Check for updates

Veterinary Ophthalmology

WILEY

ORIGINAL REPORT

Long-Term Effects of Various Therapies for Pigmentary Keratitis in Pugs

Ingrid Allgoewer

Animal Eye Practice, Berlin, Germany

Correspondence: Ingrid Allgoewer (ia@tieraugen.com)

Received: 24 November 2025 | **Revised:** 17 January 2026 | **Accepted:** 17 February 2026

Keywords: brachcephalic dogs | cornea | medial canthoplasty | medial entropion | pigment | tear film deficiency

ABSTRACT

Objective: To compare the long-term efficacy of simplified medial canthoplasty (MCP) with and without adjunctive corneal cryotherapy (CC), followed by topical therapy (TT), versus TT alone in the management of pigmentary keratitis (PK) in Pugs.

Methods: Medical records of Pugs diagnosed with PK were retrospectively reviewed and assigned to four treatment groups. Only dogs with a minimum follow-up period of 1.5 years were included. Group 1 received TT alone; Group 2 underwent MCP with TT. Group 3 was treated with MCP, bilateral CC, and TT, while Group 4 received MCP, TT, and randomized unilateral CC. Outcome measures included ocular discharge, Schirmer tear test-1 (STT-1), fluorescein staining (FS), and corneal pigmentation (density and distribution), assessed at baseline and at final follow-up.

Results: Seventy-six Pugs met the inclusion criteria. Tear film parameters (STT-1, FS, and ocular discharge) improved in all dogs following therapy. In Groups 1 and 2, regression of the PK occurred in approximately 25% of dogs. In Group 2, complete resolution of the PK was observed in two dogs. In Group 3, corneal pigmentation decreased or remained stable in most cases, and in Group 4, PK consistently regressed in CC-treated eyes.

Conclusions: Simplified MCP in combination with TT provides superior long-term control of PK compared with TT alone. In dogs presenting with advanced pigmentation, adjunctive CC offers additional therapeutic benefit, although partial recurrence of pigmentation may develop over time.

1 | Introduction

Brachycephalic ocular syndrome is characterized by a distinct combination of anatomical and physiological features of the globe and periocular structures [1]. Multiple predisposing factors and associated ocular surface disease (OSD) in brachycephalic breeds have been extensively reviewed [2]. Among these, pigmentary keratitis (PK) is of particular relevance, as it develops in Pugs at an early age and has been reported with a prevalence of up to 96% [3–14].

Keratoconjunctivitis sicca (KCS), corneal pigmentation, and corneal ulceration are interrelated sequelae of chronic ocular surface irritation and inflammation that disrupt corneal and tear film integrity [13]. In Pugs, corneal ulceration is promoted by brachycephalic conformation, macroblepharon, exophthalmos,

© 2026 American College of Veterinary Ophthalmologists.

lagophthalmos, reduced corneal sensitivity and blink rate, medial entropion–associated trichiasis, distichiasis, ectopic cilia, and tear film deficiencies [2, 11, 12, 15]. Corneal ulceration is central to the pathogenesis of pigmentary keratitis (PK) [5, 9].

Brachycephalic breeds have an increased risk of corneal ulceration, including deep stromal involvement [15–17]. In Pugs, lagophthalmos, ocular surface exposure, and medial entropion are the principal pathogenic factors for PK, while a genetic predisposition remains unconfirmed [3, 18]. Figures 1 and 2 depict PK, medial entropion, and tear film deficiency.

Despite the high prevalence of PK in Pugs, only limited peer-reviewed data on therapeutic interventions are available. Reported management strategies include topical treatment of tear film deficiencies, surgical correction of eyelid malformations, and cryotherapy [3, 11, 19–21]. To date, however, only preliminary treatment outcomes have been reported [22].

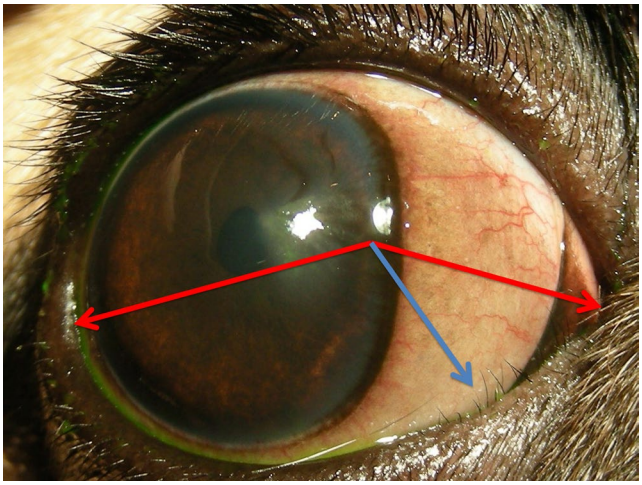

FIGURE 1 | Pug with pigmentary keratitis (PK) and epithelial ulceration, illustrating typical mechanical factors contributing to corneal irritation: exophthalmos, divergent strabismus due to a shallow orbit, macroblepharon (red arrows), and medial entropion (blue arrow). The exposed conjunctiva is pigmented. The upper eyelid is slightly elevated, revealing a part of the unpigmented conjunctiva that is otherwise covered by the eyelid.

This study aimed to retrospectively evaluate and compare the long-term effects of different treatment methods for PK in Pugs: topical therapy alone versus a combination of modified medial canthoplasty (MCP) with or without cryotherapy, as well as to determine the impact of various physical and physiologic factors on disease severity and response to treatment. Therefore, the working hypothesis of this study was that PK in Pugs is a chronic inflammatory disease resulting from multifactorial chronic irritation.

