CASE REPORT

Penetrating sclerokeratoplasty and autologous pinnal cartilage and conjunctival grafting to treat a large limbal melanoma in a dog

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Abstract

A four-year-old neutered male Labrador retriever presented to Portland Veterinary Specialists Ophthalmology Service for evaluation of a pigmented mass oculus sinister (OS) of approximately 4-month duration. Complete ophthalmic examination revealed a large, pigmented, raised, well-demarcated, epibulbar mass appearing to originate from the nasodorsal limbal region. The mass was smooth and roughly circular, extending approximately 4 mm into the sclera and 14 mm into the nasodorsal cornea. Gonioscopy directly under the mass was not possible due to mass size. The visible iridocorneal angle was normal. High-resolution B-scan ultrasound showed mass extension to Descemet’s membrane and deep sclera, but no intraocular invasion. Penetrating sclerokeratoplasty was performed followed by autologous pinnal cartilage and conjunctival grafting to repair the corneoscleral defect (20 mm x 19 mm) and to restore globe integrity and function. Histopathology confirmed the mass to be a benign limbal melanoma with complete excision. The surgery site healed without complication, and the pinnal cartilage became fully incorporated into the globe. Twelve months postoperatively, the patient remains visual with a normal intraocular and fundic examination. The pinnal harvest site on the right ear healed without complication. To the authors’ knowledge, this is the first reported case of corneoscleral grafting using autologous pinnal cartilage. This may represent a viable alternative to other corneoscleral grafting procedures for large defects and is an attractive treatment option due to lack of host rejection, readily available source of donor cartilage, and provision of tectonic support to the globe.

Key Words: cartilage, corneal transplant, limbal melanoma, melanoma, penetrating sclerokeratoplasty, pinna

INTRODUCTION

Limbal melanomas or melanocytomas, tumors arising from limbal melanocytes, are commonly reported benign neoplasms of the canine eye.1-3 Some discussion exists in the literature regarding whether the term ‘melanoma’ or ‘melanocytoma’ is the most appropriate descriptor of these tumors.3,5 Regardless, limbal melanocytic neoplasia has a typical appearance and behavior, resulting in these masses being categorically grouped together. Limbal melanomas are typically pigmented, well-circumscribed, raised masses arising from the limbus most commonly in the dorsonasal to ventrotemporal arc dorso-temporally.6,7 Amelanotic limbal melanomas have also been described.8 While limbal melanomas are usually benign, they may be locally aggressive,5 with intraocular invasion being reported in 16% of total cases and 33% of incompletely excised tumors.4 Even without intraocular invasion, progressive tumor expansion into the cornea may result in vision loss for the patient.2,4 This is especially true for the corneal region usually affected by limbal melanomas as the dorsal and temporal quadrants of the cornea are more significant for vision than the ventral and nasal quadrants.9 This is due to globe position within the canine orbit, large canine nose, and longer canine pars plana in the dorsal and temporal quadrants affecting the peripheral nasal and ventral visual hemifields.10,11

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A breed predisposition exists for limbal melanoma development with Labradors, golden retrievers, and German shepherds being at higher risk compared with other breeds.1,7 An association between heavily pigmented dogs and tumor development has also been described;4 however, one study showed no relationship between coat color and tumor formation.7 A bimodal age distribution has been reported with a peak incidence of tumor formation occurring at 3–4 years of age and 7–10 years of age.7 It has also been suggested that limbal melanomas affecting younger dogs may be more rapidly growing and aggressive than those affecting older dogs.3

Therapeutic approaches are quite varied regarding limbal melanomas with descriptions of benign neglect,8 plesiotherapy,6 cryotherapy,12 laser treatment,13,14 partial surgical removal,15 complete excision,15,16 or a combination of therapies being purported in the literature. No consensus exists regarding the single best treatment approach for these tumors. Factors affecting treatment protocol include patient age, aggressiveness or perceived aggressiveness of the tumor, growth rate, location of the mass, presence of vision, extent of the mass, and length of duration. Thus, the varied clinical presentation of limbal melanoma dictates that the most appropriate therapeutic approach differs among cases.

