema. This can occur because the surgical procedure can lead to dysfunction of the draining capacity of the corneal endothelium surrounding the graft, leading to corneal edema. [38] In some cases, there may be a degree of loosening of the layers above the endothelium. This separation can lead to the formation of bullae. In patient 11, keratitis was observed during the second examination. This condition can arise due to the continuous exposure of the outer cornea and conjunctiva to harmful materials, such as microorganisms, dust, and other sources of irritation. This creates a portal of entry for bacteria, for example. Surgical trauma can cause iatrogenic issues which may lead to keratitis.

Long-term follow-up

During the long-term follow-up, it became clear that the grafts were properly incorporated in the corneoscleral tissue. However, it was noticeable that some grafts were slightly raised and the donor cornea had a clouded appearance in 4 out of the 5 patients. In patient 1, the scleral graft was slightly raised but the donor cornea remained remarkably clear over the years. The same preservation technique was used for all the grafts. It is possible for heavy shaking during transport to have a detrimental effect on the preservation of endothelial cells, leading to stromal edema. [39] Theoretically, it is possible that some donor grafts were handled in a shaking like manner more so than others. However, it is unlikely that this is the cause of the differences in corneal clarity, as the transport distance is minimal. It does emphasize the importance of safe transport and careful manipulation. Lipid deposits along the edge of the graft were frequently observed at the long-term follow-up. Lipid deposits may arise due to abnormal vascularization of the cornea, where the lipids classically deposit adjacent to these vessels. It is possible that these lipids form at the transition from the graft to the cornea due to atypical vascularization at this junction, potentially leading to opacification.

Limitations

This study has several limitations. The study represents a retrospective analysis, this means that not all pre-operative examinations, surgeries, and follow-up visits were performed in an identical manner, which can make the results more challenging to interpret. For some patients, not all information was available, potentially introducing information bias. Additionally, the surgeries were performed by different surgeons, each with their own follow-up protocols. For example, patient 2 had their first follow-up at 19 days and the second at 103 days post-operatively, while the average second follow-up was at 33 days. In contrast, the follow-ups for patient 10 occurred relatively quickly, as the owners had travelled from abroad for the surgery and could not stay long, resulting in the first follow-up at 1 day and the second at 6 days post-operatively. The first follow-up for this patient was brought forward, as the owner had visited the emergency clinic the evening after surgery due to noticing signs of lethargy, mild hemorrhagic discharge, and blepharospasm. For a prospective study, it would be beneficial to note the used donor material in the surgical rapport, including date of freezing and origin of breed. It would be interesting to experiment with the cryopreservation protocol. A different protocol might lead to reduced loss of endothelium and consequently a reduction in corneal edema and increase in corneal clarity.

CONCLUSION

Despite the technical challenges associated with this surgical technique, it represents a worthwhile approach for managing epibulbar melanocytomas. The benefits of stable graft placement, vision preservation, low tumor recurrence rates, and minimal long-term side effects make this procedure a valuable option for ophthalmologists and their patients.

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