2 | Animals Studied and Methods

A retrospective analysis was conducted. The Pugs included in this study were presented by their owners to the Animal Eye Practice in Berlin, Germany, for ophthalmic examination. After diagnosing PK, treatment consisting of TT and MCP was recommended. In dogs with advanced PK, additional CC was offered. Dog owners subsequently made the final decision regarding therapy. Depending on their preference, owners would choose TT alone, TT combined with MCP, or, in cases of advanced corneal pigmentation, additional uni- or bilateral CC. These choices were made individually by the owners and were independent of the study. Inclusion criteria were as follows: (1) clinical signs of pigmentary keratitis with or without tear film disorder; (2) consistent adherence to prescribed treatment and scheduled follow-up appointments; and (3) a follow-up period of more than one and a half years after initiation of therapy. Exclusion criteria were: the presence of additional corneal or intraocular diseases diagnosed at the initial examination and/or the occurrence of severe intraocular disease during the observation period.

Owner consent was obtained prior to examination and treatment. All examinations were performed in accordance with the GERVO Declaration on the Use of Animals in Eye and Vision Research and were approved by the Berlin State Office for Health and Social Affairs (approval no. StN. 22/25). Owners also provided consent for the use of their dogs' data and photographs for research and publication purposes.

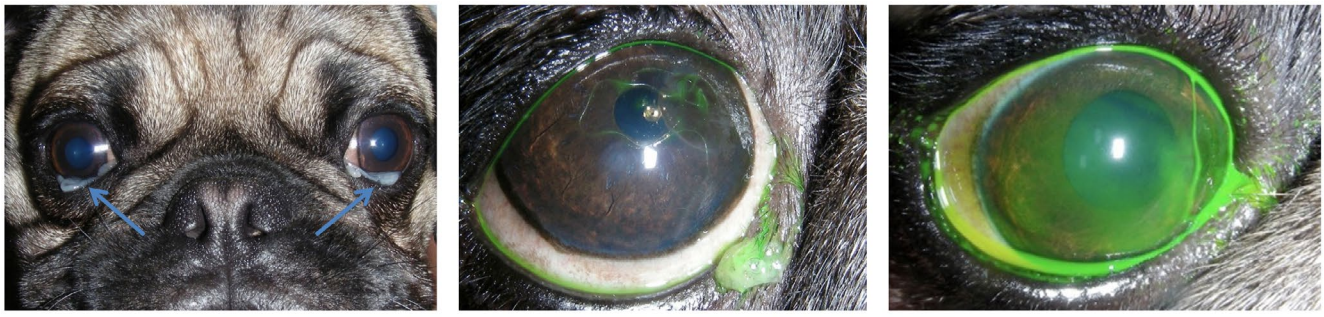

FIGURE 2 | Typical clinical presentations of qualitative tear film deficiency in Pugs. (a) Pug showing accumulations of thick, grayish mucus at the medial canthus and along the medial lower eyelid (blue arrows). (b) The right eye of a Pug with pigmentary keratitis (PK), exhibiting macroblepharon and medial entropion of both the upper and lower eyelids, along with characteristic grayish mucus on the medial lower eyelid. Macroblepharon and lagophthalmos are evidenced by the presence of hairs and debris on the cornea within the area of corneal pigmentation, indicating that the eyelids are unable to remove them effectively. (c) Right eye of a Pug with PK, macroblepharon, and medial entropion, showing diffuse fluorescein uptake across the entire cornea and a strand of mucus extending over the corneal surface, consistent with qualitative tear film deficiency.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

All dogs underwent a comprehensive ophthalmic examination performed by either a board-certified veterinary ophthalmologist or a veterinarian holding national specialization in ophthalmology. Examinations included menace response, dazzle reflex, pupillary light reflex, eyelid reflex, slit-lamp biomicroscopy (Kowa SL-17; Kowa, Tokyo, Japan), rebound tonometry (TonoVet, iCare, Vantaa, Finland), indirect ophthalmoscopy (Video Omega 2C; Optotechnik GmbH & Co. KG, Gilching, Germany), Schirmer tear test I (STT-I; Tear Touch Blu, Madhu Instruments Pvt. Ltd.) and fluorescein staining (Fluoro-Touch, Madhu Instruments Pvt. Ltd., India). Several photographs were taken during each examination, including portraits and individual eye images.

The treatment protocol for PK in Pugs included surgical correction of macroblepharon and medial entropion using modified medial canthoplasty (MCP) [23]. The surgery aimed to cover 90% of scleral show and eliminate the entropion. In cases where corneal cryotherapy was performed, it was done immediately after MCP. For cryotherapy, an eyelid speculum was placed to ensure wide exposure of the cornea. The procedure was carried out as described [24], using the Askina Skin Freeze kit (B Braun Medical SAS), originally developed for human dermatology. The kit contains an aerosol canister with liquid cryogen (95% dimethyl ether, 3% isobutane, and 2% propane) and disposable plastic foam applicators. The liquid cryogen was applied to the foam tip, which was then allowed to evaporate for 15 s, reducing the applicator temperature to −55°C before application to the cornea. Depending on the extent and density of corneal pigmentation, two to four applications of 50 s each (two to four freeze–thaw cycles) were performed by gently rolling the foam tip across the cornea. Once the cornea was frozen and turned white, the applicator was removed. Following cryotherapy, a simple temporary tarsorrhaphy was performed using polyglactin sutures (Vicryl 6–0, Ethicon, Johnson & Johnson Surgical Technologies). In dogs that underwent MCP, topical therapy was initiated 8–10 days postoperatively after suture removal. In dogs that received combined MCP and corneal cryotherapy, tarsorrhaphy sutures were removed 2–3 days postoperatively, followed by fluorescein testing. Topical therapy with dexamethasone-containing eye drops was initiated once the cornea tested negative for fluorescein.

