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# Evaluation of ocular surface parameters before and after cryo- and laser therapy for distichiasis in dogs: A pilot study

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## Abstract

**Objective:** The aim of this pilot study was to establish a satisfactory, scientific approach to effectively compare quantitative measurements of various ocular surface parameters before and after surgical treatment in dogs suffering from distichiasis.

**Methods:** An ophthalmic examination was conducted on 12 dogs (23 eyes) before and after surgical treatments for distichiasis, at four different time points, ($t_0$ = before surgery, $t_1$ = 1–2 h after surgery, $t_2$ = 1 week after surgery, $t_3$ = 1 month after surgery, and $t_4$ = 6 months after surgery) between 2021 and 2022, and analyzed retrospectively. The examination included Schirmer tear test-1 (STT-1), interferometry, noninvasive tear film break-up time (NIBUT), tear meniscus size (TMS), and meibography.

**Results:** No statistically significant changes were found in STT-1 ($t_0$: 22.2 ± 6.5 mm, $t_2$: 22.5 ± 5.7 mm, $t_3$: 20.8 ± 5.1 mm, and $t_4$: 22.6 mm ± 4.8 mm) before and after surgery. Mean interferometry scores showed a slight, not statistically significant, decrease from $t_0$ to $t_3$ and $t_4$ ($t_0$: 2.1 ± 0.8, $t_1$: 2.1 ± 0.7, $t_2$: 2.1 ± 0.9, $t_3$: 1.8 ± 0.6, and $t_4$: 1.9 ± 1.1). Mean NIBUT did not change significantly between time points ($t_0$: 3.9 ± 1.3 s, $t_1$: 4.0 ± 1.3 s, $t_2$: 4.0 ± 1.4 s, $t_3$: 3.5 ± 0.7 s, and $t_4$: 3.5 ± 0.9 s). TMS showed a slight, not statistically significant increase ($t_0$: 0.5 ± 0.3 mm, $t_1$: 0.5 ± 0.3, $t_2$: 0.5 ± 0.3 mm, $t_3$: 0.6 ± 0.2 mm, and $t_4$: 0.7 ± 0.3 mm). There were no changes in the gross morphology of the meibomian glands (MG).

**Conclusion:** This pilot study could not detect a negative effect of different forms of treatment of distichiasis on the precorneal tear film parameters in dogs. However, due to the study's retrospective nature and small sample size, no definitive conclusion can be drawn regarding the changes at the different time points.

**KEYWORDS**

cryotherapy, distichiasis, dogs, lasertherapy, ocular surface analyzer, ocular surface parameters

## 1 | INTRODUCTION

Distichiasis is the occurrence of aberrant, cilia-like hair along the free lid margin of the eyelid.[1] The hair follicle of the distichia is typically located either within the meibomian gland (MG) or next to it.[2] Particularly short and bristly distichia may irritate the surface of the cornea, causing epiphora and blepharospasm. In some severe cases these aberrant eyelashes can cause ulceration or even perforation of the cornea.[3] In dogs, distichiasis can occur in any breed; however, there is a predisposition in American and English Cocker Spaniels, Welsh Springer Spaniels, Cavalier King Charles Spaniels, Flat-coated Retrievers, Boxers, English Bulldogs, Pekinese, Shih Tzu, and other breeds.[1,4]

Several treatment options are available for distichiasis in dogs, including cryo- (CT) and laser therapy (LT) to destroy the hair follicle. These methods have been established for both human and veterinary patients.[5–8] Although CT and LT are regularly used to treat distichiasis, the sequela of the heavy manipulation of the eyelid and therefore the MGs during these procedures might be underestimated. The authors of this pilot study hypothesize that these treatments have a negative effect on the precorneal tear film. The MGs are essential for the formation of the outer lipid layer of the precorneal tear film and maintaining a healthy cornea.[9,10] Decreased or even loss of function of MGs can therefore lead to a variety of ocular surface diseases.[11] Previous studies have explored ocular surface disorders and MG dysfunction in veterinary ophthalmology, particularly in dogs.[11–14] Therefore, this study investigated the impact of distichiasis treatment on the MGs and on the precorneal tear film. There are only a few reports of the effect of distichiasis treatment on the precorneal tear film in dogs.[15–17] While there are only a few reports on the effect of distichiasis treatment on the canine precorneal tear film, this is a pilot study to assess precorneal tear film parameters using an ocular surface analyzer before and after CT and LT in the same individuals.

