

# Adjunctive cryotherapy for pigmentary keratitis in dogs: a study of 16 corneas

Thierry Azoulay

Clinique vétérinaire des Halles, 28 Faubourg de Saverne, 67000 Strasbourg, France

Address communications to:

T. Azoulay

Tel.: +33388323332

Fax: +33388751789

e-mail: azoulayt@wanadoo.fr

## Abstract

**Objective** To assess whether soft cryotherapy (dimethylether, isobutene, and propane) can remove pigmentation of the cornea that has accumulated under different conditions when conventional therapy has been unsuccessful.

**Animals studied** Nine dogs with unilateral or bilateral corneal pigmentation (16 eyes) were included based on progressive corneal pigmentation that was unresponsive to long-term etiological treatment. The dogs had keratoconjunctivitis sicca or chronic superficial keratitis.

**Procedures** A cryogen of 95% dimethylether, 3% isobutane, and 2% propane was applied to the pigmented areas of each cornea under anesthesia. Initial corneal pigmentation and changes were documented over the entire study period using a grading scheme and clinical photographs.

**Results** Most of the pigment deposits were gone by 5–15 days after cryosurgery. Postoperatively, the dogs showed some corneal edema and corneo-conjunctival inflammation, and three dogs had superficial corneal ulcers; these symptoms had resolved by 1 month after the procedure. Follow-up for more than 90 days was available in five dogs (nine corneas), and we observed total or partial repigmentation when the underlying disease was not controlled. A new cryotherapy procedure was successfully performed in two of these dogs.

**Conclusions** Given the sensitivity to cold of melanocytes, cryotherapy is a viable adjunctive treatment for refractory severe corneal pigmentation. Etiological treatment remains necessary to prevent pigmentation from rapidly reappearing. Only a few dogs were followed for more than 90 days; further study is necessary to evaluate the long-term safety and efficacy of soft cryotherapy.

**Key Words:** chronic superficial keratitis, cryotherapy, dog, keratoconjunctivitis sicca, pannus

## INTRODUCTION

Superficial corneal pigmentation results from melanin deposition in the corneal epithelium or more deeply in the corneal stroma. Migration of pigment into the superficial cornea occurs after irritation or chronic inflammation of the cornea from a variety of causes, including distichiasis, ectopic ciliae, nasal fold trichiasis, insufficient tear production (either qualitative or quantitative),<sup>1–3</sup> or exposure associated with conformational or neurologic abnormalities of eyelid function. In brachycephalic breeds, little inflammation may be observed and corneal melanosis may be pigmentary or epithelial dystrophy. Usually, melanocytic cells originate from the limbal conjunctiva and are

deposited in the cornea via neovascularization, but melanin may also be deposited by macrophages and fibroblasts.<sup>4</sup>

Chronic superficial keratitis (CSK) is an immune-mediated keratitis usually seen in German shepherds, Belgian shepherds, greyhounds, and more rarely in beaucerons, collies, poodles, and Siberian huskies.<sup>5–8</sup> In CSK, histopathology reveals superficial corneal inflammation with an accumulation of pigments in the basal layers of the epithelium and the anterior third of the corneal stroma.<sup>8–10</sup> The specific therapy chosen for pigmentary keratitis, medical or surgical, depends on the etiology.<sup>11</sup> When treatment is insufficient or delayed, pigmentation can progress, leading to visual impairment or even blindness.

In severe CSK, superficial keratectomy may be performed to excise heavily pigmented corneal stroma.<sup>6,12</sup> Radiotherapy using beta irradiation<sup>13,14</sup> or soft X-rays<sup>15</sup> has also been used.

Given the sensitivity of melanocytes to cold, cryotherapy may be an alternative technique to decrease corneal pigmentation. Cryosurgery has been used in veterinary ophthalmology to treat distichiasis and ectopic ciliae, glaucoma, lens luxation, neoplasia, and retinal detachment.<sup>16–20</sup> Because melanocytes are highly cold-sensitive, cryosurgery can also be used to treat corneal pigmentation in dogs and conjunctival or scleral melanomas.<sup>21–27</sup> Two studies exploring the use of cryosurgery to treat corneal pigmentation in canine pannus have previously been published.<sup>28,29</sup> In all of the reported cases, there was an initial deterioration in the appearance of the eye during the first postoperative days, with epithelial thinning and stromal edema with vacuolation. With freezing by evaporation of liquid nitrogen, clinical signs and histopathologic lesions were more severe in eyes exposed to two 30-s freezes than in eyes exposed to two 15-s freezes. However, reduction in corneal pigmentation and improvement in vision occurred in all cases in both groups.

The aim of this study was to assess whether soft cryotherapy (dimethylether, isobutane, and propane) can successfully remove pigmentation of the cornea that had accumulated under different conditions, when prior conventional therapy had been unsuccessful.

## MATERIALS AND METHODS

### *Inclusion criteria*

Nine dogs (16 eyes; 7 right eyes and 9 left eyes) with unilateral or bilateral corneal pigmentation secondary to CSK

or KCS were enrolled between January 2008 and December 2011. Only dogs with clinically significant and progressive corneal pigmentation that was unresponsive to long-term etiological treatment were selected for cryotherapy. Dogs being treated for CSK had previously been given topical cyclosporine 0.2% (Optimmune, Intervet, France) and dexamethasone 0.1% (Maxidex<sup>®</sup>, Alcon, France). Dogs being treated for keratoconjunctivitis sicca (KCS) had previously been given topical cyclosporine 0.2% (Optimmune), antibiotic ointment (tobramycin, Tobrex<sup>®</sup>, Alcon, France), and hyaluronic acid (Viskyl<sup>®</sup>, TVM, Lempdes, France). Tacrolimus was not used in these cases because of its potential effects on corneal pigmentation (I Balicki, personal communication, ECVO 2011). Dogs with pigmentation that might have been caused by unmanaged adnexal conditions were excluded. Breeds represented were two German shepherds, two Belgian shepherds, two American cocker spaniels, and one shih-tzu, one Yorkshire terrier, and one shepherd-cross (Table 1). There were five females and four males, and the mean age was 7 years (range 3–14).

