THE COMBINED USE OF SURGICAL DEBULKING AND DIODE LASER PHOTOCOAGULATION FOR LIMBAL MELANOMA TREATMENT: A RETROSPECTIVE STUDY OF 20 DOGS AND 1 CAT.

Valentina Andreani, DMV, Adolfo Guandalini, DMV, PhD, DECVO, Nunzio D'Anna, DMV, PhD, DECVO, Chiara Giudice, DMV, DECVP, Roberta Corvi, DMV, Nicola Di Girolamo, DVM, John S. Sapienza, DVM, DACVO

From the Department of Ophthalmology (Andreani, Guandalini, D'Anna, Di Girolamo), Centro Veterinario Specialistico (CVS), Via Sandro Giovannini 53, 00137 Rome, Italy; Department of Veterinary Sciences, University of Teramo (Corvi), Strada Provinciale 18 snc, Piano d'Accio, 64100 Teramo, Italy; Department of Veterinary Sciences and Public Health (DiVet), University of Milan (Giudice), via Celoria 10, 20133 Milano, Italy; Long Island Veterinary Specialists (Sapienza), Plainview, Long Island, New York, USA.

The authors disclose any financial interests with companies that manufacture products that are the subject of the present research or with companies that manufacture competing products.

Address correspondence to:
Dr. Valentina Andreani
Tel.: (+39) 3339999062
e-mail: valeand27@gmail.com

Abstracts

Objective: To evaluate effectiveness and safety of debulking and diode laser photocoagulation (DPC) for the treatment of limbal melanomas (LMs).

Animal studied: Twenty dogs (12 females and 8 males) and 1 cat were included in this study. The dogs' average age was 5 years (range: 7 months to 12 years).

Procedure: Retrospective multi-institutional case series. Medical records of animals diagnosed with LM at the Centro Veterinario Specialistico (CVS) and at the Long Island Veterinary Specialists from 1994 to 2013 were retrieved. Signalment, location, extent of tumors, recurrence rate, and early and late complications were reported. Patient follow-up information was obtained from veterinary ophthalmologists, primary care veterinarians, and, when appropriate, owners.

Results: The follow-up period ranged from 1 to 72 months after the surgery. Long-term follow-up was obtained by telephone interviews in 6/21 cases and by clinical re-evaluations in 15/21 cases. The most common early complications were a moderate anterior uveitis peripheral corneal edema (21/21 eyes). Late complications included corneal fibrosis and/or pigmentation (21/21). In one case, a severe bullous keratopathy associated with an extended corneal fibrosis was observed. The vision was maintained in 19 eyes (19/21).

Conclusions: Debulking associated with diode laser photocoagulation was technically straightforward to perform, minimally invasive, well-tolerated and highly successful in this case series.

Key Word: debulking, diode laser, limbal melanoma, dog, cat

INTRODUCTION

Melanocytic tumors represent the most common ocular tumors in small animals and seem to occur more commonly in dogs than in cats.[1-3] Melanocytic neoplasias may be classified according to the involved ocular structures [intraocular melanoma (localized in the anterior uvea, and choroid) or limbal melanoma]. Epibulbar or limbal melanomas (LMs) represent 10-34% of all canine melanocytic ocular tumors, and they are recognized as solid and circumscribed masses arising in the sclera or subconjunctival connective tissue at the limbus.[4-5] These masses are characterized by strong pigmentation, but they can occasionally be amelanotic.[6] LMs originate from melanocytes of the corneoscleral junction and have slow growth in sclera, corneal stroma and conjunctival tissue.[5,7-9]

Predisposing factors for limbal melanomas are scattered throughout the literature. Highly pigmented dogs seem to be more predisposed to developing LM.[9] Several studies have confirmed a high incidence of this tumor in breeds such as the German Shepherd, Labrador Retriever, and Golden Retriever.[6,9-11] Previous reports suggested that LM, anterior uveal melanoma and ocular melanosis are presumed to be hereditary in Golden and Labrador Retrievers and that the same genetic mutation may be associated with melanocytic disease at different ocular sites.[9] The difference in the incidence of LM between sexes is currently considered irrelevant.[5,8,9,11] With regard to anatomical sites, although the LM can develop anywhere on the limbus, the dorsolateral quadrant is the most frequently affected site.[5,6,8-10]

The most frequent primary intraocular tumor in cats is a malignant melanoma of the anterior uvea.[12,13] In the literature, a few cases of LMs are reported in cats.[14,15] LM occurs more often in older cats[15] but can also affect younger cats.[16-18] In contrast to dogs, metastasizing melanomas of the limbus have been reported in cats.[16,17,19]