Topical therapy (TT) included ciclosporin (CsA) (0.2% compounded CsA eye drops or Optimmune, Intervet Deutschland GmbH, Germany), tacrolimus (0.2% compounded eye drops or Protopic 0.1%, Astellas Pharma, Germany), dexamethasone (Dexapos Comod, Ursapharm Arzneimittel GmbH, Germany, or Dexagel, Dr. Mann Pharma, Germany), or a combination of dexamethasone and antibiotics (dexamethasone/neomycin/polymyxin B eye drops, Maxitrol, Novartis, Germany). Frequency of administration was two to three times daily, depending on the severity of ocular surface disease (OSD) and aqueous-deficient or evaporative dry eye disease (ADED, EDED). Dogs that underwent corneal cryotherapy received dexamethasone-containing eye drops three times daily for an extended period until corneal vascularization had regressed.

The study population was divided into four groups:

1. Pugs treated with topical therapy only (TT).
2. Pugs treated with MCP followed by TT.
3. Pugs with bilateral severe PK and extensive corneal pigmentation treated with MCP and bilateral corneal cryotherapy, followed by bilateral TT.
4. Pugs with extensive corneal pigmentation treated with bilateral MCP in combination with unilateral corneal cryotherapy, followed by bilateral TT.

Follow-up examinations were performed at least 6 weeks and 3 months after initiation of TT, and subsequently every 6 months. Parameters evaluated for statistical analysis included head and eyelid conformation, corneal pigment density and distribution, corneal fibrosis, ocular discharge, STT-I, and fluorescein staining.

At the initial examination, head and eyelid conformation was graded on a scale of 1–3, based on comparison of the patient's eye position and eyelid shape with reference photographs (Figure 3a). The presence of nasal entropion and corneal fibrosis was also recorded (Figure 3b,c).

Pigment extension was graded on a scale of 1–6 (Figure 4a,b). Pigment density was graded 1–3 (Figure 4c):

- Grade 1: pigment density allowed full visualization of the iris.
- Grade 2: pigment partially obscured iris inspection.
- Grade 3: pigment completely obscured the iris.

For the evaluation of the severity of the tear film deficiency (ADED/EDED), the Schirmer tear test, Fluorescein stain grading, and mucous discharge grading were used.

The characteristic slimy, translucent, gray-white, elastic ocular discharge was graded 1–3 (Figure 5a):

- Grade 1: small amount of discharge present at the medial canthus or lower eyelid.
- Grade 2: discharge spread across the cornea during blinking, leaving strands on the surface.
- Grade 3: abundant discharge covering and adhering to the cornea.

Schirmer tear test I (STT-1) results were graded 1–4:

- Grade 1: > 20 mm/min.
- Grade 2: > 15 mm/min.
- Grade 3: 5–15 mm/min.
- Grade 4: 0–5 mm/min.

Fluorescein staining was graded 1–4 (Figure 5b):

- Grade 1: Fluorescein-negative.
- Grade 2: Focal weak epithelial uptake.
- Grade 3: Diffuse epithelial uptake.
- Grade 4: Fluorescein-positive defect.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