### *Presurgical assessment and treatment*

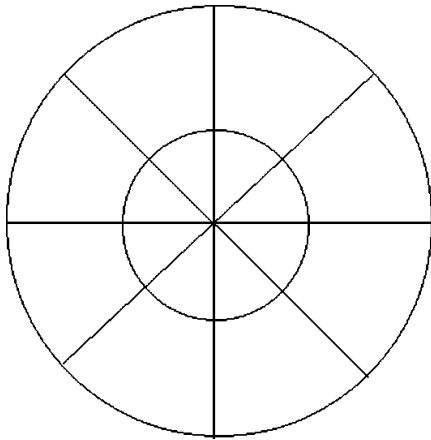
Prior to being subjected to cryotherapy, each dog underwent an ophthalmic examination, including visual assessment, Schirmer tear test, fluorescein stain, tear breakup time, and intraocular pressure measurement (Tono-Pen VET, Medtronic Solan, Jacksonville, FL, USA). The adnexa, the cornea, and the anterior segment were examined by slit-lamp biomicroscopy. After dilation with tropicamide (Mydriaticum<sup>®</sup> 0.5%, 1 drop/5 min for 20 min; Thea Laboratories, France), indirect ophthalmoscopy was attempted. The cause and the extent of pigmentation in each cornea as well as visual function were noted. The

**Table 1.** Dogs breed, gender and age, underlying disease of and duration of clinical signs

Dog no.	Breed	Sex	Age (years)	History and treatment prior to presentation
1	Belgian shepherd	F	5	CSK for 18 months treated with dexamethasone 0.1% (Maxidex <sup>®</sup> ) and topical cyclosporine 0.2% (Optimmune)
2	American cocker spaniel	FC	6	KCS for 1 year treated with hyaluronic acid (Viskyl <sup>®</sup> ), topical cyclosporine 0.2% (Optimmune), and tobramycin (Tobrex <sup>®</sup> )
3	German shepherd	F	7	CSK for 3 years treated with dexamethasone 0.1% (Maxidex <sup>®</sup> ) and topical cyclosporine 0.2% (Optimmune)
4	German shepherd	M	8	CSK for 18 months treated with dexamethasone 0.1% (Maxidex <sup>®</sup> ) and topical cyclosporine 0.2% (Optimmune)
5	American cocker spaniel	F	8	KCS for 2 years treated with hyaluronic acid (Viskyl <sup>®</sup> ), topical cyclosporine 0.2% (Optimmune), and tobramycin (Tobrex <sup>®</sup> )
6	Shepherd-cross	FC	8	CSK for 2 years treated with dexamethasone 0.1% (Maxidex <sup>®</sup> ) and topical cyclosporine 0.2% (Optimmune)
7	Yorkshire terrier	FC	16	KCS for 4 years with hyaluronic acid (Viskyl <sup>®</sup> ), topical cyclosporine 0.2% (Optimmune), and tobramycin (Tobrex <sup>®</sup> )
8	Belgian shepherd	FC	6	CSK for 3 years treated with dexamethasone 0.1% (Maxidex <sup>®</sup> ) and topical cyclosporine 0.2% (Optimmune)
9	Shih-tzu	M	10	KCS for 2 years treated with hyaluronic acid (Viskyl <sup>®</sup> ), topical cyclosporine 0.2% (Optimmune), and tobramycin (Tobrex <sup>®</sup> )

CSK, Chronic superficial keratitis; KCS, keratoconjunctivitis sicca.

cornea was divided into 24 sectors for documentation (Fig. 1), as previously described by Grüning<sup>13</sup> and Allgoewer.<sup>14</sup> The extent of corneal pigmentation in each sector was graded from 0 (absence) to 3 (sector mostly pigmented). The final grade documenting pigmentation of the cornea was obtained by adding the sector grades (Table 2, Fig. 2). Visual function was assessed for each eye individually (with the contralateral eye occluded) with the menace response test, cotton ball test, and maze test.



**Figure 1.** Division of the cornea in 24 sectors for documentation of corneal pigmentation. The extent of pigmentation was scored in each sector (0 = no pigmentation, 1 = pigmented area <30% of the sector area, 2 = pigmented area >30%–60% of the sector area, 3 = pigmented area >60% of the sector area). Scores of each sector were re-added to obtain a final pigmentation grade with a maximum of 72 (24 sectors with a maximal grade of 3 per sector).

Vision was thus rated as normal (positive response to all three tests and score of 2), decreased (insufficient response to one or two tests and score of 1), or absent (negative response to all three tests and score of 0) (Table 3, Fig. 3).