## 2 | MATERIALS AND METHODS

### 2.1 | Ophthalmic examination and assessment of tear film parameters

This retrospective pilot study included 12 canine patients between 2021 and 2022, which were treated for distichiasis in at least one eye at the Department of Ophthalmology of the Veterinary University Vienna. Owner consent was given for the examination as well as for the use of data. An ophthalmic examination via slit lamp biomicroscopy (Kowa Portable Slit-Lamp SL-17), including a STT-1 (MSD Sac Schirmer tear test) was conducted. After a minimum of 5 min following manipulation of the eye with the tear strip, the precorneal tear film was assessed with the hand-held Ocular Surface Analyzer (OSA; OSA Vet SBM Sistemi®, Torino, Italy).

The OSA was used to measure the thickness of the lipid layer of the precorneal tear film with interferometry. Values were determined using the grading scale, as described by Vinas et al. Grade 0 for a thickness of 0–15 nm, Grade 1 for 15–30 nm, Grade 2 for 31–60 nm, Grade 3 for 61–100 nm, and Grade 4 for a thickness over 100 nm.[11]

The noninvasive tear film break-up time (NIBUT), measuring the time in seconds (sec) between a blink and the occurrence of tear film discontinuity, was analyzed by video assessment of the tear film recorded with the OSA.

In addition, the size of the tear meniscus (TMS), which describes the space between the lower eyelid and the cornea on the OSA images, was examined immediately after a spontaneous blink and measured in millimeters (mm).

Infrared meibography was performed with the OSA. Changes of morphology and dropout of MGs, documented by the infrared pictures of the OSA, were analyzed using the grading system according to Arita et al. Grade 0 describing no dropout of MGs in the total MG area, Grade 1 in less than one third, Grade 2 between one third and two thirds, and Grade 3 in more than two thirds.[18] During the use of the automated detection system, it became apparent that accurate classification was not possible. Due to varying exposures of the infrared images and light reflections, the system incorrectly identified areas without MGs as having MGs and vice versa. As a result, the manufacturer's automated detection system was not used to identify MG areas and MG counts were performed manually.

All tear film parameters, except dropout rate of MGs, were analyzed and graded using the OSA Vet SBM Sistemi software ICP for Windows by SBM Sistemi SRL (OSA Vet SBM Sistemi®, Torino, Italy).

All parameters were assessed during the first ophthalmic examination before surgical treatment ($t_0$). Only eyes of patients with STT-1 values above 15 mm/min were included in this study.

### 2.2 | Distichiasis treatment

For the treatment of distichiasis, either CT or LT was chosen depending on its extent. Dogs with less than three lashes were treated with LT, while those with three or more distichia were treated with CT. In cases of severe distichiasis, where there were more than 10 distichia on the upper and lower lid, a combined therapy with CT and LT was chosen. All dogs underwent treatment under general anesthesia. The lids were

everted using the Graefe forceps after aseptic preparation. The ERBOKRYO® AE cryosurgical system (Erbe Elektromedizin GmbH, Tübingen, Germany) was used for CT. The probe was positioned approximately 2 mm from the free lid margin, and two freeze and thaw cycles were conducted, each lasting 3 s, at a probe temperature of minus 80°C. For LT, the FOX 810 Ophthalmology Laser's distichiasis probe (A.R.C. Laser GmbH, Nürnberg, Germany) was used by inserting the tip of the probe into the MG opening that contained a distichia. Two cycles of 700 mW for 1.5 s were performed on each distichia. Loose distichiae were then removed from the MG orifices using Arruga forceps. Following assessment of ocular surface parameters, patients were routinely administered antibiotic ophthalmic ointment and a systemic anti-inflammatory agent.

## 2.3 | Follow-up examinations

The ocular surface parameters, excluding STT-1, were reexamined 1–2 h after the surgery ($t_1$). Follow-up ophthalmic and ocular surface examinations, including an analysis of the precorneal tear film and STT-1, were performed at approximately 1 week ($t_2$), approximately 1 month ($t_3$), and around 6 months ($t_4$) after surgery.

## 2.4 | Statistical analysis

The findings of the ophthalmic examination, including the STT-1, were documented in the facility's own data software system. The ocular surface parameters were documented in the OSA software program, and later exported into an excel file. An excel file was created to tabulate MG dropout. The mean values of the right and left eye were calculated and the values at the different time points were compared. The assumption of normal distribution was assessed using the Shapiro–Wilk test. Additionally, the values of the different interferometry grades at different time points were compared using the Mann–Whitney *U*-test. For all other parameters the difference between the two groups and the differences between the time points were analyzed using linear mixed effects models for every measured parameter. Time points were compared using Sidak's alpha correction procedure. As this is a pilot study, we also provide effect sizes according to Cohen where an effect size $d < 0.8$ indicates medium effects, effect size $d < 0.5$ small effects. Effect sizes below 0.1 are considered to have no practical relevance.[19] All statistical analysis were performed using IBM SPSS v29. A *p*-value below 5% ($p < .05$) was seen as statistically significant. Following these calculations, a post hoc sample size calculation was conducted to provide information for further studies. This calculation was carried out using G*Power v3.1 for various effect sizes (0.2–0.8) always under the assumption that the values behave in the same way as in the pilot study. The case numbers were calculated for the various effect sizes for group comparisons and for comparisons between the time points.