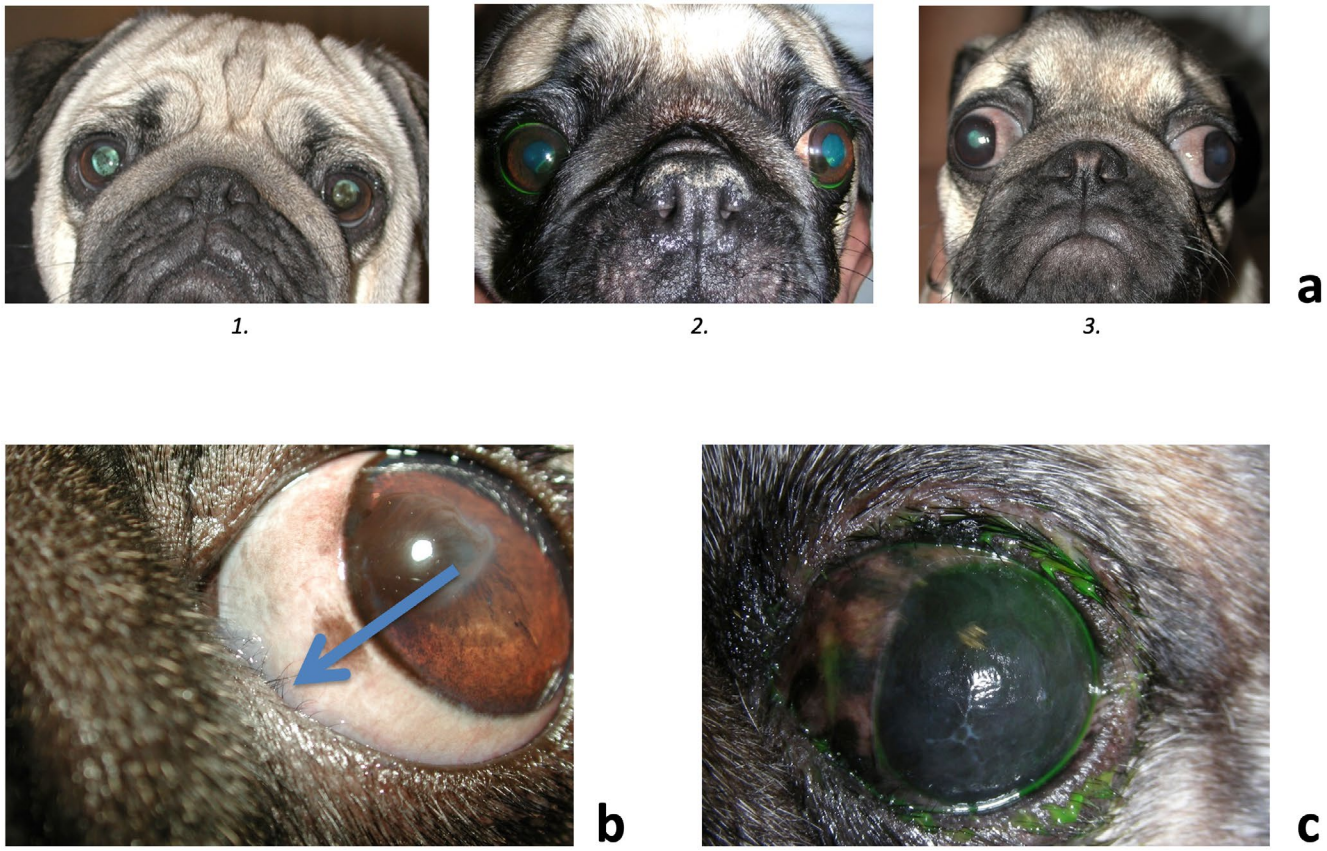

FIGURE 3 | (a) Three types of head and lid conformation were used for comparison. All Pugs were graded 1–3 by comparing their appearance with the photos of the Pugs displayed. (b) Medial entropion and trichiasis resulting in conjunctival pigmentation and PK. (c) PK with corneal fibrosis.

2.1 | Statistical Analysis

The software packages R (R Core Team, 2018) and RStudio (version 2025.5.1.513; RStudio Team, 2016) were used to perform all statistical analyses and graphical visualizations. A significance level of < 0.05 was set for all statistical analyses. To describe the dataset, demographic variables and target parameters were evaluated descriptively. To summarize both eyes, an index was calculated from the arithmetic mean for each characteristic.

Metric variables were described using the mean, minimum, maximum, and standard deviation (SD). Cross-tables with percentages and absolute values were used for ordinally scaled variables. For metric variables, multiple testing was conducted by ANOVA and a Tukey test as implemented in RStudio. For ordinally scaled variables, multiple testing was conducted by a Kruskal–Wallis test, Jonckheere–Terpstra test, or a pairwise Wilcoxon rank-sum test (p-value adjustment method: Holm) as implemented in RStudio. To assess the relationship between the parameters, correlations were tested using correlation tests as implemented in RStudio. Given that both variables were measured on a metric scale and met the assumptions of normality, Pearson's correlation coefficient was applied. Normal distribution was checked using the Shapiro–Wilk test. Alternatively (for non-normally distributed or ordinally scaled data), the Kendall–Tau test was used as a non-parametric method for ordinal data that accounts for tied ranks to evaluate whether the distributions of the two groups differ significantly. Analyses were conducted by Novustat GmbH, Wollerau, Switzerland.

3 | Results

Seventy-six Pugs met the inclusion criteria, including 41 females (22 intact, 19 spayed) and 35 males (26 intact, 9 neutered). The mean age (years) at the start of treatment was: Group 1, 4.4; Group 2, 2.6; Group 3, 4.5; and Group 4, 4.4. The mean follow-up duration (years) was: Group 1, 2.3; Group 2, 3.0; Group 3, 1.9; and Group 4, 3. All groups were comparable regarding age and follow-up time.

3.1 | Evaluation of Parameters Before Treatment

No significant differences were observed between groups in eyelid position, eyelid closure, exophthalmos, corneal fibrosis, tear film parameters, or demographic characteristics such as gender, age, and follow-up duration (Kruskal–Wallis and Jonckheere–Terpstra tests), indicating a homogeneous distribution of participants.

All eyes showed dense limbal pigmentation, with nasal accentuation. Before treatment, both pigment extension and density correlated positively with the grading of discharge (r 0.21, p 0.03; r 0.18, p 0.04). Older dogs exhibited more severe PK, with age positively correlated with pigment extension (r 0.26, p 0.002) and pigment density (r 0.29, p 0.001; Group 2: r 0.38, p 0.03) (Figures 6A, 7A, and 8A). In addition, pigment