Surgical management is currently considered the treatment of choice in the case of limbal melanomas in dogs and cats. In dogs, LMs are slowly progressive benign tumors.[5,2] Nevertheless, their growth may be locally invasive, and their extensions can impair vision and compromise the internal structures of the globe.[5,6,9,10] In the past, surgical management was advised in young dogs, whereas in older dogs, periodic surveillance was considered satisfactory.[6,10] This practice was based upon two retrospective studies in which limbal melanomas had different biological behavior at different ages, with younger dogs (2-4 years-old) developing more invasive melanomas than older dogs (8 to 11 years old).[6,10] A recent report demonstrated that growth rate of limbal melanomas is faster in older animals compared to younger ones.[9] This has led to a change in current clinical recommendations, with current recommendations suggesting a more aggressive surgical treatment should be pursued, regardless of the age of the animal. Several surgical techniques for treatment of LMs were described in the literature, including a full thickness en bloc resection associated with fresh or frozen corneoscleral homologous graft, partial removal approach combined with cryosurgery, photocoagulation, and strontium-90 plesiotherapy.[6,9,10,15,17,20,26-28] The objective of the present retrospective case series is to evaluate the effectiveness of debulking associated with diode laser photocoagulation for treatment of LM in dogs and cats.

MATERIALS AND METHODS

The medical records of 21 patients presented for evaluation of a unilateral pigmented limbal mass, with a clinical diagnosis of LM and treated with debulking and diode laser photocoagulation at the Ophthalmology Department of the CVS (Centro Veterinario Specialistico, Rome, Italy) or at the Long Island Veterinary Specialists (Plainview, Long Island, New York) between 1994 and 2013 were included in this retrospective study. Information recorded from each case included breed, age, sex, affected eye, presence of lipid keratopathy and location of the lesion in the eye (Table 1). Veterinary ophthalmologists, primary care veterinarians, and occasionally owners were contacted via phone for patient follow-up information.

All patients received a complete ophthalmic examination evaluating the menace response, pupillary light reflex (PLR), dazzle reflex, tear production (Schirmer Tear Test, Schering-Plough Animal Health, Union, New Jersey), fluorescein staining (HS HAAG-STREIT International Fluorescein, Switzerland) and intraocular pressure (IOP) (TonoPen Vet, Reichert Inc., Depew, NY). All of the patients underwent a slit-lamp biomicroscopy (SL-14 Biomicroscope, Kowa Co Ltd, Tokyo, Japan), gonioscopic evaluation of the iridocorneal angle (ICA) (Koeppe lens, Ocular Instruments, Bellevue, Washington, USA), and indirect ophthalmoscopy (Omega 500, Heine Optotechnik, Herrsching, Germany) for the evaluation of the fundus. Ocular ultrasound with a 12.5 MHz. probe (Linscan Systems Inc., Rolla, Mo, Missouri, USA, or E-Technology unit, Devemport, IA, USA) was performed for the evaluation of the mass extension within the eye. All patients received a thorough physical examination prior to general anesthesia (chest radiograph, abdominal ultrasound, and complete blood work).

Surgery was performed by a board-certified ophthalmologist (AG, JS, ND). Prior to surgery, a drop of tropicamide+phenylephrine hydrochloride solution (Visumidriatic with Fenilefrina, Visufarma S.p.a., Rome, Italy) was topically administered. The eyes were subsequently aseptically prepared, and patients were positioned in dorsal recumbency. Lateral canthotomy was created to allow access to the globe in all patients except cases 10, 11, 15 and 18, depending on the surgeon's preference. The conjunctiva above the melanoma was incised at the limbus (Fig. 1 a), and it was separated from episclera below to obtain a fornix-based conjunctival flap (Fig. 1 b). After adequate exposure of the neoplastic tissue, an incision was made using a 64 beaver blade (Eagle Labs, Rancho Cucamonga, CA, USA) peripherally to the melanoma. If lipid keratopathy was present, it was included in the excision. A lamellar sclerectomy and keratectomy was performed, including the removal of a 1-2 mm margin of healthy tissue. The excessive bleeding was controlled using an Absorbent Stik (Cell Sponge) (Becton, Dickinson and Company, Waltham, MA, USA) or with fine wet-field cautery prior to the ablation of the pigmented base of the melanoma with a diode laser (Iris Medical Diovet 810 nm, Mountain View, CA, USA). A 25 gauge straight endoprobe (Iridex, Mountain View, CA, USA) was used to destroy any residual pigmentation and was positioned 2 mm from the operated site (Fig. 1 c). The laser settings are shown in Table 2. Three settings of energy were applied: low ($\leq$200J), medium (201-399J) and high ($\geq$400J) from 1994 to 2013. After the treatment with the diode laser, a conjunctival flap was sutured over the surgical site using a 8-0 or 9-0 polyglactin (Vicryl, Ethicon, Johnson & Johnson Intl, St-Stevens-Wolnwe, Belgium) with a simple interrupted pattern (Fig. 1 d). A-Cell graft tissue (A-Cell Vet, A-Cell Inc., Jessup, MD) was used in 3 eyes, and a Biosis membrane (Vet BioSis, Cook Veterinary Products) was used in 1 eye. Instead of the conjunctival graft A-cell or Biosis material was sutured to the corneoscleral defect using a 8-0 polyglactin (Vicryl, Ethicon, Johnson & Johnson Intl, St-Stevens-Wolnwe, Belgium) (Table. 2). In all cases, tissue removed from the mass was formalin-fixed and sent for histopathological examination at the Department of Veterinary Pathology, Hygiene and Public Health, School of Veterinary Medicine, State University of Milan, Italy.