This report describes a penetrating sclerokeratoplasty with pinnal cartilage transplantation to treat a large growing limbal melanoma in a dog. To the authors’ knowledge, this is the first reported case of pinnal cartilage transplantation to repair a scleral or corneal ocular defect in a dog.17,18

CLINICAL REPORT
Initial presentation
A four-year-old neutered male black Labrador retriever presented to Portland Veterinary Specialists Ophthalmology Service for evaluation of a nasodorsal pigmented limbal mass OS. On initial presentation, the mass was roughly circular, well-circumscribed, smooth, and raised, measuring approximately 8 × 9 mm (Fig. 1). There was an arc of corneal lipidosis at the leading edge of the tumor, as has been well described.1,12 The remainder of the ophthalmic examination was normal at this time as determined by slit-lamp biomicroscopy (Kowa SL-15, Kowa Optimed, Torrance, CA, USA) and indirect ophthalmoscopy (Keeler Vantage Plus, Dan Scott and Associates, Westerville, OH, USA) performed by a board-certified veterinary ophthalmologist. The mass had been present for approximately 1 month. A presumptive diagnosis of limbal melanoma was made. The patient had a concurrent ruptured right anterior cruciate ligament that had recently occurred, and repair of the cruciate rupture was planned with a board-certified surgeon. Limbal surgery was planned after cruciate surgery as the cruciate tear was affecting patient quality of life more significantly than the limbal mass, and concurrent surgeries were not feasible or warranted.

Second presentation
Three months after initial presentation, the patient presented for surgical removal of the limbal mass. The presumed limbal melanoma had substantially grown without changing in overall gross appearance, measuring approximately 17 × 18 mm (Fig. 2). The patient had since recovered uneventfully from cruciate surgery, and all other findings on physical examination were unremarkable.

A complete ophthalmic examination was performed by slit-lamp biomicroscopy and indirect ophthalmoscopy again at representation. The ophthalmic examination was otherwise normal save for the previously described mass.

Figure 1. The left eye is depicted from the patient at the time of presentation. A well-circumscribed, pigmented, roughly circular, raised mass is present at the dorsonasal limbus extending into the cornea and sclera (scleral portion visible on excyclotorsion and abduction of the eye) measuring 8 × 9 mm. A crescent of corneal lipid is present at the leading edge of the mass. The ring flash artifact distortion is caused by the mass being raised off the globe surface.

Figure 2. The left eye is depicted from the patient 3 months after initial presentation and at the time that surgical excision was performed. The mass is raised, pigmented, well-circumscribed, and had grown substantially since initial presentation measuring 17 × 18 mm. There is a crescent of corneal lipid at the leading edge of the mass.
Both globes were in a normal position with normal motility. Schirmer tear test values were 18 mm/min OD and 20 mm/min OS (Schirmer Tear Test, Merck and Company, Inc., Whitehouse Station, NJ, USA), and intraocular pressures were 18 mmHg OD and 19 mmHg OS measured by applanation tonometry (Tonopen XL, Reichert Technologies, Depew, NY, USA). There was no corneal or conjunctival fluoroscein stain retention OU. The patient was visual in the left eye as determined by menace response, tracking and navigating with the right eye covered.

Gonioscopy was performed OS (G3 Three-Mirror Glass Gonio Fundus Lens; Volk Optical, Inc., Mentor, OH, USA). All visible regions of the iridocorneal angle were normal; however, the angle deep to the mass centrally could not be evaluated due to mass size. Peripheral to the mass, pigment could be seen within the overlying cornea and sclera, but did not extend into the iridocorneal angle. Ultrasound biomicroscopic imaging (Linear 50 mHz UBM probe; Aviso, Quantell Medical, Bozeman, MT, USA) was obtained and showed a large, limbal-based, homogenous, soft tissue, anechoic mass. The mass extended full thickness through the cornea and sclera, but did not extend into the globe or iridocorneal angle.