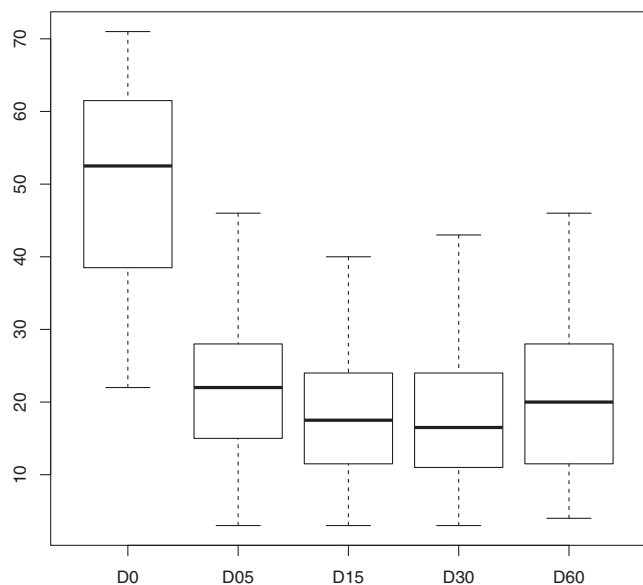
#### *Surgery, postsurgical management, and clinical follow-up*

All cryotherapy procedures were conducted by the same veterinary ophthalmologist. Dogs were anesthetized using ketamine at 5 mg/kg (Ketamine1000<sup>®</sup>, Virbac France) and medetomidine at 20 µg/kg (Domitor<sup>®</sup>, Pfizer France) intravenously. Oxybuprocaine (Cebesine<sup>®</sup>) was applied topically to the eye before treatment. The eye was exposed, and cryotherapy was performed with the Askina Skin Freeze<sup>®</sup> kit (B Braun Medical SAS) originally designed for dermatology in humans. The kit contains an aerosol bottle of liquid cryogen (95% dimethylether, 3% isobutane, and 2% propane) and single-use plastic foam applicators. The liquid is delivered to the applicator and the surgeon waits 15 seconds for the cryogen to evaporate, so the tip reaches the application temperature of –55 °C before application to the cornea. We performed two applications of 50 s each (two freeze–thaw cycles) by slowly rolling the foam tip over the cornea. Once the cornea was white, the applicator was moved to another place. We began with the most pigmented areas and progressed to the edges of the lesion. Initial treatment was continued, and antibiotic ointment (Fucidic acid, Fucithalmic<sup>®</sup>) was used twice daily, if not already used for KCS. Artificial tears were applied 6–10 times a day after treatment. Follow-up included examinations 5, 15, 30, and 60 days after surgery (D5, D15, D30, and D60, respectively) and

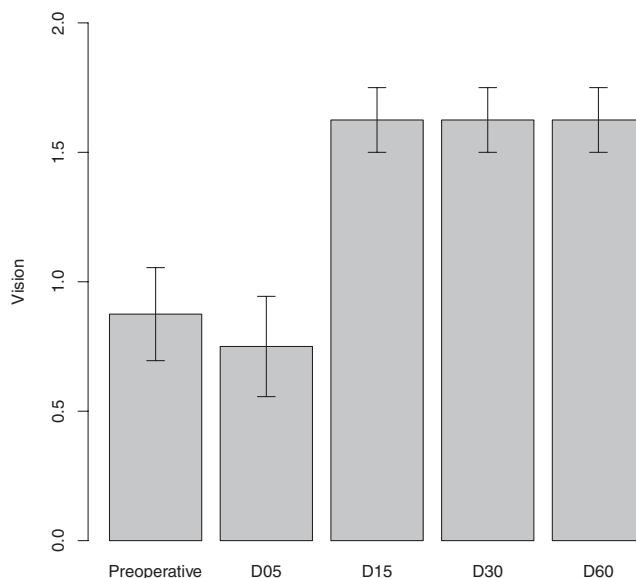
**Table 2.** Extent of corneal pigmentation before and after cryotherapy

DOG	Cause	Affected eye	First cryo procedure						Second cryo procedure									
			D0	D5	D15	D30	D 60	D90	D180	D270	D300	D600	D0	D5	D15	D30	D180	
1	CSK	OD	55	22	18	17	28	28						28	19	15	14	14
	CSK	OS	50	18	13	13	21	21						21	17	15	15	11
2	KCS	OS	71	14	10	8	6				38							
	CSK	OD	57	46	40	40	46											
3	CSK	OS	67	48	43	43	46											
	CSK	OD	29	12	10	9	9			10								
4	CSK	OS	40	18	14	14	14			6								
	KCS	OD	67	24	20	18	16				38	42						
5	KCS	OS	22	16	16	16	14				22	23						
	CSK	OD	43	32	27	27	29											
6	CSK	OS	47	33	29	28	28											
	KCS	OS	64	23	21	20	20											
7	KCS	OS	64	23	21	20	20											
	CSK	OD	59	22	17	15	20		58					58	18	15	14	31
8	CSK	OS	37	23	21	21	22		54					54	17	14	14	29
	KCS	OD	58	12	8	8	8		15									
9	KCS	OS	35	3	3	3	4		4									
	Average		50.1	22.88	19.4	19	20.7											
	SD		12.7	13.44	10.6	10	17											
	Max		71	48	43	43	46											
	Min		22	12	10	8	6											

CSK, Chronic superficial keratitis; KCS, keratoconjunctivitis sicca.



**Figure 2.** Extent of corneal pigmentation during the first 60 days after cryosurgery. Mean scores (/72) and standard deviations over time are presented with a box plot.



**Figure 3.** Vision during the first 60 days after cryosurgery. Mean scores (/2) and standard deviations over time are presented with histograms.