# 3 | RESULTS

## 3.1 | Animal based data

The medical records of 12 dogs were analyzed. The mean age was 3.4 ± 2.5 years, ranging from 7.4 months to 7.8 years. At the time of the first examination, 6 dogs were male (6/12; 50%), 1 male castrated (1/12; 8.3%), 4 were female (4/12; 33.3%), and 1 was female and spayed (1/12; 8.3%). The breeds included in the study were brachycephalic (7/12; 58.3%), dolichocephalic (4/12; 33.3%), and mesocephalic dogs (1/12; 8.3%). The breeds consisted of two French Bulldogs and two Boxers as well as two Shetland Sheepdogs and one Shih Tzu, Chihuahua, Pomeranian, Berger Blanc Suisse, Flat-coated Retriever, and Miniature Dachshund each (Table 1).

## 3.2 | Eye-based data

As one eye from one dog was excluded, due to keratoconjunctivitis sicca, only 23 eyes of 12 dogs were investigated in the study. Fifteen eyes of eight dogs were treated with CT (15/23 eyes; 65.2%), 4 eyes of 2 dogs were treated with LT (4/23 eyes, 17.4%), and 4 eyes of 2 dogs were treated with a combination of CT and LT (4/23 eyes, 17.4%). All 23 eyes were examined preoperative ($t_0$). Twenty eyes (20/23; 87.0%) were analyzed immediately postoperative ($t_1$). In 3 eyes (Dogs 2 and 3) the postoperative examination was not possible due to the application of eye ointment, by the attending anesthesiologist. All other patients received eye ointment after the postoperative examination. First follow-up examination ($t_2$) was carried out in 21 eyes (21/23; 91.3%) 8.9 ± 2.3 days postoperative. Fifteen eyes (15/23; 65.2%) were examined at the second follow-up examination ($t_3$) 36.9 ± 10.2 days postoperative and 13 eyes (13/23; 56.5%) at the third follow-up examination ($t_4$) 179.0 ± 34.3 days postoperative (Table 1).

## 3.3 | Schirmer tear test (STT-1)

The mean STT-1 values of both eyes were 22.2 ± 6.5 mm at $t_0$, 22.5 ± 5.7 mm at $t_2$, 20.8 ± 5.1 mm at $t_3$, and

**TABLE 1** Signalment of the dogs, treatment form, eye, and time in days postoperatively.

| Dog | Breed | Sex | Age | Treatment | Eye | Postoperatively $t_1$ | Days postoperatively $t_2$ | Days postoperatively $t_3$ | Days postoperatively $t_4$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Boxer | M | 7 years and 12 days | CT | OU | Analyzed | 8 | n.a. | n.a. |
| 2 | Shih Tzu | F | 2 years and 148 days | CT and LT | OU | n.a. | 13 | 52 | 131 |
| 3 | French Bulldog | F | 7 years and 276 days | CT | OD | n.a. | 13 | 37 | n.a. |
| 4 | Miniature Dachshund | FS | 5 years and 44 days | CT | OU | Analyzed | 6 | n.a. | n.a. |
| 5 | Chihuahua | M | 5 years and 14 days | CT and LT | OU | Analyzed | 8 | n.a. | n.a. |
| 6 | Boxer | F | 225 days | CT | OU | Analyzed | n.a. | n.a. | n.a. |
| 7 | Pomeranian | M | 319 days | CT | OU | Analyzed | 8 | 33 | 181 |
| 8 | Shetland Sheepdog | M | 356 days | LT | OU | Analyzed | 8 | 29 | 190 |
| 9 | Flat-coated Retriever | MC | 5 years and 107 days | LT | OU | Analyzed | 9 | 30 | 212 |
| 10 | Berger Blanc Suisse | M | 2 years and 15 days | CT | OU | Analyzed | 8 | 36 | 183 |
| 11 | French Bulldog | F | 3 years and 51 days | CT | OU | Analyzed | 8 | 44 | n.a. |
| 12 | Shetland Sheepdog | M | 346 days | CT | OU | Analyzed | 8 | 30 | 181 |

Abbreviations: CT, cryotherapy; CT and LT, cryo- and lasertherapy; f, female; fs, female spayed; LT, lasertherapy; m, male; mc, male castrated; n.a., not analyzed; OD, oculus dexter; OU, oculus uterque.