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

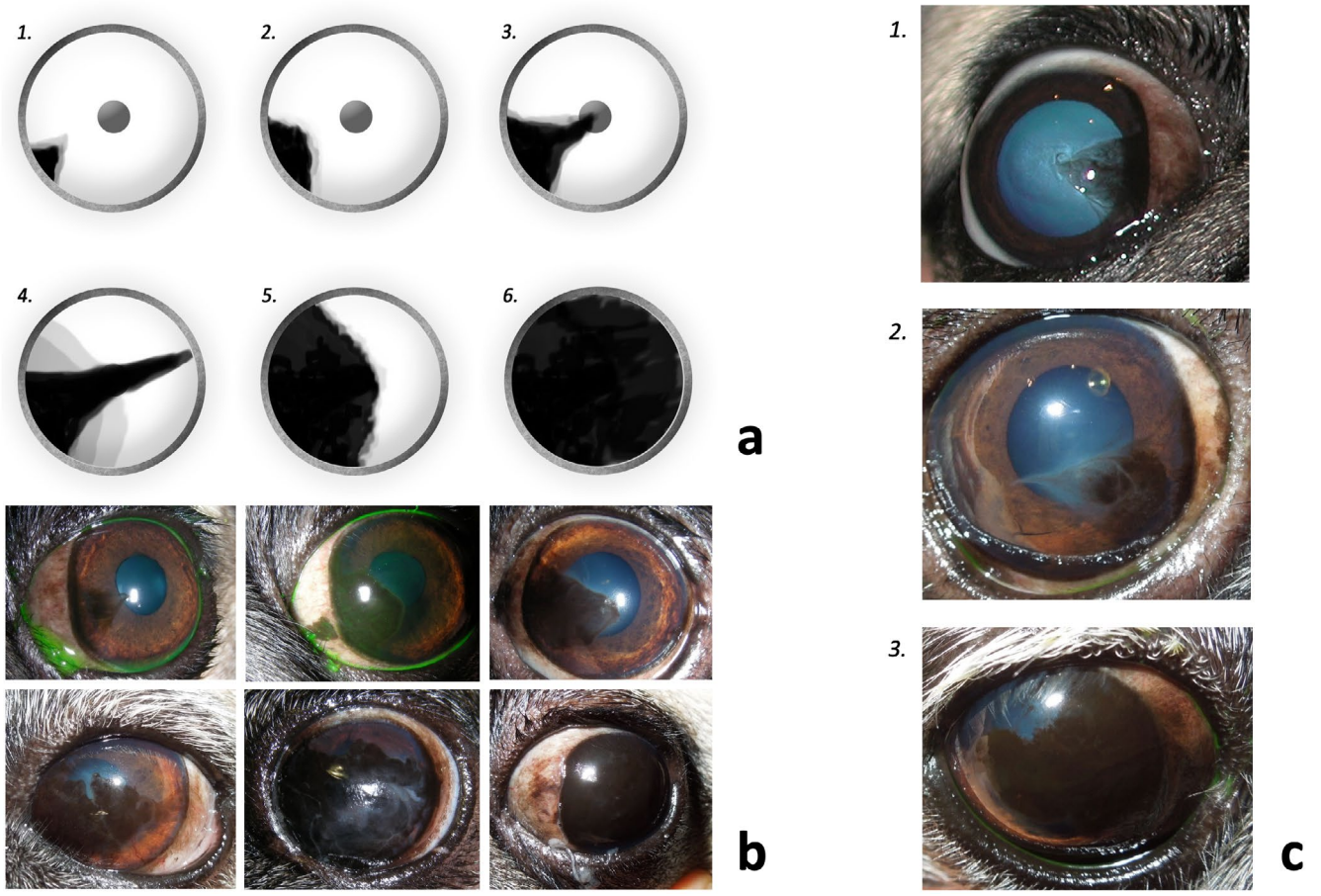

FIGURE 4 | Corneal pigmentation in Pugs: (a) schematic of pigment extension Grades 1–6. Grade 1: Focal triangular pigmented area (< 3 mm) nasally adjacent to the limbus. Grade 2: Focal triangular pigmented area (≥ 3 mm) nasally adjacent to the limbus. Grade 3: Pigmented area extending from the nasal limbus to the center of the cornea. Grade 4: Pigmented area extending from the nasal limbus across the cornea. Grade 5: Pigmented area extending nasally over more than half of the corneal surface. Grade 6: Diffuse pigmentation involving the entire corneal surface. (b) Photographic examples of pigment extension. (c) Pigment density Grades 1–3.

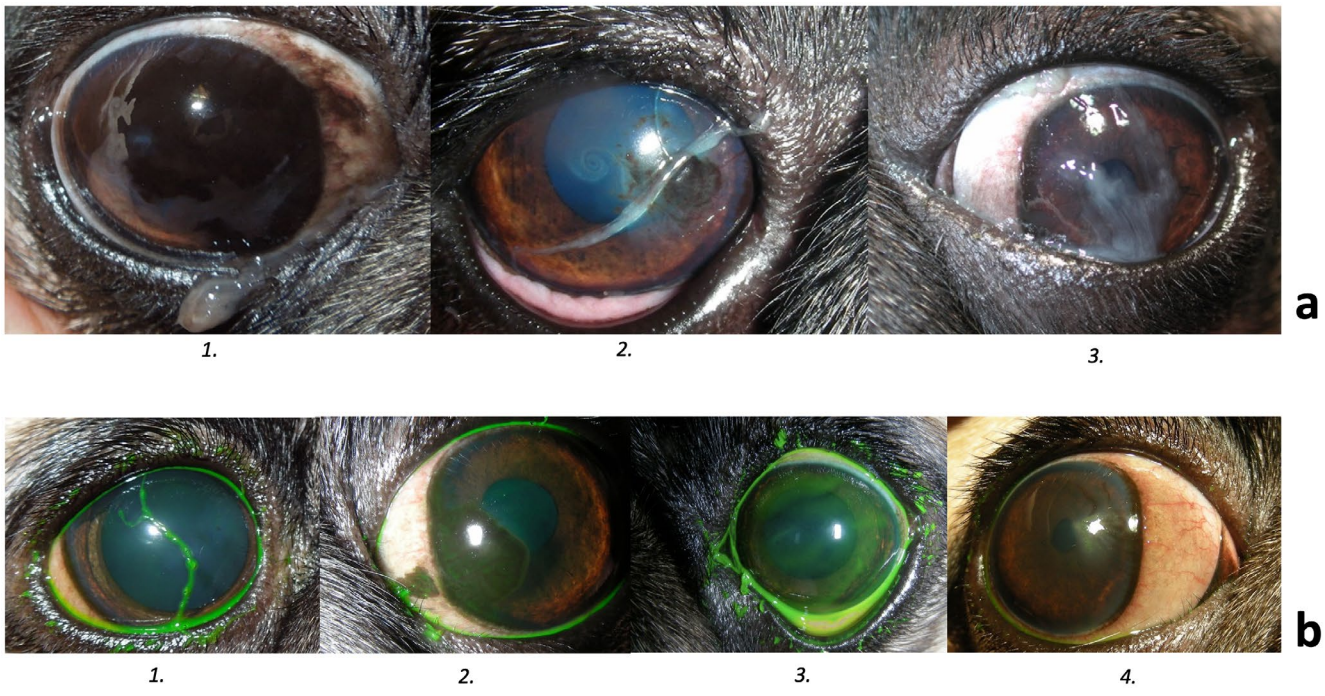

FIGURE 5 | Ocular signs in Pugs. (a) Ocular discharge Grades 1–3. (b) Fluorescein test Grades 1–4.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