Based on these diagnostics and the owners’ desire to preserve the globe and vision, a penetrating sclerokeratoplasty with pinnal cartilage grafting was planned to treat the large, pigmented, limbal mass.

**Pinnal cartilage harvest, penetrating sclerokeratoplasty, and corneal grafting**

The patient was premedicated prior to general anesthesia with acepromazine (0.02 mg/kg IV; Fort Dodge, Fort Dodge, IA, USA) and hydromorphone (0.1 mg/kg IV; West Ward, Eatontown, NJ, USA). Anesthetic induction was achieved with PropoFlo (4 mg/kg IV; PropoFlo, Abbott Animal Health, North Chicago, IL, USA) to effect. General anesthesia was maintained after endotracheal intubation with inhalant isoflurane 1%–2% in oxygen (Isoflo, Abbott Animal Health, North Chicago, IL, USA). After endotracheal intubation and anesthesia maintenance, cefazolin (22 mg/kg IV; WG Critical Care LLC, Paramus, NJ, USA) and dexamethasone sodium phosphate (0.5 mg/kg IV, Bimeda-MTC Animal Health, Cambridge, Ontario, Canada) were administered.

The central convex left pinnal surface was clipped and prepped routinely. Two 20-mm linear connecting skin incisions were made 90 degrees to each other with a #15 blade. The skin and fibrous connective tissue were undermined from the central pinnal cartilage for a distance of 22 mm using Mayo scissors. The pinnal cartilage was incised with a #15 blade, and the concave surface of the cartilage was undermined from the skin for a distance of approximately 22 × 22 mm. The cartilage incision was extended using Mayo scissors, and a 21 × 21 mm square of cartilage was harvested from the central pinnal with measurements based on the planned sclerokeratoplasty. The cartilage section was flushed with cefazolin (100 mg/mL) and dilute betadine (1:1000; Butler Animal Health, Melville, NY, USA) and placed in sterile saline until grafting surgery. The skin of the pinnal harvest site was closed with 4-0 Ethilon (Ethicon, J&J, New Brunswick, NJ, USA) simple interrupted skin sutures. Multiple-staggered vertical full-thickness simple interrupted pinnal sutures were placed with 4-0 Ethilon in the left pinna parallel to the long axis of the pinna avoiding major auricular blood vessels to prevent aural hematoma formation. A Penrose drain was placed through the cartilage defect emerging through the dorsal and ventral portions of the skin incision and secured with 4-0 Ethilon (Fig. 3a).

The patient was repositioned in dorsal recumbency, and the left eye was prepped routinely for surgery. Intraoperative neuromuscular paralysis was achieved with rocuronium (0.1 mg/kg IV Q15 min; SAGENT Pharmaceuticals, Schaumburg, IL, USA) to decrease extraocular globe forces and provide ideal globe position for surgical exposure. A peripheral neuromuscular stimulator (Sun-Stim, SunMed, Largo, FL, USA) with TOF (train-of-four) monitoring was used to determine the frequency of rocuronium dosing required to maintain ideal globe position. The patient was also provided time-cycled, volume constant, pressure-limited mechanical ventilation (Model 2002, Hallowell EMC, Pittsfield, MA, USA) with respiratory parameters calculated using patient weight and adjusted, if warranted, based on capnograph, PCO2 and SPO2 monitoring during neuromuscular paralysis.