Post-surgical care consisted in topical ofloxacin 0.3% (for times a day for 21 d; Exocin, Allergan Spa, Rome, Italy), tropicamide 1% (twice daily for 3 d and once daily for 7 d; Visumidriatic 1%, Visufarma S.p.a., Rome, Italy), and Fluorometolone Acetate (twice daily for 15 d; Flarex 0.1%, Alcon Spa, Milano, Italy). Systemic therapy included amoxicillin-clavulanate (12.5 mg/kg, PO, twice daily; Synulox; Pfizer Animal Health, Sandwich, Kent, UK) and carprofen (2 mg/kg, PO, once daily; Rimadyl; Pfizer Animal Health, Sandwich, Kent, UK) for 7-10 days.

Statistical analysis

The Freeman-Halton extension of the Fisher exact test (Freeman and Halton, 1951) was used to determine whether the total Joule provided could significantly influence recurrence of LM. Three ranks of energy were considered: low (≤200J), medium (201-399 J) and high (≥400J). Recurrences were considered only for the first combined surgical treatment. Data were analyzed using commercial software (IBM SPSS Exact tests, SPSS Inc., Chicago, Ill). Two-sided *P* values of less than 0.05 were considered significant.

RESULTS

A total of 20 canine cases (20 eyes) and 1 feline case (1 eye) met the inclusion criteria, and they were treated with partial lamellar resection, diode photocoagulation, and conjunctival grafting, A-cell or Biosis grafts for unilateral LM. The mean age at the diagnosis was 5 years, with a range from 7 months to 12 years. Eleven breeds of dogs were represented, including 6 German Shepherds, 1 Dachshund, 1 Labrador Retriever, 1 Golden Retriever, 1 Bull Mastiff, 1 Newfoundland, 1 Irish Setter, 1 Poodle, 1 Pug, 1 Leonberger and 5 mixed breed dogs. Nine intact females, two spayed females, seven intact males and two castrated males were included in this series of cases. The only cat included was an intact Domestic Short Hair (DSH) male. Gonioscopy showed that ICA was compressed but not invaded. No fundoscopic abnormalities were observed in any of the cases. Ocular ultrasonography did not show any evidence of extension of the mass into the globe in any of the eyes. Routine metastasis evaluation (complete blood work, abdominal ultrasound, X-ray chest) failed to reveal abnormalities precluding surgery.

The right eye (OD) was affected in 13/21 cases, and the left eye (OS) was affected in 8/21 cases. Tumor localization was dorsolateral in 11/21 eyes, dorsal in 3/21 eyes position, dorsomedial in 2/21 eyes, ventrolateral in 2/21 eyes, ventromedial in 2/21 eyes and medial 1/21 eyes. The most common location of the mass was the dorsolateral position (11/21; 52%). Corneal endothelial pigmentation associated with the mass was observed in all patients (21/21), and corneal degeneration (lipid keratopathy) at the periphery was noted in 7/21 patients (Fig. 2 and 3). The size of the tumor never exceeded 1/3 of the entire surface of the globe. No other significant ophthalmic findings were noted in any patient, with the exception of case number 17, which showed negative direct and indirect PLR, negative swinging flash light test, negative menace response in both eyes and flat electroretinographic response (RetinoGraphics BPM-200 System, Retinographics, Norwalk, CT, USA). The dog was diagnosed with sudden acquired retinal degeneration (SARD).

The most remarkable intra-operative complications included moderate to severe scleral hemorrhage and scleral thinning at the limit of globe perforation.

Histopathological analysis confirmed the diagnosis of limbal melanocytoma. Tumors were composed of two main cell types, a bland population of spindle or star shaped melanocytes, characterized by minimal anisocytosis and anisokaryosis and no or occasional mitotic figures and a second population of large, non-cohesive, round cells with small dense peripheral nuclei and abundant, melanin laden cytoplasm (Fig. 4).

Follow-up information was obtained by standard re-examination of patients at referral centers at 24 h and 7, 14, 21, 31, 93, 186, and 365 days after the surgery. Long-term follow-up information (≥12–72 months) was collected by clinical re-evaluations in 15/21 pets or completed by a telephone interview with the owner in 6/21 subjects.

The short-term complications noted during the first two weeks were a slight anterior uveitis and a moderate corneal edema, both in 21/21 cases (Fig. 2 and 3). In one case (n. 15), necrosis of the dorsal sclera was noticed. This healed uneventfully with medical therapy over time.