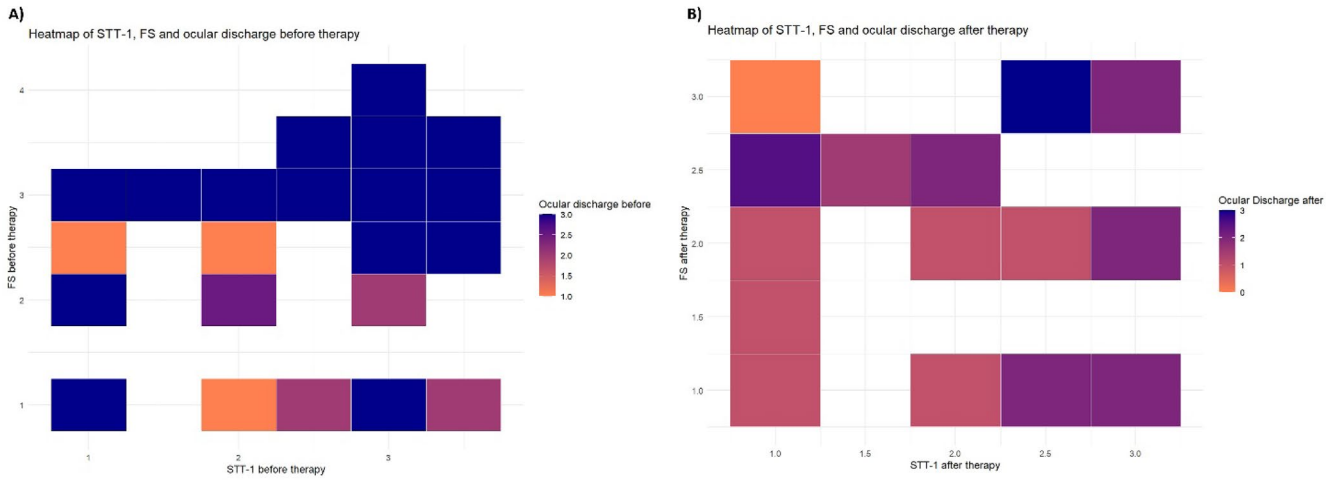

FIGURE 6 | Heat maps of STT-1, FS, and ocular discharge before treatment (A) and following treatment (B) show a generalized improvement in tear-film quality at the conclusion of the observation period. Grading of STT-1: Grade 1: > 20 mm/min; Grade 2: > 15 mm/min; Grade 3: 5–15 mm/min; Grade 4: 0–5 mm/min. Grading of FS: Grade 1: fluorescein-negative; Grade 2: focal weak epithelial uptake; Grade 3: diffuse epithelial uptake; Grade 4: fluorescein-positive defect. Grading of ocular discharge: Grade 1: small amount of discharge present at the medial canthus or lower eyelid; Grade 2: discharge spread across the cornea during blinking, leaving strands on the surface; Grade 3: abundant discharge covering and adhering to the cornea.

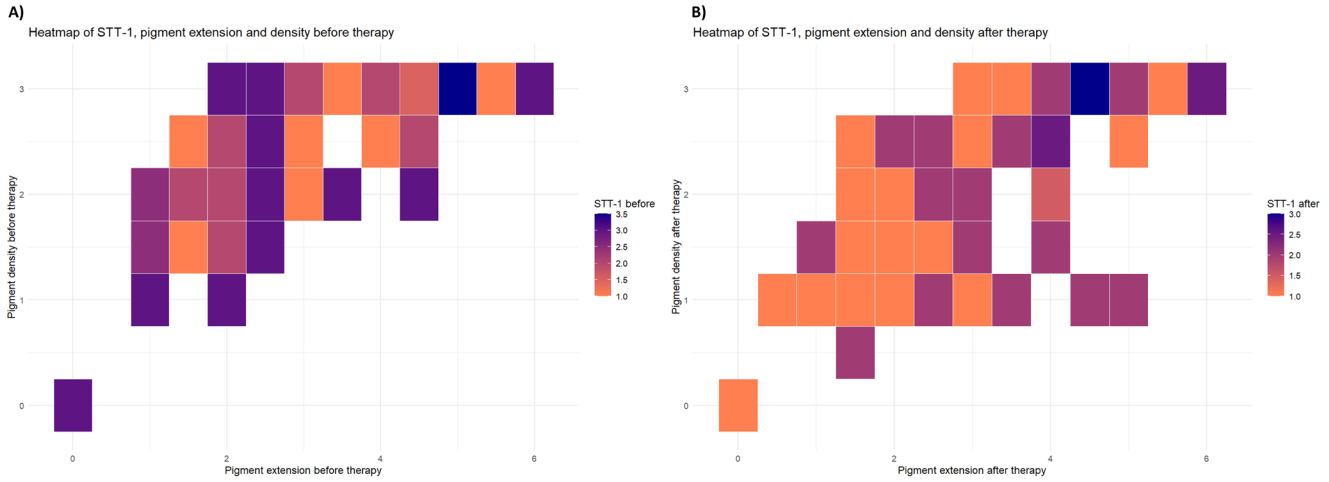

FIGURE 7 | Comparative heat maps of pigment extension, pigment density, and STT-1 acquired before (A) and after treatment (B) reveal a clinically meaningful improvement in ocular surface parameters by the end of the observation period. Grading of pigment extension: Grade 1: Focal triangular pigmented area (< 3 mm) nasally adjacent to the limbus; Grade 2: Focal triangular pigmented area (≥ 3 mm) nasally adjacent to the limbus; Grade 3: Pigmented area extending from the nasal limbus to the center of the cornea; Grade 4: Pigmented area extending from the nasal limbus across the cornea; Grade 5: Pigmented area extending nasally over more than half of the corneal surface; Grade 6: Diffuse pigmentation involving the entire corneal surface. Grading of pigment density: Grade 1: Pigment density allowed full visualization of the iris; Grade 2: Pigment partially obscured iris inspection; Grade 3: Pigment completely obscured the iris.

density was positively associated with entropion across all dogs (r 0.21, p 0.046). These findings suggest that age is linked to increased pigmentation and that pigment density may be influenced by entropion.