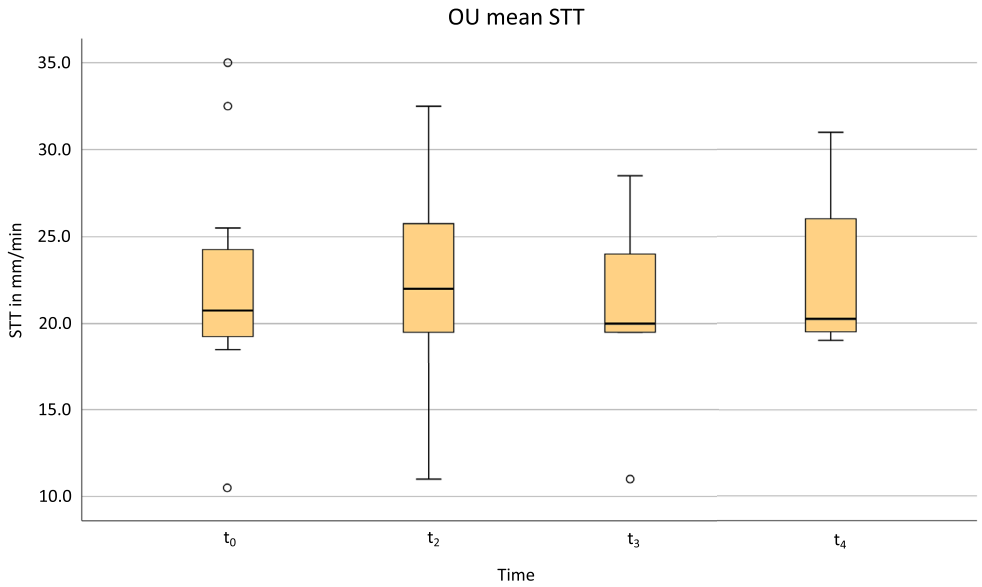


**FIGURE 1** Mean STT in mm/min of both eyes over different time points (OU, oculus uterque; STT, Schirmer tear test).

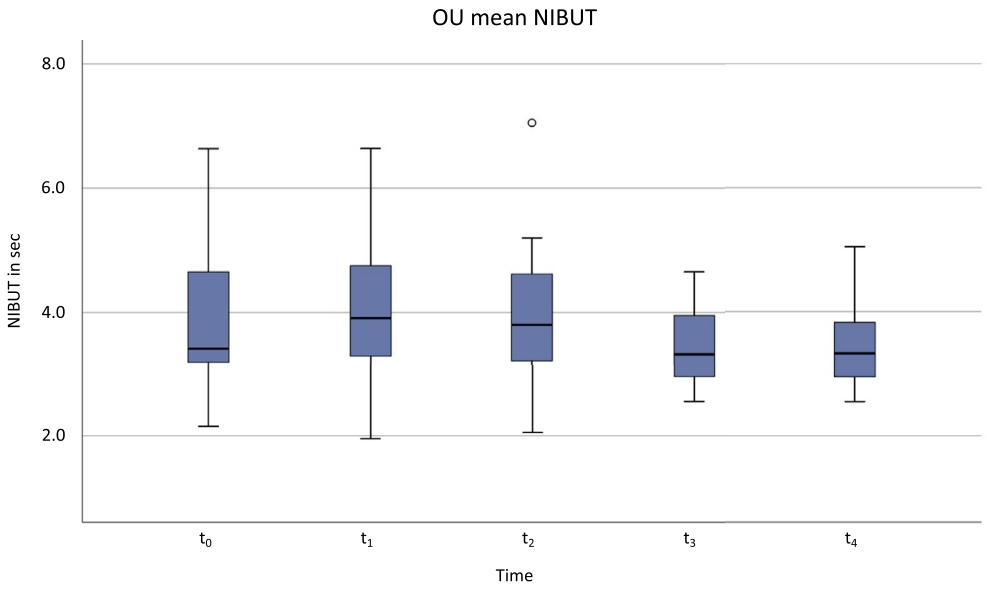


**FIGURE 2** Mean interferometry grades of both eyes over different time points (OU, oculus uterque).

22.6 mm ± 4.8 mm at $t_4$, shown in Figure 1. The mean STT-1 values across all time points for dogs treated with CT was 23.3 ± 1.2 mm and for dogs treated with LT alone or in combination with CT it was 20.3 ± 1.4 mm.

There was no statistically significant difference between the mean STT-1 value for eyes treated with CT before treatment ($t_0$) and all other follow-up examinations ($t_2$, $t_3$, and $t_4$), with p-values of 0.753, 0.711, and 0.420 respectively. For eyes treated with LT or LT and CT the *p*-values were .845, .763, and .736 respectively. The effect size according to Cohen showed no practical relevance between $t_0$ and $t_2$ ($d < 0.1$) as well as a small effect between $t_0$ and $t_3$ ($d = 0.2$), and $t_0$ and $t_4$ ($d = 0.3$).