The long-term complications recorded from the 72 month after surgery showed corneal fibrosis and corneal pigmentation in 21/21 eyes (Fig. 2, 3) bullous keratopathy in 1/21 eyes (case number 2), lipid keratopathy in 2/21 eyes, and a mature cataract in 1/21 eyes. There were no recurrences of the LM in 17/21 eyes. Three of the four recurrences underwent a second similar surgery. Case number 12 underwent successful surgery one month after the first surgical procedure. Case number 2 had 2 recurrences at 6 months of each other. Case number 13 showed a recurrence 1 year after the first treatment. After the second surgery, no recurrences were observed. Case number 9 had a recurrence 4 years after surgery, but the owner declined any other surgical procedure. In one case (number 8), a mature cataract was noticed five years after the surgery. Two cases showed a lipid keratopathy after surgery (numbers 9 and 21) in an eccentric corneal position.

In 19/21 cases, the eyes were visual, comfortable and off all treatment at the time of the final re-examination. The recurrence rate of LMs was significantly lower in patients exposed to high laser setting then in medium and low group (P=0.04). Tumors treated by laser energy in the range of 201 to 399J were more prone to recurrence. There were 3/5 recurrences with 201-399 J (60%) versus 1/11 with low energy (9%) and 0/6 with high energy (0%).

DISCUSSION

During the last decades, several techniques have been described for the management of LMs. In general, surgical approaches to the mass may be into full thickness or partial lamellar resections. In the case of partial removal, different additional treatments (photocoagulation, cryotherapy and plesiotherapy) have been suggested to improve effectiveness and to minimize recurrences.[6,9,15,17,18,20-28] Based on this case series, a combined use of debulking and diode laser photocoagulation was technically easier to perform, minimally invasive and well-tolerated in the animal, with mild postoperative discomfort and a relatively short anesthesia time. This technique also showed an acceptable long-term success, as 19/21 eyes (90,47%) were visual 72 months after the surgery.

The only use of photocoagulation without any surgical reduction of the neoplastic tissue has been described.[10] Removal of the eye was usually reserved for cases of extensive intraocular tumor growth or in cases of secondary glaucoma or intractable uveitis.[15,29-31] Full thickness resection of LM is the ideal surgical option to minimize any recurrence of the mass,[6,13,20-26] but is technically difficult to perform and requires fresh or frozen corneoscleral homologous graft [6,20] or other tectonic support material that are not always readily available [21-26] and may be associated with complications such as intraocular bleeding, blindness, cataract formation, marked uveitis, fibrin in the anterior chamber and synechiae formation. *Martin* described the graft to develop a bulge at 34 weeks postoperatively, which was surgically explored and excised from the scleral base.[6] *Blogg et al.* reported an iris bulging through the corneal wound at the limbus that required another surgery to replace the iris.[22] A ventromedial distortion of the pupil due to an iris adhesion to the site of the graft was the main complication in the *Lewin* study.[25] Another possible complication may be the failure of the neovascularization of the graft, as noticed by *Wilkie et al.* 7 weeks after surgery.[24] Suture dehiscence was reported by *Maggio et al*.[20] All of the eyes in these studies were visual post-operatively, and no recurrence was noted., but the use of the "en bloc" resection is a complex surgical approach and requires both appropriate surgical skill and the availability of specialized surgical equipment and donor tissue.

Another surgical approach consists of primary debulking of the mass. This partial removal approach has been combined with cryosurgery in cats [15,17] and dogs,[27] photocoagulation with Nd:YAG laser and A-Cell bio-scaffold material,[18] equine pericardium,[28] and strontium-90ß plesiotherapy.[9] In the Donaldson study, 30 dogs were treated with strontium-90 β plesiotherapy. Results revealed a low rate of recurrence (3%) but an overall complication rate of 53%. This procedure required multiple general anesthetic episodes and was not readily available due to health and safety implications for personnel.[9]

The use of cryotherapy involves the destruction of residual tissue after partial lamellar resection by low temperatures, which induces crystallization of the cytosol and subsequent cell death. Previous publications have reported that a temperature of -20°C is able to induce tissue cryonecrosis.[15,32] Both nitrous oxide and liquid nitrogen are commonly used in veterinary ophthalmology for cryotherapy. [15] [21] This technique exploits the capacity that freezing has to induce the formation of crystals inside and outside the cell, which causes water outflow and tissue dehydration. Within a few hours ischemic necrosis occurs. Melanocytes are particularly cryosensitive due to a high water content, and corneal melanocytes are as cryosensitive as dermal melanocytes.[33,34] The most common complication of cryotherapy is the development of lipid keratopathy at the treatment site, but recurrences were not clinically detected in study of *Featherstone et al.*[27] In the present study, the number of lipid keratopathy after surgery was 2/21 (case number 9 and 21) compared with those observed before treatment (7/21); only case 21 showed this deposit of crystals before treatment and the recurrence at one year after. In comparison with the cryotherapy, the use of the diode photocoagulation in this report has a smaller number of corneal lipidosis as well as extension neither to exceed the visual axis nor to impair vision, as in the *Featherstone et al.* report.[27] Nevertheless, the low number of cases makes this result not significant and it is difficult to assess which are the causes of lipid keratopathy that could be linked to other factors (nature of the tumor, surgery, pre-existing corneal lipidosis, type of graft, or postoperative use of topical steroid).