3.2 | Comparison of Parameters Before and After Therapy

The groups were compared with respect to pigment extension, pigment density, and ocular discharge, indexing both eyes based on the arithmetic mean. Pairwise comparisons were performed using the Wilcoxon Rank Sum Test with Holm adjustment. Pigment density showed significant improvement from pre-treatment to final status ($p < 0.001$), and all tear film parameters demonstrated significant post-treatment changes (STT-1, p 8×10^{-8}; FS, p 1×10^{-6}; discharge, p 5×10^{-10}) (Figures 6–11).

Clinical outcomes varied by treatment group. Group 2 (MCP + TT) outperformed Group 1 (TT only), showing a significant reduction in ocular discharge (p 0.008), indicating that MCP enhances the efficacy of TT. Group 3 (MCP + CC + TT) achieved the most pronounced improvements, exceeding the results of Groups 1 and 2 ($p < 0.001$ and p 0.003, respectively). In Group 4, eyes treated with cryotherapy showed superior

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

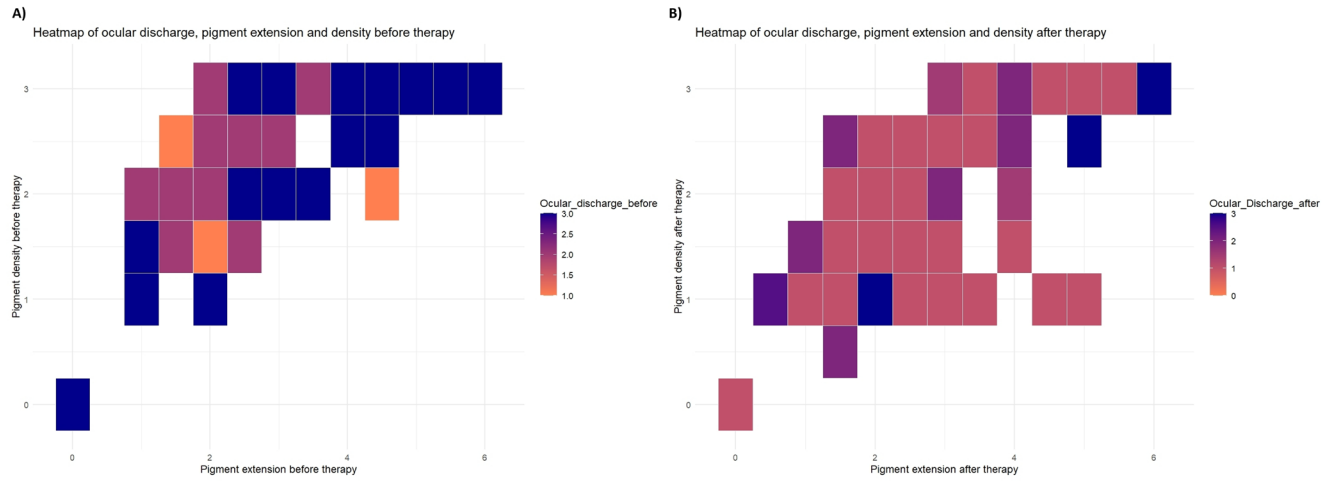

FIGURE 8 | Heat maps illustrating pigment extension, pigment density, and ocular discharge before treatment (A) and after treatment (B) show substantial improvement by the conclusion of the observation period.