## 3.4 | Interferometry

Figure 2 demonstrates the mean lipid layer thickness grades of both eyes, 2.1 ± 0.8 at $t_0$, 2.1 ± 0.7 at $t_1$, 2.1 ± 0.9 at $t_2$, 1.8 ± 0.7 at $t_3$, and 1.9 ± 1.1 at $t_4$. The mean lipid layer thickness grade across all time points for dogs treated with CT was 1.7 ± 0.2 mm and for dogs treated with LT alone or in combination with CT was 2.4 ± 0.2 mm. The mean lipid layer thickness showed no statistically significant difference at various time points. Comparing the mean values of lipid layer thickness at $t_0$ with $t_1$, $t_2$, $t_3$, and $t_4$ for eyes treated with CT each, the *p*-values were .755, .915, .583, and .612, respectively. For eyes treated with LT

or laser- and CT the *p*-values were .595, 1.000, .383, and .692, respectively.

### 3.5 | Noninvasive tear film break-up time (NIBUT)

The mean NIBUT values of both eyes were 3.9 ± 1.3 s at $t_0$, 4.0 ± 1.3 s at $t_1$, 4.0 ± 1.4 s at $t_2$, 3.5 ± 0.7 s at $t_3$, and 3.5 ± 0.9 s at $t_4$, displayed in Figure 3. The mean NIBUT values across all time points were 3.4 ± 0.2 mm in dogs treated with CT and 4.2 ± 0.3 mm in dogs treated with LT alone or in combination with CT. The mean NIBUT did not change statistically significantly throughout all time points, although a slight decrease across time was noted. Comparing the preoperative values ($t_0$) with the values of follow-up examinations $t_1$, $t_2$, $t_3$, and $t_4$ for eyes treated with CT the *p*-values were .972, .497, .249, and .089, respectively. For eyes treated with LT or LT and CT, the *p*-values were .846, .266, .900, and .770, respectively. The effect size according to Cohen showed no practical relevance between $t_0$ and $t_1$ ($d < 0.1$), no effect between $t_0$ and $t_2$ ($d = 0.0$), no practical relevance between $t_0$ and $t_3$ ($d < 0.1$) as well as $t_0$ and $t_4$ ($d < 0.1$).

### 3.6 | Tear meniscus size (TMS)

Figure 4 demonstrates the mean TMS of both eyes, 0.5 ± 0.2 mm at $t_0$, 0.6 ± 0.2 mm at $t_1$, 0.6 ± 0.3 mm at $t_2$, 0.6 ± 0.2 mm at $t_3$, and 0.7 ± 0.3 mm at $t_4$. The mean TMS across all time points for dogs treated with CT was 0.6 ± 0.1 mm and for dogs treated with LT alone or in combination with CT was 0.6 ± 0.1 mm. The mean TMS did show a slight increase across time. However, the differences between the first examination ($t_0$) and $t_1$, $t_2$, $t_3$, and $t_4$ for eyes treated with CT were also not statistically significant, with *p*-values of .452, .890, .420, and .215, respectively. For eyes treated with LT or LT and CT the *p*-values were .589, .626, .675, and .884, respectively. The effect size according to Cohen showed a medium effect between $t_0$ and $t_1$ ($d = -0.6$), a small effect between $t_0$ and $t_2$ ($d = -0.2$), and a large effect between $t_0$ and $t_3$ ($d = -0.9$) and $t_0$ and $t_4$ ($d = -0.9$).

### 3.7 | Meibography (MB)

Preoperatively ($t_0$), 10 eyes showed Grade 0 MG dropout (10/23 eyes, 43.5%), 8 eyes showed Grade 1 MG dropout (8/23 eyes, 34.8%) and in 5 eyes (5/23 eyes, 21.7%) it was not possible to assess the MG dropout due to pigmentation of the conjunctiva. Due to postoperative chemosis, the grade of dropout could not be assessed directly postoperative. There was no change noted, neither in the gross morphology nor in the MG dropout grades during any time points ($t_2$, $t_3$, and $t_4$) in any dog. Table 2 shows a detailed overview of dropout and ocular changes affecting MG dropout assessment (Table 2).