**Table 3.** Vision before and after cryotherapy

Dog	Eye	Vision before and after cryotherapy Visual score (/2)				
		Preoperative vision	D5	D15	D30	D60
1	OD	1	1	2	2	2
	OS	1	1	2	2	2
2	OS	0	1	2	2	2
	OD	0	0	1	1	1
3	OD	0	0	1	1	1
	OS	0	0	1	1	1
4	OD	2	2	2	2	2
	OS	2	2	2	2	2
5	OD	0	0	1	1	1
	OS	1	2	2	2	2
6	OD	1	1	2	2	2
	OS	1	1	2	2	2
7	OS	0	0	1	1	1
	OD	1	0	1	1	1
8	OS	1	0	2	2	2
	OD	1	0	1	1	1
9	OS	2	1	2	2	2
	OD	1	0	1	1	1
Average		0.875	0.75	1.625	1.625	1.625
SD		0.718795288	0.774596669	0.5	0.5	0.5

later when possible. If the fluorescein test was negative at D5, a corticosteroid ointment (dexamethasone 0.1%, Maxidex<sup>®</sup>) was applied to limit postoperative corneo-conjunctival inflammation. If corneal erosion was apparent, the steroid treatment was delayed and then started on D15 if the ulcer had resolved.

The extent of corneal pigmentation and visual status was documented until the final checkup as in the preoperative examination. Signs of secondary effects (i.e. Photophobia, blepharospasm, epiphora, corneal erosion or

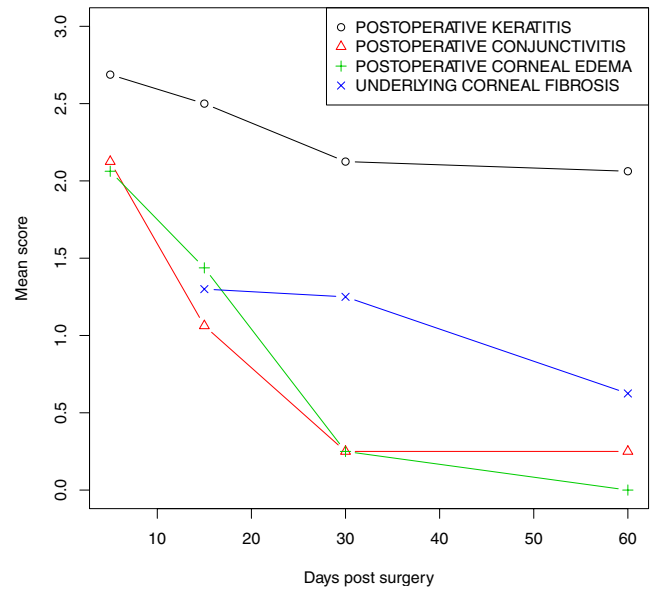
edema, ocular inflammation, and corneal fibrosis) were recorded if present (Table 4, Fig. 4). Postoperative conjunctivitis was defined by the presence of hyperemia, swelling, and conjunctival discharge and graded as follows: 0 (no signs), 1 (mild), 2 (moderate), or 3 (severe). Keratitis was noted if corneal vascularization was observed and graded as follows: 0 (no vessels visible), 1 (mild superficial vascularization, thin vessels visible with magnification), 2 (profuse superficial vascularization visible to the naked eye), or 3 (extensive vascularization with thick vessels originating from all quadrants). Corneal edema was characterized by corneal thickening and diffuse opacity; it was graded as follows: 0 (no signs), 1 (mild corneal haze), 2 (marked corneal opacity, anterior chamber still visible), or 3 (severe corneal opacity, anterior chamber not visible). Underlying corneal fibrosis was defined as the presence of corneal scarring visible when overlying pigmentation had disappeared; it was recorded from D 15 and graded as follows: 0 (no signs), 1 (slight: nubecula), or 2 (marked: leukoma).<sup>30</sup>

#### Statistical analysis

Results are presented as means and standard deviations. We used descriptive statistics to analyze the results as is appropriate for the relatively small number of subjects. Corneal pigmentation was analyzed by fitting a generalized linear mixed model for Poisson data. The number of days following surgery was set as a qualitative variable (4 time points: D5, D15, D30, and D60) and considered as a fixed effect. A random intercept was considered to deal with replications among corneas. Analyses were conducted with R software (lme4 package).<sup>31</sup>

**Table 4.** Side effects during the first 60 days after cryotherapy

Dog	Eye	Postoperative side effects															
		Postoperative corneal edema				Underlying corneal fibrosis				Postoperative conjunctivitis				Postoperative keratitis			
		D5	D15	D30	D60	D15	D30	D60	D5	D15	D30	D60	D5	D15	D30	D60	
1	OD	2	1	0	0	1	1	0	2	1	0	0	3	2	2	2	
	OS	2	1	0	0	1	1	0	2	1	0	0	3	2	2	2	
2	OS	2	1	0	0	1	1	0	2	0	0	0	3	2	2	2	
3	OD	2	1	0	0	1	1	0	2	1	0	0	3	3	2	2	
	OS	2	1	0	0	1	1	1	2	1	0	0	3	3	2	2	
4	OD	1	1	0	0	1	1	0	2	1	0	0	2	2	1	0	
	OS	1	1	0	0	1	1	0	2	1	0	0	2	2	1	1	
5	OD	3	2	0	0	2	2	2	2	1	0	0	2	2	2	2	
	OS	1	1	0	0	2	2	1	2	1	0	0	3	2	2	2	
6	OD	1	0	0	0	0	0	0	2	1	0	0	3	3	2	2	
	OS	1	0	0	0	0	0	0	2	1	0	0	3	3	2	2	
7	OS	3	3	1	0	2	2	1	2	1	1	1	3	3	3	3	
8	OD	3	3	1	0	2	2	1	2	1	1	1	3	3	3	3	
	OS	3	2	1	0	2	2	1	2	1	1	1	3	3	3	3	
9	OD	3	2	0	0	2	2	2	3	2	1	1	3	3	3	3	
	OS	3	3	1	0	1	1	1	3	2	0	0	2	2	2	2	
Average		2.06	1.438	0.25	0	1.25	1.25	0.625	2.13	1.1	0.25	0.25	2.69	2.5	2.1	2.063	
SD		0.85	0.964	0.45	0	0.68	0.68	0.719	0.34	0.4	0.45	0.447	0.48	0.52	0.6	0.772	



**Figure 4.** Side effects during the first 60 days after cryosurgery. Mean scores of postoperative keratitis, conjunctivitis, corneal edema, and underlying corneal fibrosis over time are presented.