A penetrating sclerokeratoplasty was performed in the left eye as previously described.1,8 Briefly, a partial-thickness free-hand roughly square corneal and scleral incision was made outlining the lesion and 1–2 mm of surrounding healthy cornea and sclera to include the total mass. The anterior chamber was entered, evacuated, and maintained with a viscoelastic substance (I-Visc-Vet 1.8% HA; Dollard-dex-Ormeaux, Quebec, Canada). Viscoelastic material was also used to gently dissect the ciliary body and rostral choroid from the scleral component of the excised tissue. The viscoelastic substance was left in place throughout the procedure and replaced as required to maintain the anterior chamber. The viscoelastic was not removed at the completion of surgery. Hemorrhage was controlled with disposable microcautery (Bovie; Aaron Medical, St. Petersburg, FL, USA) and topical preservative-free epinephrine (1:1000; Hospira Inc, Lake Forest, IL, USA). The excised mass was placed immediately into 10% buffered formalin.

The previously harvested pinnal cartilage section was placed in the defect and trimmed to the size of the defect plus 1 mm to account for graft shrinkage. Once placed within the defect, the cartilage section did not conform to the globe curvature due to thickness and inherent cartilage stiffness. Thus, the cartilage section was sectioned in half...
along the length using a 6500 Beaver blade to reduce the cartilage plate overall thickness. This created a section of partial-thickness fibrous and cartilaginous autologous tissue that was pliable and would conform to the globe curvature (Fig. 3c). This was sutured into the corneoscleral defect using 7-0 Vicryl (Ethicon; J&J, New Brunswick, NJ, USA) simple interrupted sutures (Fig. 3c). Topical atropine (Akorn, Inc., Lake Forest, IL, USA) was administered intra-operatively to decrease ciliary spasm. After graft placement, a pedicle conjunctival graft 10 mm in width and 21 mm in length was harvested from the dorso-temporal bulbar conjunctiva as previously described\(^1\) to provide a vascular supply to the corneal portion of the free auricular cartilage graft (Fig. 3d). The conjunctival graft was not large enough to cover the entire transplant site due to the large size.

Figure 3. Schematic diagrams of the pinnal surgery site (a), limbal mass (b), cartilage surgery transplant (c), and conjunctival graft (d) are depicted. Figure A depicts the left pinnal at the time of skin closure after harvesting of the central free cartilage graft. Two 20-mm linear connecting skin incisions were made 90 degrees to each other on the convex left central pinnal surface as depicted by the upside down ‘L’ in the diagram. After the skin was undermined from the cartilage and the cartilage plate was harvested as depicted by the dotted square, a Penrose drain was placed through the cartilage defect emerging through the dorsal and ventral portions of the skin incision and secured with 4-0 Ethilon on the convex surface as depicted by the two parallel lines to close the skin defect. Multiple-staggered vertical full-thickness simple interrupted pinnal sutures were placed with 4-0 Ethilon in the right pinnal to prevent aural hematoma formation as depicted by the staggered hash lines in the diagram. (a). A penetrating sclerokeratoplasty was performed to excise a limbal-based full-thickness simple interrupted pinnal defect using 7-0 Vicryl in the right pinnal to prevent aural hematoma formation as depicted in the diagram (b). The dotted line depicts the penetrating sclerokeratoplasty incision to remove the temporal melanoma. A split-thickness pinnal cartilage section was sutured into the full-thickness limbal defect after temporal mass excision (c). Finally, a temporally harvested conjunctival graft was sutured to the leading edge of the temporal portion of the cartilage graft as depicted (d). The conjunctival graft was sutured temporally to the cornea and nasally directly to the cartilage graft as the conjunctival graft was not large enough to cover the entire transplant site due to the large size.
Histopathology
A moderately well-demarcated but unencapsulated, nodular mass was present at the limbus (Fig. 4), surrounded by 1–2 mm of tumor-free cornea or sclera. The mass primarily infiltrated and replaced corneal stroma with less extensive infiltration of sclera, conjunctiva, and corneal epithelium. This densely cellular mass was composed of clusters and cords of large, round to polygonal cells admixed with occasional spindle-shaped cells (Fig. 5). Neoplastic cells had variably distinct borders and moderate to abundant cytoplasm with numerous, dark-brown granules that often obscured the nuclei. When visible, nuclei were oval and finely stippled with 1–2 distinct nucleoli. Anisokaryosis was mild, and anisocytosis was mild to moderate. Mitotic figures were not observed. Multifocally, there were coalescing, oval to serpentine areas of necrosis within the mass containing free melanin, lipid vacuoles, and cholesterol clefts mixed with necrotic debris. These foci were partially bordered by cuffs of lymphocytes and epithelioid macrophages with fewer plasma cells and rare neutrophils. Within the corneal epithelium, neoplastic cells were occasionally mixed with eosinophilic fluid. Surrounding the neoplasm were numerous melanin laden macrophages. Neoplastic cells did not extend to the surgical margins.