### 3.8 | Sample size calculation

Based on the results of this study, a sample size calculation for future research was carried out. The power

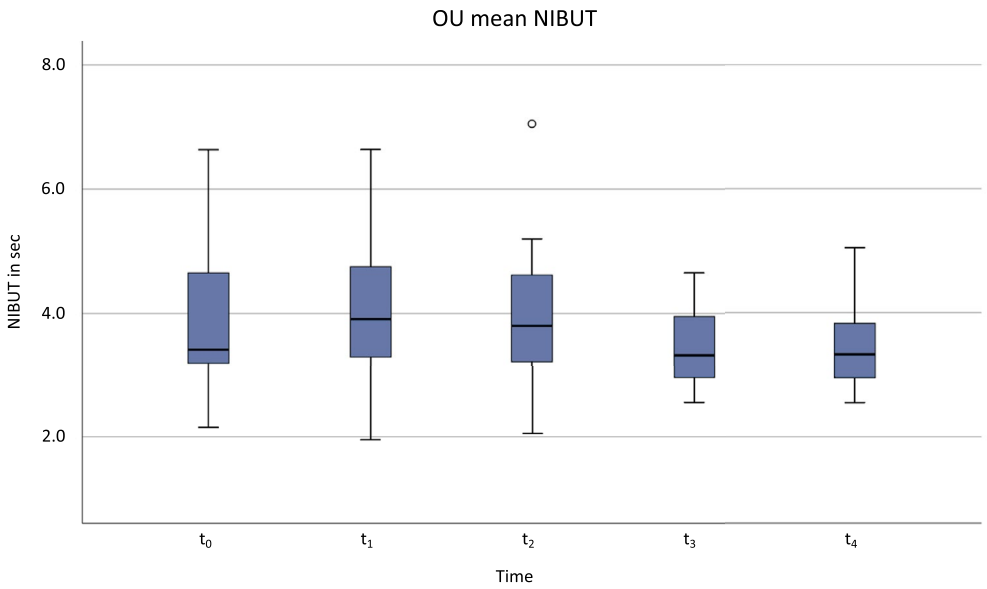


**FIGURE 3** Mean NIBUT in seconds of both eyes over different time points (OU, oculus uterque; NIBUT, non-invasive tear film break-up time).

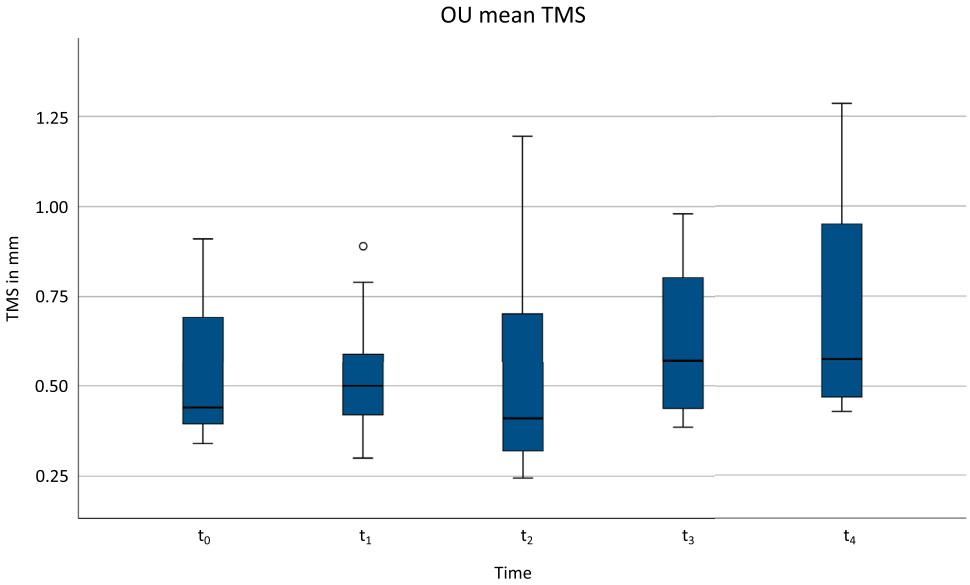


**FIGURE 4** Mean TMS in mm of both eyes over different time points (OU, oculus uterque; TMS, tear meniscus size).

**TABLE 2** Meibomian gland dropout grade according to Vinas et al. of each dog at all time points.[11]

| | $t_0$ | | $t_1$ | | $t_2$ | | $t_3$ | | $t_4$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dog** | **OD** | **OS** | **OD** | **OS** | **OD** | **OS** | **OD** | **OS** | **OD** | **OS** |
| 1 | 1 | Pigment | Chemosis | Chemosis | 1 | Pigment | n.a. | n.a. | n.a. | n.a. |
| 2 | 0 | 0 | n.a. | n.a. | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 | n.a. | n.a | n.a. | 1 | n.a. | 1 | n.a. | n.a. | n.a. |
| 4 | 1 | 1 | Chemosis | Chemosis | 1 | 1 | n.a. | n.a. | n.a. | n.a. |
| 5 | Pigment | Pigment | Chemosis | Chemosis | Pigment | Pigment | n.a. | n.a. | n.a. | n.a. |
| 6 | 1 | 1 | Chemosis | Chemosis | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 7 | 0 | 0 | Chemosis | Chemosis | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 1 | 1 | Chemosis | Chemosis | 1 | 1 | 1 | 1 | 1 | 1 |
| 9 | Pigment | Pigment | Chemosis | Chemosis | Pigment | Pigment | Pigment | Pigment | Pigment | Pigment |
| 10 | 0 | 0 | Chemosis | Chemosis | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | Chemosis | Chemosis | 0 | 0 | 0 | 0 | n.a. | n.a. |
| 12 | 0 | 0 | Chemosis | Chemosis | 0 | 0 | 0 | 0 | 1 | 0 |