Photocoagulation represents an effective and available option in destroying melanoma cells. In veterinary ophthalmology, both the Nd:YAG and diode laser are commonly used for treatment of glaucoma, uveal pigmented cysts, iris melanoma and retinopexy.[10,18,35-38] The diode laser provides energy in the infrared electromagnetic spectrum (810 nm). At such wavelengths, the main target chromophore is the melanin. Melanin-rich tissues show a greater tendency to absorb the light that determines a localized photocoagulative necrosis.[39] Diode laser has 1.3 times a superior penetration rate of tissues compared with the Nd:YAG laser. Consequently, the destructive effect of the diode laser is achieved with less energy in comparison to the Nd: YAG laser.[39] Treatment of LM with the Nd: YAG laser alone has also been described by *Sullivan et al.* in dogs and cats.[10] This treatment method was associated with only mild postoperative discomfort, but the recurrence rate was higher (20%; 3/15) compared with the recurrence rate with the full thickness surgical resection and grafting techniques. A second treatment was necessary for all three recurrences, and in two eyes, the tumor did not successfully regress. Additionally, a complication reported with Nd:YAG laser was corneal lipidosis, which was noted in 3/15 cases, and in one case, was marked compared with the use of debulking and diode laser photocoagulation (2/21), in which the presumed lipid opacity was considered to be mild and peripheral. In this presently described technique, the recurrences rate was 4/21: all recurrent LMs were associated at the low and medium laser settings (200J and 201-399J, respectively) and in only one case was a second treatment not curative (case #2).

In the present study, diode photocoagulation was combined with the lamellar removal of the LM. Surgical debulking of tumors ensures an adequate tumor exposure to the laser. Partial lamellar resection was continued until the maximum amount of tumor had been safely excised as judged by the surgeon. In this case series, globe perforation did not occur. Recurrences (cases 2, 9, 12, 13) could be caused by a too superficial of a dissection or an insufficient applied energy, as suggested by the statistical analysis. As shown in Table 2, the number of treated sites increased during the years. The authors increased the laser power settings to obtain a more intense treatment of the bed of the debulked mass. In the future, we would advocate the use of higher laser setting (≥400J) to avoid recurrences. Further investigations should consider the power setting related to the tumor size. The surgical technique limits the size evaluation of the LM at the time of histopathological examination due to the partial resection of the neoplasia (provided by the surgeons subjectively). The inability to perform a complete measurement of the tumor size impair to correlate data about the power setting vs. mass dimension and any other possible association with recurrences rate.

Loss of vision occurred in 2/21 eyes (cases 2 and 5). In one of these cases (case 5), a mature cataract that caused blindness was noted 5 years after surgery. In the literature, focal cataractous changes were observed at equatorial and posterior polar regions of the lens after the use of Nd: YAG laser[37] The exact etiology of the cataract formation in this only case is unknown and may not be due to the surgical procedure or laser therapy.

Case 2 developed a severe bullous keratopathy after a second treatment with DPC within the first year of age. The aggressive LM growth in younger dogs it already described by Martin and Sullivan. [6,10] In this subject, enucleation was suggested to relieve the pain in this blind eye. Because of the limited number of cases, it is not possible to correlate the age with the tumor growth and the recurrences but it could be possible that subjects with melanomas very invasive could be more prone to recurrences and to failure of surgical treatment. However, there are many causes of corneal edema, including endothelial dystrophy, age-related degeneration, endothelial damage associated with persistent pupillary membranes (PPMs), mechanical trauma, toxic reactions, anterior uveitis, endotheliitis, glaucoma, neovascularization, and ulceration.[40] *Sapienza et al*. reported stromal inflammation and consequent destruction of the normal lamellar corneal composition as an effect of Nd: YAG laser therapy in 60% of the eyes treated to reduce aqueous humor production[36] Repeated treatments with the use of laser might have predisposed to a more serious corneal inflammation which could have led to the development of a bullous keratopathy.

Other complications such as slight anterior uveitis, moderate corneal edema and necrosis of the sclera occurred, but they resolved uneventfully with medical therapy.