Clinical outcome and results
Immediately postoperatively, the left pupil was mydriatic with a formed and clear anterior chamber. The lens was normal in the visible region. The morning after surgery, the patient was visual and comfortable with pharmacologic mydriasis, trace flare within the anterior chamber, and normal indirect ophthalmoscopic fundic examination in regions visible (Fig. 6a). The patient was discharged with instructions to have the pinnal Penrose drain removed at the referring veterinarian’s office in 3–5 days.

The patient represented to Portland Veterinary Specialists Ophthalmology Service fourteen days after surgery. The conjunctival graft site was stable and vascular with vascular bridging noted into adjacent corneal stroma (Fig. 6b). There was no fluorescein stain retention, and the intraocular pressure measured 8 mmHg. Pharmacologic mydriasis was present, and a few pigmented punctate lesions were noted on the anterior lens capsule. The intraocular examination was otherwise unremarkable. The pinna cartilage harvest site was healed, and all sutures were removed.

Five weeks after surgery, full cartilage and conjunctival graft incorporation with the cornea was present (Fig. 6c). The intraocular examination was unremarkable. Slight wrinkling of the pinna was noted over the cartilage harvest site, but ear carriage and facial symmetry were normal. The conjunctival pedicle was trimmed dorsally as previously described.19

Two months after surgery, the cartilage graft and conjunctival graft remained incorporated (Fig. 6d). The intraocular examination was normal. The patient was comfortable and visual in the left eye. Twelve months after surgery, telephone conversation confirmed that no changes were noted in the left eye. The patient remains visual and comfortable with no evidence of mass regrowth.

DISCUSSION
The case herein describes a corneoscleral grafting procedure using pinnal cartilage. Therapeutic approaches to limbal melanoma treatment are widely varied. Cogent rationality would dictate that the best treatment approach should counter the perceived aggressiveness of the tumor or projected ability of the tumor to affect patient vision in the long term. Maintenance of long-term vision, if possible, for the patient should be a goal when treating these tumors. In our case, the patient had a clearly demonstrable rapidly growing tumor with the immediate potential to affect vision in the left eye. For this reason, full-thickness excision with grafting was considered superior to
debulking or adjunctive therapy options. In addition, it was not considered reasonable to remove the left eye completely as large grafting, if successful, would result in vision for the patient.

Although this technique was used to treat a limbal melanoma after excision, this technique could be used for any large eyewall defect. Described corneoscleral grafting techniques are numerous and include autologous grafts using conjunctiva, third eyelid cartilage, fascia lata, biosynthetic grafts, homologous cornea or cornea with sclera, amniotic membrane from a variety of species and prosthetic grafts. Autologous grafts are desirable for corneal surgery because they do not elicit a donor-host rejection reaction. Corneal immune rejection is the leading cause of human allograft failure. Increased rejection rates are also associated with large defects. Studies are lacking on canine corneal graft rejection; however, allograft rejection has been identified in the horse. Although homologous grafting was considered, difficulty in obtaining donor tissue and possible graft rejection made this option unattractive. The sheer size of the defect rendered third eyelid cartilage and prosthetic grafting unrealistic. Amniotic membrane and biosynthetic grafting with A-cell was considered but would likely not result in sufficient tectonic support for the globe. Fascial lata grafting was the most promising described grafting technique for our patient, but procurement of a section large enough to support the defect was questionable after a conversation with a board-certified surgeon. Many of these published grafting techniques require extensive efforts from either the practitioner or support staff to obtain the donor material, as well. Thus, a novel grafting technique was sought.