**RESULTS**

*Preoperative data*

Preoperatively, 5/16 eyes were blind (score = 0), 8/16 had reduced vision (score = 1), and 3 had normal vision (score = 2). The mean visual score for the 16 eyes preoperatively was  $0.875 \pm 0.72$ . The average preoperative corneal pigmentation score was  $50.1/72 \pm 14.6$  (range, 29–71/72).

*Postoperative data*

Upon recovery from anesthesia, the presence or absence of photophobia, blepharospasm, conjunctival inflammation, and epiphora was noted.

The first postoperative examination was conducted 5 days after surgery. Corneal edema (mean score,  $2.06/3 \pm 0.85$ ), conjunctival inflammation (mean score,  $2.13/3 \pm 0.34$ ), and keratitis (mean score,  $2.69/3 \pm 0.48$ ) were observed systematically (Table 4, Fig. 4). Of 16 eyes, 4 eyes initially showed impaired vision due to severe corneal edema (edema score, 3), including 3 eyes (dog 8 OD and OS, dog 9 OD) that had become blind (visual score, 0) and 1 eye (dog 9 OS) that had reduced vision (visual score, 1) in comparison with preoperative status (visual score, 2). Visual status was maintained or improved in the other dogs. Superficial corneal erosions were observed in three cases (dog 5 OD, dog 8 OD, dog 9 OD) and treated topically with tobramycin solution and ointment (Tobrex®). Corneal pigmentation scores were decreased in all dogs, and the mean score dropped from  $50.1/72 \pm 12.7$  at D0– $22.9/72 \pm 13.4$  at D5.

The second postoperative examination was conducted 15 days after surgery. Corneal edema, signs of conjunctivi-

tis, and keratitis were decreased at D15 relative to D5. The mean edema, conjunctivitis, and keratitis scores at D15 were  $1.44/3 \pm 0.96$ ,  $1.06/3 \pm 0.44$ , and  $2.5/3 \pm 0.52$ , respectively. All ulcers (dog 5 OD, dog 8 OD, and dog 9 OD) were healed by D15, and topical steroids were started. In six corneas (dog 5 OD and OS, dog 7 OS, dog 8 OD and OS, dog 9 OD), the disappearance of pigmentation had unmasked marked underlying corneal fibrosis (score, 2) that may have been impairing vision. However, vision was either maintained (visual score unchanged) or improved (score increased from 1 to 2) in all dogs that could see before treatment, and vision had been restored or improved in dogs that could not see before the operation (score increased from 0 to 1 or 2). The mean visual score was  $1.625 \pm 0.5$ , and none of the eyes were blind at D15. The mean corneal pigmentation score at D15 was  $19.4 \pm 10.6/72$ .

Thirty days after surgery, ocular inflammation had continued to decrease with reduced signs of conjunctivitis (mean score,  $0.25 \pm 0.45/3$ ) and keratitis (mean score,  $2.125 \pm 0.62/3$ ). Functional assessments of all eyes at D30 showed the same results as D15 (mean visual score,  $1.625 \pm 0.5$ ). The extent of corneal pigmentation was similar to that observed on D15 (mean score,  $18.7 \pm 9.9/72$ ).

Two months after the initial procedure, the mean corneal pigmentation score was  $20.7 \pm 16.97/72$ . All dogs had a pigmentation score lower than their initial D0 score. The mean decrease in pigmentation score from D0 to D60 was 29.4. However, 8/16 had started a repigmentation process between D30 and D60 as evidenced by a higher pigmentation score at D60 than at D30. Days since surgery had a significant effect on pigmentation ( $\chi^2 = 369.68$ ,  $df = 4$ ,  $P < 0.001$ ).

Follow-up beyond 90 days was available in 5 of the 9 dogs (9/16 corneas). After D90, 4/9 corneas showed repigmentation with a score  $>36/72$ . The longest term pigmentation score data were as follows: dog 2 OS, 38/72 300 days postprocedure; dog 5 OD, 42/72 at 600 days postprocedure; and dog 8 OD 58/72 and OS 54/72 180 days postprocedure. The remaining 5/9 corneas showed no or partial repigmentation with pigmentation scores  $<29/72$ .

In the case of dog 8 with CSK, pigmentation reappeared completely within 6 months of cryosurgery (OD 58/72 and OS 54/72 180 days postprocedure), despite treatment with 0.2% cyclosporine, corticosteroids, and artificial tears. The dog was not able to ambulate without colliding with objects. A new cryo procedure was performed and well tolerated; it resulted in the removal of most of the corneal pigmentation (OD 14/72 and OS 14/72 30 days after the second procedure), but revealed extensive underlying fibrosis. Nonetheless, the dog regained enough vision to ambulate safely.