The only cat included in this study was operated at 17 years and examined without any recurrence or metastasis until death at 20 years. Although, the biological behavior of LM is different in cats than in dogs, in the one feline case that we treated, debulking and diode laser photocoagulation (DPC) were effective in controlling the disease process. Future studies should be made on a higher number of cases to confirm the efficacy of this procedure in cats. One report described a 12.5-year-old cat from which the tumor was removed but he died 6 month after initial diagnoses with local recurrence and apparent distant metastases.[16] In another report, metastasis occurred 32 months after the initial presentation,[17] with minimal local invasiveness in the feline patient. Therefore, some authors have proposed that a better description for this neoplasia in cats may be malignant melanoma with late metastasis.[19] Due to the latency of the melanoma, those patients should be subjected to complete physical examination, chest radiographs, and abdominal ultrasound every 3-6 months starting 6 months after the first diagnosis.[17]

Disadvantages of the current surgical technique include the need of the relatively expensive diode laser and the possible recurrences (additional surgery was required in 4 of 21 eyes (19.0%). Case number 9 had a recurrence 4 years after surgery, and the owner refused further surgical intervention. The other 3 dogs with recurrences underwent a further surgery. Of these cases, only one (case 2) had vision-threatening complications that required an enucleation Futures studies could be conducted on a larger population using the last laser setting (≥400J) for verify the effective against the tumor regrowth.

According to the authors' knowledge, this is the first report of the combined use of debulking associated to diode laser photocoagulation in veterinary medicine post-operative long-term follow-up in 6/21 cases were obtained by telephone interview, and the presence of a recurrence was based on owner judgement and not on a clinical examination. Furthermore, the relatively low number of cases may have influenced the obtained results. Future studies should include longer and more consistent updates, a larger population of patients and different laser settings. Ideally, a randomized controlled trial to compare this technique to previous techniques is welcome.

In conclusion, lamellar debulking with DPC appeared to be an acceptable, simple, and effective treatment for LMs in dogs and cats and may be considered as a suitable surgical alternative to en-bloc resection and donor grafting especially in cases of extensive melanomas, which are too large for full thickness resection.

REFERENCES

1. Goldschmidt MH. Benign and malignant melanocytic neoplasms of domestic animals. *American Journal of Dermatopathology* 1985; 7 Suppl: 203-12.
2. Smith SH, Goldschmidt MH, M cManus PM. A comparative review of melanocytic neoplasms. *Veterinary Pathology* 2002; 39: 651-678.
3. Patnaik AK, Mooney S. Feline melanoma: a comparative study of ocular, oral, and dermal neoplasms. *Veterinary Pathology* 1988; 25: 105-112.
4. Dubielzig RR, Ketring KL, McLellan GJ *et al.* Diseases of the cornea and sclera. In: *Veterinary Ocular Pathology: A Comparative Review* (eds Dubielzig RR, Ketring KL, McLellan GJ, Albert DM) Saunders Elsevier, Edinburgh, 2010; 201-243.
5. Diters RW, Dubielzig RR, Aguirre GD, *et al*. Primary ocular melanoma in dogs. *Veterinary Pathology* 1983; 20: 379-395.
6. Martin CL. Canine epibulbar melanomas and their managment. *Journal of the American Animal Hospital Association* 1981; 17: 83-90.
7. Ryan AM, Diters RW. Clinical and pathologic features of canine ocular melanomas. *Journal of the American Veterinary Medical Association* 1984; 184: 60-67.
8. Wilcock BP, Peiffer RL. Morphology and behavior of primary ocular in 91 Dogs. *Veterinary Pathology* 1986; 23: 418-424.
9. Donaldson D, Sansom J, Scase T, *et al*. Canine limbal melanoma: 30 cases (1992-2004). Part 1. Signalment, clinical and histological features and pedigree analysis. *Veterinary Ophthalmology* 2006; 9(2): 115-119.
10. Sullivan TC, Nasisse MP, Davidson MG, *et al*. Photocoagulation of limbal melanoma in dogs and cats: 15 cases (1989-1993). *Journal of the American Veterinary Medical Association* 1996; 15, 208(6): 891-894.
11. Giuliano EA, Chappell R, Fischer B, *et al*. A Matched observational study of canine survival with primary intraocular melanocytic neoplasia. *Veterinary Ophthalmology* 1999; 2: 185-

190.

12. Williams LW, Gelatt KN, Gwin RM. Ophthalmic neoplasms in the cats. *Journal of the American Veterinary Medical Association* 1981; 17: 999-1008.
13. Harris BP, Dubielzig RR. Atypical primary ocular melanoma in cats. *Veterinary Ophtalmology* 1999; 2: 121-124.
14. Duncan DE, Peiffer RL. Morphology and prognostic indicators of anterior uveal melanomas in cats. *Progress in Veterinary & Comparative Ophthalmology* 1991; 1: 25-32.
15. Harling DE, Peiffer RL, Cook CS. Feline limbal melanoma: four cases. *Journal of the American Animal Hospital Association* 1986; 22:795-802.
16. Day MJ, Lucke VM. Melanocytic neoplasia in the cat. *Journal of Small Animal Practice* 1995; 36: 207-213.
17. Betton A, Healy LN, English RV, *et al*. Atypical limbal melanoma in cat. *Journal of Veterinary Internal Medicine* 1999 Jul-Aug; 13: 379-81.
18. Plummer CE, Kallberg ME, Ollivier FJ, *et al*. Use of biosynthetic material to repair the surgical defect following excision of an epibulbar melanoma in a cat. *Veterinary Ophthalmology* 2008; 11(4): 250-254.
19. Ogilvie GK, Moore AS. Tumors of the eye in cats. *Managing the Veterinary Cancer Patient: A Practice Manual. Treaton. NJ: Veterinary Learning Systems Co Inc*; 1995: 308-310.
20. Maggio F., Pizzirani S., Pena T., *et al*. Surgical treatment of epibulbar melanocytomas by complete excision and homologous corneoscleral grafting in dogs: 11 cases. *Veterinary Ophthalmology* 2013; 16(1): 56-64.
21. Norman JC; Urbanz JL, Cavarese ST. Penetrating keratoplasty and bimodal grafting for treatment of limbal melanocytoma in a dog. *Veterinary Ophtalmology* 2008; 11(5): 340-345.
22. Blogg JR, Dutton AG, Stanley RG. Use of third eyelid grafts to repair full-thickness defects in the cornea and sclera. *Journal of the American Animal Hospital Association 1989*; 25:

505-512.

23. Kanai K, Kanemaki N, Matsuo S, *et al*. Excision of a feline limbal melanoma and use of nictitans cartilage to repair the resulting corneoscleral defect. *Veterinary Ophthalmology* 2006; 9: 255-258.

24. Wilkie DA, Wolf ED. Treatment of epibulbar melanocytoa in a dog, using full-thickness eye wall resection and synthetic graft. *Journal of the American Veterinary Medical Association* 1991 Mar; 198,6: 1019-1022.

25. Lewin GA,. Repair of full thickness corneoscleral defect in a german shepherd dog using porcine small intestinal submucosa. *Journal of Small Animal Practice* 1999; 40: 340-342.

26. Bussiers M, Krohne SG, Stiles J, *et al*. The use of porcine small intestinal submucosa for the repair of full-thickness corneal defects in dogs, cats and horses. *Veterinary Ophthalmology* 2004; 7(5): 352-359.

27. Featherstone HJ, Renwick P, Heinrich CL, *et al*. Efficacy of lamellar resection, cryotherapy, and adjunctive grafting for the treatment of canine limbal melanoma. *Veterinary Ophthalmology* 2009 Nov-Dec; 12 suppl 1: 65-72.

28. Barros PS, Safatle AM, Rigueiro M. Experimental lamellar corneal graft in dogs using preserved equine pericardium. *Brazilian Journal of Veterinary Research and Animal Science* 1995; 36: 304-307.

29. Donoso LA, Shields JA, Nagy RM. Epibulbar lesions simulating extraocular extension of uveal melanomas. *Annuals of Ophthalmology* 1982; 14: 1120-1123.

30. Gow JA, Spencer WH. Intraocular extention of an epibulbar malignant melanoma. *Archives of Ophthalmology* 1973; 90: 57-59.

31. Varga M, DaroczyJ, Toth J. Intraocular invasion of recurrent epibulbar malignant melanoma. *Acta Morphologica Hungarica* 1986; 34: 127-140.

32. Guandalini A. La terapia chirurgica del glaucoma. *Veterinaria* 2008; 22(5): 19-32.

33. Vestre WA. Cryosurgical techniques in veterinry ophthalmology. *The Compendium on*

Continuing Education 1984; 6: 481-487.

34. Hidayat AA, LaPiana FG, Kramer KK. The effect of rapid freezing on uveal melanomas. *American Journal of Ophthalmology* 1987; 103: 66-80.
35. Nasisse MP, Davidson MG. Laser therapy in veterinary ophthalmology: perspective and potential. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 1988 Feb; 3(1): 52-61.
36. Sapienza JS, Miller TR, Gum GG, *et al*. Contact transscleral cyclophotocoagulation using a neodymium: yttrium aluminium garnet laser in normal dogs. *Progress in Veterinary & Comparative Ophthalmology 1992*; 2(4): 147-153.
37. Pizzirani S, Davidson MG, Gilger BC. Transpupillary diode laser retinopexy in dogs: ophthalmoscopic, fluorescein angiographic and histopatologic study. *Veterinary Ophthalmology* 2003; 6(3): 227-235.
38. Cook C, Wilkie D. Treatment of presumed iris melanoma in dogs by diode laser photocoagulation: 23 cases. *Veterinary Ophthalmology* 1999; 2(4): 217-225.
39. Cook C, Mike D, Brinkmann M, *et al*. Diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in dogs: results of six and twelve month follow-up. *Veterinary & Comparative Ophthalmology* 1997; 7: 148-154.
40. Gelatt KN, Gilger BC, Kern TJ. *Veterinary Ophthalmology,* 5th edition. Wiley-Blackwell 2013; 18: 981-982.