Abbreviations: n.a., not analyzed; OD, oculus dexter; OS, oculus sinister.

analysis revealed a minimum number of 104 individuals necessary to demonstrate an effect size *d* 0.5, to show a difference between groups as well as a minimum number of 28 individuals to illustrate an effect size *d* 0.5, to show a difference between time points. All results of the power analysis are presented in Table 3.

## 4 | DISCUSSION

Ocular surface parameters and MG dysfunction are of increasing interest in veterinary ophthalmology. As CT and LT strongly manipulate the eyelids, it is unclear whether the sequelae of these treatments could have a negative impact on the MGs and thus the precorneal tear film in dogs. This pilot study showed no significant changes in STT, lipid layer thickness, NIBUT, TMS and meibography in dogs undergoing CT and LT.

In this pilot study, the STT-1 did not significantly change at different time points. The STT-1 values collected in this study are comparable to previously published STT-1 values in healthy dogs.[20–22]

Concerning lipid layer thickness, a comparison with previously published results turns out to be rather difficult due to its inconsistent classification throughout the literature. In 2019, Vinas et al., whose classification system

**TABLE 3** Estimated sample size for nonparametric one tailed comparison of groups or timepoints. Assuming a power of 80% and alpha = 5%. The effect size *d*, provides information about the practical relevance of the research findings.

| Difference between groups | | Difference between timepoints | |
|---|---|---|---|
| **Effect size *d*** | **Total sample size (difference between two groups) (*n*1 = *n*2)** | **Effect size *d*** | **Total sample size *N* (difference between timepoints)** |
| 0.2 | 648 | 0.2 | 164 |
| 0.3 | 288 | 0.3 | 74 |
| 0.4 | 162 | 0.4 | 42 |
| 0.5 | 104 | 0.5 | 28 |
| 0.6 | 72 | 0.6 | 20 |
| 0.7 | 54 | 0.7 | 16 |
| 0.8 | 42 | 0.8 | 14 |

was used for this study, determined interferometric values in their retrospective study of 81 dogs (155 eyes) suffering from ocular surface disorders. The most frequent grade observed was Grade 1 (15–30 nm, 49.3%), followed by Grade 2 (31–60 nm, 22%), Grade 0 (0–15 nm, 19.4%), Grade 3 (61–100 nm, 7.3%), and Grade 4 (>100 nm, 2%).[11] These findings show lower interferometric values in dogs suffering from ocular surface disorders than the values from dogs treated for distichiasis, found in this pilot study. Reynolds et al. found a similar range of results, although they used a slightly different grading system (0–14, 15–30, 31–60, 61–100, or>100 nm). They were able to detect a difference in values between the group of dogs that received distichiasis treatment and a reference group. The mean lipid layer thickness was 36.8 ± 19 nm in the treated group, compared to a lipid layer thickness of 59.2 ± 34.9 nm in the healthy, reference group.[15]

Other studies have examined lipid layer thickness in healthy dogs and dogs with various ocular surface diseases, with values from studies of healthy dogs mimicking the values found in this study, except for healthy brachycephalic dogs, which had lower lipid layer thickness than in this study.[21] Values from studies of diseased dogs were lower than in the current study.[12,22]

Regarding NIBUT values, no significant changes were observed in this study before and after distichiasis treatment. However, the values of the NIBUT were lower than those previously described in healthy dogs. Kim et al. describe a mean NIBUT of 19.1 ± 9.5 s in 10 healthy beagles.[22] Jeong et al. compared the NIBUT of healthy dogs and dogs suffering from MG dysfunction and found a mean NIBUT Grade 2 (4–8 s) in dogs suffering from MG dysfunction and Grade 3 (8–12 s) in healthy dogs.[12] Mengher et al. (1986) describe that a deficit of goblet cells as well as MG dysfunction lead to reduced NIBUT in humans.[23] Comparing the results of this study with these two studies the NIBUT values are more similar to those of dogs suffering from MG dysfunction.

In addition to the noninvasive measurement of the break-up time (BUT), the BUT can also be determined by fluorescein staining of the tear film (TFBUT). Published TFBUT values, except those collected by Faghihi and Rajaei, are higher than published NIBUT values.[20,21,24] However, Moore et al. (2011) found no correlation between TFBUT and goblet cell numbers or function in humans.[25] Other authors report that only MG function influences the TFBUT in dogs and humans.[21,26] Since the NIBUT values did not change before and after treatment in the present study, it is possible that these dogs did not only suffer from distichiasis, but also from MG dysfunction.