Dog 1 also suffering from CSK with an initial pigmentation score of 55 for the right eye and 50 for the left had

partially repigmented after 3 months but not to the initial level (OD 17/72 and OS 13/72 at D30; OD 28/72 and OS 21/72 at D90). We performed an additional cryotherapy on both eyes, after which the corneal pigmentation score was 14/72 (OD) and 15/72 (OS) after 1 month, and 14/72 (OD) and 11/72 (OS) after 6 months.

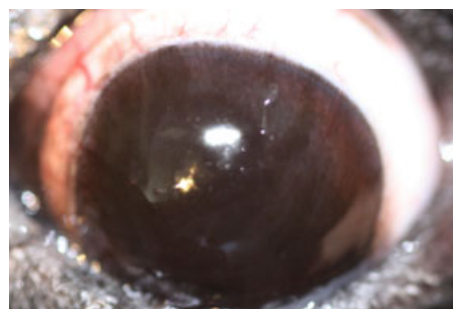
Dog 2 that was monophthalmic with KCS maintained vision after 10 months but had repigmentation. Before cryotherapy, dog 2's pigmentation score was 71/72, at D60 it was 6/72, and at the final checkup 300 days after the procedure it was 38/72 (Figs 5-9). Cyclosporine was discontinued by the owner 2 months postoperatively, and artificial tears were administered four times a day during the subsequent 8 months.

Dog 5 became completely blind 2 years after the procedure due to combined pigmentation and corneal fibrosis. The owners declined a new cryo procedure because of other health problems.

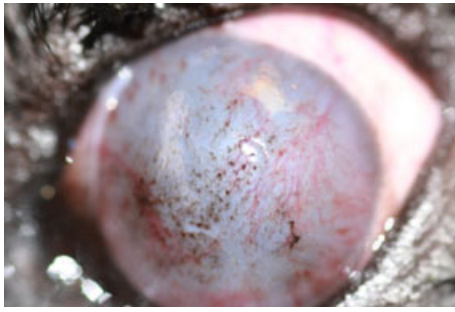
## DISCUSSION

The mechanism of action of cryotherapy has an immediate, a delayed, and a late phase. First, freezing causes ice crystal formation. The freezing rate determines where crystals form: with slow freezing, ice crystals are formed extracellularly, and with rapid freezing, they are predominantly intracellular and cause maximal cell death as they expand during a slow thaw. Simultaneously, osmotic alterations rupture the cell membrane. The second phase occurs a few hours later as vascular stasis leads to destruction of the microcirculation and tissue ischemia. The late phase consists of an immune reaction that is particularly interesting in cancer therapy because cryosurgery increases the specific immunity following tumor necrosis.<sup>8,19,32,33</sup> The greatest cellular destruction is obtained with a fast freeze/slow thaw cycle.<sup>16,34</sup> The destructive effect is cumulative, but the frequency of complications increases as the number of cycles increases.<sup>35-37</sup> Therefore, most studies limit treatment to two freeze-thaw cycles, because more than three cycles are not beneficial.<sup>38,39</sup>

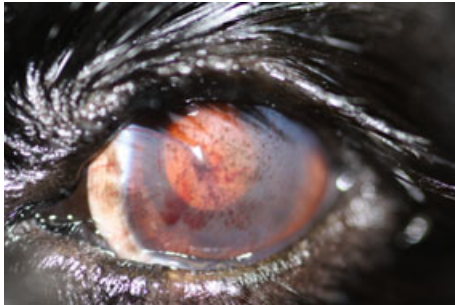
Moreau and Haut described the effect of cold on the cornea in 1971.<sup>40</sup> Epithelial and stromal cells are



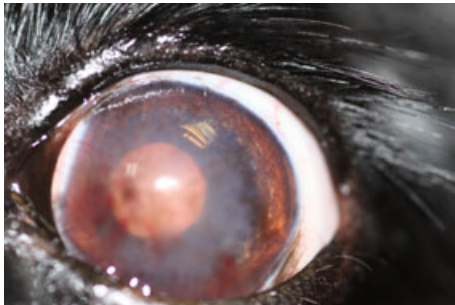
**Figure 5.** Clinical appearance of OS of dog 2 before cryosurgery (D0). Grade = 71/72. 1371 × 912 mm (72 × 72 DPI).



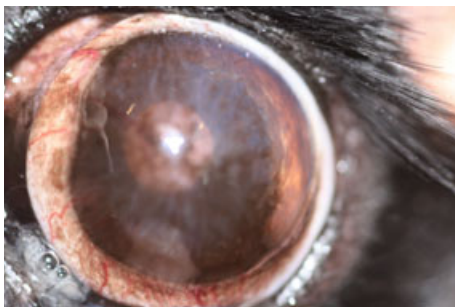
**Figure 6.** Presentation of OS of dog 2 5 days after cryotherapy. Grade = 14/72. 1371 × 914 mm (72 × 72 DPI).



**Figure 7.** Presentation of OS of dog 2 15 days after cryotherapy. Grade = 10/72. 1371 × 914 mm (72 × 72 DPI).



**Figure 8.** Presentation of OS of dog 2 60 days after cryotherapy. Grade = 6/72. 1371 × 914 mm (72 × 72 DPI).



**Figure 9.** Presentation of the same eye 300 days after cryotherapy. 1371 × 914 mm (72 × 72 DPI).

destroyed at  $-30^{\circ}\text{C}$ , but freezing does not have a destructive effect on collagen and fundamental substances. Cellular repopulation begins with an expansion of adjacent normal cells. The corneal endothelium has a heightened cold sensitivity and does not regenerate well, suggesting that pronounced destruction would cause permanent corneal edema.