FIGURE LEGENDS

Figure 1. a) Intra-operative photograph of a limbal melanoma in the dorsolateral limbus (case 4, OS). The conjunctiva above the melanoma is excised at the limbus with a 64 beaver blade, and the dissection was completed with Castroviejo scissors. The conjunctival flap was retracted toward the fornix for an adequate exposure of the neoplastic tissue. **b)** An incision was made with a 64 beaver blade peripherally to the melanoma, and a lamellar dissection was completed using both the beaver blade and Martinez corneal dissector. **c)** A 25 gauge straight endoprobe was positioned at 2 mm from the melanoma to deliver laser energy. **d)** The retracted conjunctival flap was repositioned and sutured over the surgical site.

Figure 2. Case # 6. **a)** Preoperative photograph of a well-defined dorsolateral melanoma involving 50° of the intere limbal circumference with a mild lipid keratophaty. **b)** The same eye in a postoperative photograph 1 month after surgey with a moderate corneal fibrosis and neovascolarization.

Figure 3. Case # 17. **a)** Preoperative photograph of a large pigmented mass at the ventromedial limbus OD. A moderate lipid keratopathy associated at the limbal melanoma is present. **b)** The same eye 2 weeks after surgery shows a moderate corneal edema at the periphery of the graft and abundant neovascularization**. c)** Two months after surgey, large corneal fibrosis and vascularization are noted involving approximately half of cornea.

Figure 4. Histological section of limbal melanocytoma. The tumor is composed of a mixed population of spindle and large non-cohesive round cells, loaded by abundant intracytoplasmic melanin.

Table 1. *Signalment and clinical features*

Case	Sex	Age	Breed	lipid keratopathy	Eye	Location
1	F	3 yrs	Irish Setter	N	OD	M
2	F	7 months	German Shepherd	N	OS	D
3	M	3 yrs	Mixed breed	N	OD	VL
4	M	8 yrs	German Shepherd	N	OD	DL
5	F	6 yrs	German Shepherd	N	OD	DL
6	M	3 yrs	German Shepherd	Y	OS	DL
7	F	6 yrs	Mixed breed	N	OS	DL
8	M	2 yrs	Mixed breed	N	OS	DL
9	F	6 yrs	German Shepherd	N	OS	DL
10	MN	3 yrs	Golden Retriever	Y	OD	DM
11	MN	6 yrs	Poodle (standard)	N	OD	VL
12	F	2 yrs	German Shepherd	N	OD	DL
13	M	4 yrs	Newfoundland Labrador	Y	OD	DL
14	F	10 yrs	Retriever	N	OD	VM
15	FS	11 yrs	Pug	N	OS	DL
16	F	6 yrs	Bull Mastiff	Y	OS	DL
17	F	8 yrs	Dachshund	Y	OD	VM
18	FS	7 yrs	Terrier Mixed	Y	OS	DL
19	M	17 yrs	DSH	N	OD	DM
20	F	2 yrs	Mixed breed	N	OD	D
21	M	3 yrs	Leonberger	Y	OD	D

F = intact female; M = intact male; FS = female spayed; MN = male neutered; OD = right eye; OS = left eye; D = Dorsal; M = Medial; VL = Ventrolateral; DL = Dorsolateral; DM = Dorsomedial; VM = Ventromedial: N = no: Y = yes.

Table 2. *Treatment and recurrences*

Case	Laser setting	Total Joules delivered	Surgery	Recurrance
1	1.5W x 1S x 30 sites	45J	D, L, CF	no
2	1.5W x 1S x 30 sites	45J	D, L, CF	Twice
3	1.5W x 1S x 30 sites	45J	D, L, CF	no
4	1.5W x 1S x 30 sites	45J	D, L, CF	no
5	1.5W x 1S x 30 sites	45J	D, L, CF	no
6	1.5W x 1S x 30 sites	45J	D, L, CF	no
7	1.5W x 1S x 30 sites	45J	D, L, CF	no,
8	1.5W x 5S x 30-40 sites	225-300J	D, L, CF	no
9	1.5W x 5S x 30-40 sites	225-300J	D, L, CF	Once
10	1W x 1S x 34 sites	34J	D, L, AC (2L)	no
	1.5W x 1S x 186 sites	279J		
11	1.2W x 1.5S x 72 sites	135J	D, L, B (2L)	no
12	1.5W x 5S x 30-40 sites	225-300J	D, L, CF	Once
13	1.5W x 5S x 30-40 sites	225-300J	D, L, CF	Once
14	1.5W x 9S x 100 sites	1350 J	D, L, CF	no
15	1W x 1S x 151 sites	151J	D, L, AC	no
16	1.5W x 9S x 100 sites	1350J	D, L, CF	no
17	1.5W x 9S x 100 sites	1350J	D, L, CF	no
18	1W x 2S x 59 sites	118J	D, L, AC	no
19	1.5W x 1S x 30 sites	45J	D,L	no
20	1.5W x 9S x 100 sites	1350J	D,L,CF	no
21	1.5W x 9S x 100 sites	1350J	D,L,CF	no

D = Debulking; L = Laser; CF = Conjunctival Flap; AC = A-cell; 2L = 2Layers; B= Biosist.