The literature describes the use of TMS measurements in healthy dogs and dogs with MG dysfunction.[12,22] In 2022, Kim et al. established reference values for dry eye tests for canine patients, measuring different ocular surface values in healthy dogs. They found a mean TMS of 0.41 ± 0.21 mm.[22] Jeong et al. reported a TMS of 0.552 ± 0.273 mm in healthy dogs and 0.574 ± 0.410 mm in dogs with MG dysfunction.[12] The values of mean TMS of healthy dogs as well as dogs suffering from MG dysfunction values are similar to the results of the present study, suggesting that the treatment of distichiasis might not have a negative impact on the TMS.

Reynolds et al. (2022) described significantly higher MG drop out in dogs undergoing distichiasis treatment (64.7%) compared with an untreated reference group (9.5%), with the OSA. They suggest that CT causes MG drop out in dogs.[15] However, in our study 41.7% of all right eyes and 27.3% of all left eyes showed grade 1 MG drop out before treatment and drop out grades did not change at any time points. The reasons for these different results can be manifold, with the small and highly variable group sizes in our study precluding the detection of real differences between time points. It is possible that the treated dogs were already suffering from MG dropout before receiving treatment for distichiasis. Additionally, the dog breed might have had an influence on the

morphology of the MGs and thus on MG dropout. In the study of Reynolds et al. (2022), 77.8% (7/9 dogs) of the dogs in the treated group for distichiasis and 58.3% of the dogs in the present study were brachycephalic breeds.[15] It has been suggested that brachycephalic breeds are predisposed to ocular surface disease,[27] but Vinas et al. (2019) have not detected a significant effect of skull shape on MG dropout.[11]

This pilot study was able to demonstrate the use of the OSA in a veterinary setting. The OSA was selected to evaluate the precorneal tear film parameters (interferometry, NIBUT, TMS, and MB) because of the convenience of carrying out multiple tests with one device. The evaluation of NIBUT was sometimes difficult due to the movement of the third eyelid. Therefore a few additional videos had to be recorded. Acquisition of MB values presented the greatest challenge. Adequate assessment requires complete eversion of the upper and lower eyelids, which is especially challenging in brachycephalic dogs due to their skull formation and the resulting exposed eyeball. Pigmentation of the palpebral conjunctiva made MB assessment impossible in some dogs. Interpretation of the results is subjective in any case, but a multitude of videos and photos make repeated analysis for one or multiple examiners possible, contributing to more objectivity. However, as the inter- and intra-observer difference was not the aim of the study, the present investigations were only analyzed by one person, but this advantage should be taken into account for future studies.

Taking a closer look at the effect size, most of the calculations showed no practical relevance between the different time points of values observed in this study. These findings show that a bigger sample size is needed to find a possible negative effect of the treatments of distichiasis on the precorneal tear film in dogs. The post hoc sample size calculations conducted in this pilot study confirm that large numbers of cases would be necessary to show small effects. In this pilot study, a 1-year period of medical records was chosen, providing the study with the small number of just 12 patients. However, one of the main reasons for this small sample size was the inclusion criteria. In ophthalmic veterinary practice, dogs suffering from distichiasis do often do not solely suffer from this condition. Dogs affected by distichiasis are often presented with other ocular conditions as well. These additional diseases, such as trichiasis, ectopic cilia, KCS, and corneal defects, could also have an impact on the precorneal tear film. For future studies a multicenter study design should therefore be considered, to recruit more and reach the desired number of patients. The small sample size is the main limitation of our study. The results of STT, interferometry, and NIBUT should therefore be interpreted with caution due to the high standard deviation observed in this study. Additionally, the fact that this study is retrospective also poses a major limitation.

In conclusion, this pilot study demonstrated that treatment of distichiasis does not seem to negatively affect the precorneal tear film parameters of dogs. However, considering the small and highly variable group size, this conclusion is not definitive. Further studies have to consider bigger sample sizes in the different groups. Therefore, it remains uncertain whether CT and LT, which strongly manipulate the eyelids, have a negative impact on the MGs and, consequently, the precorneal tear film in dogs.

## AUTHOR CONTRIBUTIONS

**V. Zwiauer-Wolfbeisser:** Conceptualization; data curation; formal analysis; writing – original draft. **A. Tichy:** Formal analysis; writing – review and editing. **B. Nell:** Conceptualization; supervision; writing – review and editing.

## CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## ETHICS STATEMENT

This study complies with the Guidelines for Ethical Research in Veterinary Ophthalmology (GERVO) and is exempt from approval by an ethics committee.

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