Cryogenic agents traditionally used in medicine include liquid nitrogen, carbon dioxide, and gaseous nitrous oxide, which achieve temperatures between  $-196^{\circ}\text{C}$  and  $-89^{\circ}\text{C}$ .<sup>41</sup> They must be used with caution to obtain a satisfactory surface effect without penetrating too deeply into the stroma, to avoid reaching the endothelium.<sup>42,43</sup> Side effects with powerful cryogenic agents include epithelial bubbles, corneal melting, deep and permanent corneal edema (following endothelial destruction from the cold). Freon gases and liquid propane are usually reserved for treating superficial dermatologic lesions because they achieve a tissue temperature between  $-40^{\circ}\text{C}$  and  $-60^{\circ}\text{C}$ .<sup>41</sup> Melanocytes are more cold-sensitive than other corneal cells because of their high water content; these soft cryogens are useful for selective destruction of superficial corneal pigmentation. They can be applied directly to a given surface with a prefrozen instrument or a fine spray.

In this study, we chose an aerosol cryogen with foam tip applicators because a spray would achieve deeper penetration of the cryogen and be less precise.<sup>23,38,39</sup> Our technique allowed for very quick freezing of those cells in contact with the applicator during the first 20 s. As the cryogen's temperature increased, cellular freezing was less intense and occurred more gradually. Because of the high sensitivity of melanocytes to freezing, this method allows for a highly targeted ablation of pigmented cells, with a relatively limited effect on the less cold-sensitive corneal cells. Even without thermocoupling, the duration of treatment could be recorded and the rapid freeze–slow thaw principle applied.<sup>23</sup>

This study is the first documentation of the effects of soft cryotherapy on corneal pigmentation. Despite the poor response to previous aggressive medical therapy, corneal pigmentation decreased in all cases during the 2 months after cryosurgery. In cases where months of medical treatment had been ineffective, most pigment deposits were gone by 5–15 days after our cryo treatment. Side effects of cryotherapy included postoperative corneal edema resulting from epithelial injury caused by the cryogen and corneo-conjunctival inflammation. These symptoms resolved by about a month after the procedure. Only three dogs developed a superficial corneal ulcer, and they were resolved at day 15. However, partial repigmentation was observed in almost all cases for which long-term follow-up was possible. Cryosurgery of the cornea may have caused irritation and inflammation resulting in increased pigmentation of the cornea. The use of postoperative topical steroids may be able to control this adverse

event. Recurrence of pigmentation also resulted from the inability to control rather than lack of efficacy of the cryotherapy. Considering that corneal pigmentation is progressive in CSK and KCS, arrest in, or even delay of, progression may be regarded as treatment success. Postoperative improvement in the animal's ability to see well enough to ambulate in its environment without colliding with objects, even temporarily, could justify the use of cryotherapy as an adjunctive treatment in the management of severe corneal pigmentation.

Our cryotherapy protocol is a simple, gentle, and noninvasive procedure that can be delivered using cost-effective equipment and a brief general anesthesia. Unlike superficial keratectomy, there is no obvious thinning of the cornea and more than one procedure can be performed if needed. Given the sensitivity of melanocytes to freezing, cryotherapy is a potential adjunctive treatment of severe corneal pigmentation that is unresponsive to initial medical treatment. However, without treating or keeping the underlying disease under control, pigmentation recurs. Although this technique is promising, it should be noted that the number of dogs in this study was small and the long-term follow-up data were limited. Further studies are needed to evaluate the safety and efficacy of this procedure.