In this patient, the pinnal cartilage was a viable option owing to his large and pendulous pinnae.

Advantages of pinnal cartilage grafting include readily available autologous tissue and good provision of tectonic support to the globe. In this case, the graft site healed readily and without reaction. This grafting option is appealing due to the relative ease in obtaining tissue, tectonic support to the globe and perceived likely success. The healing response was subjectively rapid, which may have also been a product of our patient’s young age. A conjunctival graft was placed over this large, free graft to provide vascularization, which may have also contributed to the rapid healing. A conjunctival graft was deemed appropriate as the pinnal cartilage graft was not predicted to be transparent after surgery and an overlying conjunctival graft would not change the corneal opacification in the grafted region. For this reason and because the avascular cartilage graft was so large, a vascular supply was deemed appropriate to help aid healing and incorporation of the large, free graft.

Pinnal cartilage grafting does require a second surgery in the same patient, which adds anesthetic time and a second surgery site. In addition, the pinna is well-innervated, and subjectively, this site may be more painful than other regions of the body for surgical manipulations. An increase in heart rate and blood pressure under anesthesia was noted in our patient during surgery when the pinnal cartilage was undermined and incised. Intra-operative and postoperative analgesia was considered very important for our patient when taking into consideration the pinnal harvest region and large ocular surgical site. Objectively, our patient did not seem to experience undue pain postoperatively.
although subjective pain scoring was not performed. Postoperatively, the patient was interactive with the hospital staff as noted by a wagging tail, playful behavior and pursuance of human contact. He also did not resent manipulation or cleaning of the pinna and exhibited normal eating, drinking, and sleeping under hospital monitoring twenty-four hours after surgery.

The pinnal cartilage may be approached from the convex or concave surface of the pinna. In our case, the convex surface approach was chosen based on the perceived ease of surgical approach related to ‘normal’ ear carriage (e.g., the pinna rested flat in this position for surgical preparation). In retrospect, the concave surface may have been a better choice as the cartilage is closer to the skin on this side, and undermining the skin from the cartilage section blindly was a bit difficult owing to the decreased amount of connective tissue between the skin and cartilage compared with the convex side.

This grafting technique may not be ideal in small patients, patients with cropped ears, or those with erect pinnae. It is possible that the pinna may be distorted or become pendulous after pinnal harvesting. In addition, this grafting procedure renders the grafted region opaque. Thus, large defects located in the dorsetwimal quadrant, the quadrant most affected by limbal melanomas, may not be ideal candidates for this technique. Large corneoscleral defect repair is warranted if it will result in vision for the patient. If the globe integrity is maintained, but the surgical outcome results in blindness, the advantage of one such procedure is subjectively dubious. In our case, the location of the mass supplied a relatively large unaffected region of dorsal, temporal, and ventral clear cornea that resulted in vision for our patient postoperatively.

Pinnal cartilage grafting can be a reasonable and practical option for large corneoscleral grafting and adds a previously undescribed technique to the ophthalmic surgeon’s repertoire for eyelid defect repair. In this case, the neoplasm was completely excised, and the patient experienced seemingly rapid, uneventful healing of the graft site. The patient remains visual and comfortable long term. This may represent a viable alternative to other corneoscleral grafting procedures for large defects and is an attractive treatment option due to lack of host rejection, readily available source of donor cartilage, and provision of tectonic support to the globe.

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