## REFERENCES

- Westermeyer HD, Ward DA, Abrams K. Breed predisposition to congenital alacrima in dogs. *Veterinary Ophthalmology* 2009; **12**: 1–5.
- Kaswan RL, Martin CL, Chapman WL. Keratoconjunctivitis sicca: histopathologic study of nictitating membrane and lacrimal glands from 28 dogs. *American Journal of Veterinary Research* 1984; **45**: 112–118.
- Kaswan RL, Martin CL, Dawe DL. Keratoconjunctivitis sicca: immunological evaluation of 62 canine cases. *American Journal of Veterinary Research* 1985; **46**: 376–383.
- Gilger BC, Bentley E, Ollivier FJ. Diseases and surgery of the canine cornea and sclera. In *Veterinary Ophthalmology* 4th edn. (ed. Gelatt KN). Blackwell, Oxford, 2007; 690–752.
- Bedford PGC, Longstaffe JA. Corneal pannus (chronic superficial keratitis) in the German Shepherd dog. *The Journal of Small Animal Practice* 1979; **20**: 41–56.
- Stanley RG. Superficial stromal keratitis in the dog. *Australian Veterinary Journal* 1988; **65**: 321–323.
- Chavkin MJ, Roberts SM, Salman MD *et al*. Risk factors for development of chronic superficial keratitis in dogs. *Journal of the American Veterinary Medical Association* 1994; **204**: 1630–1634.
- Petrick SW, Van Rensburg BJ. Corneal anatomical differences in the aetiology of chronic superficial keratitis. *The Journal of Small Animal Practice* 1989; **30**: 449–453.
- Williams DL. Histological and immunohistochemical evaluation of canine chronic superficial keratitis. *Research in Veterinary Science* 1999; **67**: 189–193.
- Williams DL. Major histocompatibility class II expression in the normal canine cornea and in canine chronic superficial keratitis. *Veterinary Ophthalmology* 2005; **8**: 395–400.
- Williams DL, Hoey AJ, Smitherman P. Comparison of topical cyclosporin and dexamethasone for the treatment of chronic superficial keratitis. *Veterinary Record* 1995; **137**: 635–637.
- Wilkie DA, Whittaker C. Surgery of the cornea. *Veterinary Clinics of North America: Small Animal Practice* 1997; **27**: 1067–1107.
- Höcht S, Grüning G, Allgoewer I *et al*. Die Behandlung der Keratitis chronica superficialis des Hundes mit Strontium-90. *Strahlentherapie und Onkologie* 2002; **178**: 99–104.
- Grüning G, Allgoewer I, Höcht S *et al*. Zur Strahlentherapie der Keratitis superficialis chronica mit Strontium 90. *Kleintierpraxis* 2001; **46**: 389–399.
- Allgoewer I, Höcht S. Radiotherapy for canine chronic superficial keratitis using soft X-rays (15 kV). *Veterinary Ophthalmology* 2010; **13**: 20–25.
- Aragon A. *Contribution a l'étude de la cryothérapie en ophtalmologie vétérinaire. Utilisation d'un système à azote liquide: Erbcryo-derm chez les carnivores domestiques*. Thèse de doctorat vétérinaire, Lyon, 1998.
- Monin C, Campinchi F, Gazagne C *et al*. Journal Français d'Ophthalmologie, organe d'expression de la SFO. Numéro spécial. *Cryothérapie* 1993; **7**: 174–178, 189–195.
- Giaconi JA, Karp CL. Current treatment options for conjunctival and corneal intraepithelial neoplasia. *The Ocular Surface* 2003; **1**: 66–73.
- Lincoff H, Cavero R, Nadel A, McLean J. Cryosurgery of the ciliary body. Part II. *Archives of ophthalmology* 1968; **79**: 196–204.
- Merideth RE, Gelatt KN. Cryotherapy in veterinary ophthalmology. *Veterinary Clinics of North America: Small Animal Practice* 1980; **10**: 837–846.
- Vestre WA. Cryosurgical techniques in veterinary ophthalmology. *The Compendium on Continuing Education* 1984; **6**: 481–487.
- Hidayat AA, LaPiana FG, Kramer KK. The effect of rapid freezing on uveal melanomas. *American Journal of Ophthalmology* 1987; **103**: 66–80.
- 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* 2009a; **12**: 65–72.
- Bosch G, Klein WR. Superficial keratectomy and cryosurgery as therapy for limbal neoplasm in 13 horses. *Veterinary Ophthalmology* 2005; **8**: 241–246.
- Cayce KA, Feldman SR, McMichael AJ. Hyperpigmentation: a review of common treatment option. *Journal of Drugs in Dermatology* 2004; **3**: 668–673.
- Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. *Cryobiology* 1998; **37**: 171–186.
- Hoyt RF, Seim HB. Veterinary cryosurgery: mechanisms of cell death, cryosurgical instrumentation and cryogens. Part I. The Compendium on. *Continuing Education* 1981; **3**: 426–432.
- Rickards DA, Carter KL. Cryosurgery in canine pannus. *Canine Practice* 1978; **5**: 48–52.
- Holmberg DL, Scheifer HB, Parent J *et al*. The cryosurgical treatment of pigmentary keratitis in dogs. An experimental and clinical study. *Veterinary Surgery* 1986; **15**: 1–4.
- Nell B, Walde I, Billich A *et al*. The effect of topical pimecrolimus on keratoconjunctivitis sicca and chronic superficial keratitis in dogs: results from an exploratory study. *Veterinary Ophthalmology* 2005; **8**: 39–46.
- R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2011.
- Farrant J, Walter CA. The cryobiological basis for cryosurgery. *The Journal of Dermatologic Surgery and Oncology* 1977; **3**: 4.
- Whittaker DK. Mechanism of tissue destruction following cryosurgery. *Annals of the Royal College of Surgeons of England* 1984; **66**: 313–318.

34. Neel HB. Immunotherapeutic effect of cryosurgical tumor necrosis. *Veterinary Clinics of North America: Small Animal Practice* 1980; **10**: 763–769.
35. Wilkes TDI, Fraunfelder FT. Principles of cryosurgery. *Ophthalmic Surgery* 1979; **10**: 21–30.
36. Gage AA, Guest K, Montes M *et al.* Effect of varying freezing and thawing rates in experimental cryosurgery. *Cryobiology* 1985; **22**: 175–182.
37. Liu D, Natiella J, Schaeffer A *et al.* Cryosurgical treatment of the eyelids and lacrimal drainage ducts of the rhesus monkey. Course of injury and repair. *Archives of Ophthalmology* 1984; **102**: 934–939.
38. Seim HB. Mechanisms of cold-induced cellular death. *Veterinary Clinics of North America: Small Animal Practice* 1980; **10**: 755–762.
39. Withrow SJ. General principles of cryosurgical technique. *Veterinary Clinics of North America: Small Animal Practice* 1980; **10**: 779–785.
40. Moreau PG, Haut J. *Cryo-ophtalmologie. Rapport présenté à la Société française d'ophtalmologie le 4 mai 1971.* Masson, Paris, 1971; 16–52.
41. Byrne MD. Cryosurgical instrumentation. *Veterinary Clinics of North America: Small Animal Practice* 1980; **10**: 771–778.
42. Wingfield DL, Fraunfelder FT. Possible complications secondary to cryotherapy. *Ophthalmic Surgery* 1979; **10**: 47–55.
43. Wood JR, Anderson RL. Complications of cryosurgery. *Archives of Ophthalmology* 1981; **99**: 460–463.