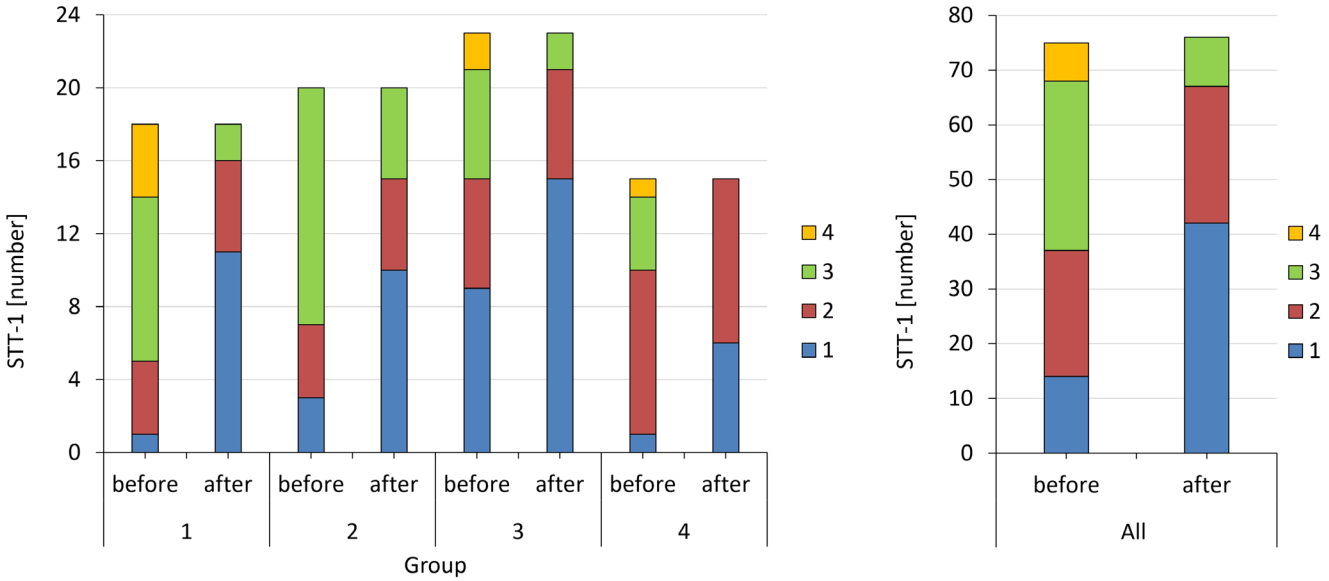

FIGURE 9 | Stacked bar charts for SST-1 in all groups before and after therapy illustrate the changes in SST-1 over time. Grading of STT-1: Grade 1: > 20 mm/min; Grade 2: > 15 mm/min; Grade 3: 5–15 mm/min; Grade 4: 0–5 mm/min.

outcomes, with a significant reduction in pigment density ($p < 0.001$, signed-rank test).

Age influenced post-treatment pigment extension across all groups, with older dogs exhibiting greater increases (r 0.27, p 0.002). Within-group analysis revealed that pigment extension changed significantly only in Group 1 (p 0.014), while Groups 2 and 3 showed no significant changes (p ns) (Figure 12). Pigment density improved clinically in Group 2 without reaching statistical significance, whereas Group 1 remained unchanged or increased (p ns) (Figure 13). In contrast, Group 3 demonstrated a significant decrease in pigment density post-treatment ($p < 0.001$), highlighting the additive benefit of CC in combination therapy. Notably, PK was fully reversed in two dogs from Group 2, underscoring the potential for substantial clinical improvement with MCP-based interventions.

Structural factors, including head, exophthalmos, and corneal fibrosis, did not influence outcomes (Jonckheere–Terpstra test; Figure 14). Representative cases from Groups 1–3 are presented in Figures 15–20. The photos serve to illustrate the progression of representative cases in each group.

Group 4 ($n = 15$) included Pugs with extensive, dense corneal pigmentation at initial presentation, treated with bilateral MCP and simultaneous unilateral cryotherapy. The eye selected for cryotherapy was randomized. Clinically, outcomes in the cryotherapy-treated eyes were superior, though the difference was not statistically significant (signed-rank test; Figures 21 and 22). Figures 23 and 24 show representative cases from Group 4.

4 | Discussion

Our hypothesis that pigmentary keratitis (PK) in Pugs represents a multifactorial chronic inflammatory disease was supported by the findings of this study. The elimination or reduction

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

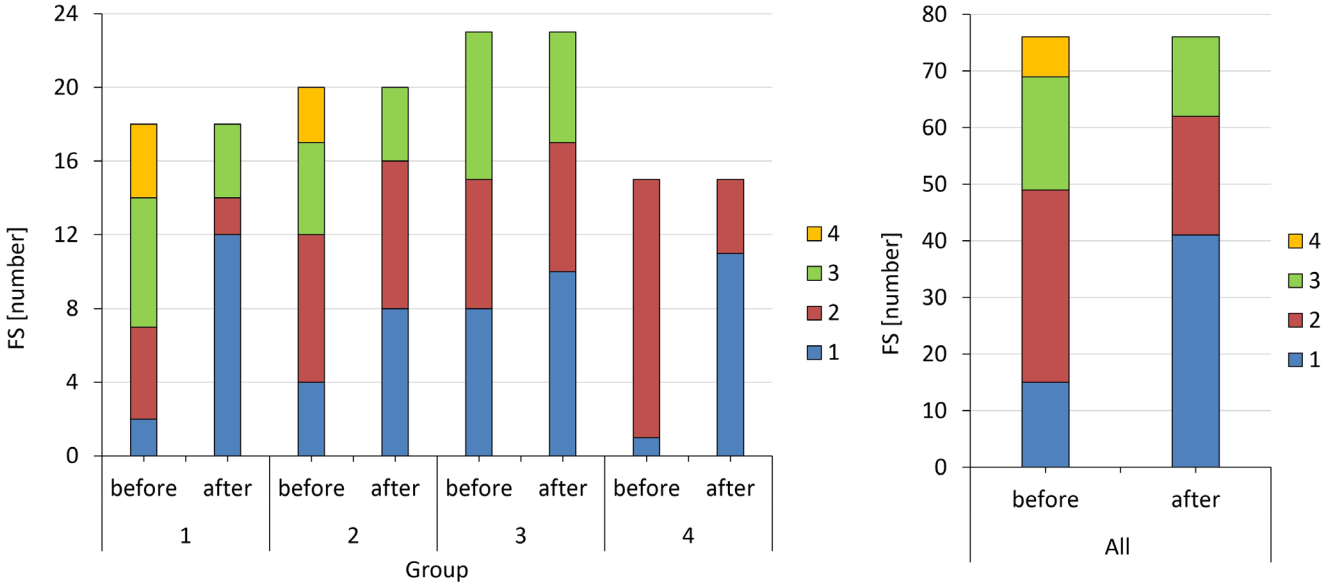

FIGURE 10 | Stacked bar charts depicting FS levels across all study groups before and after therapy. The visualization highlights group-specific changes and overall trends in FS following treatment. Grading of FS: Grade 1: Fluorescein-negative; Grade 2: Focal weak epithelial uptake; Grade 3: Diffuse epithelial uptake; Grade 4: Fluorescein-positive defect.

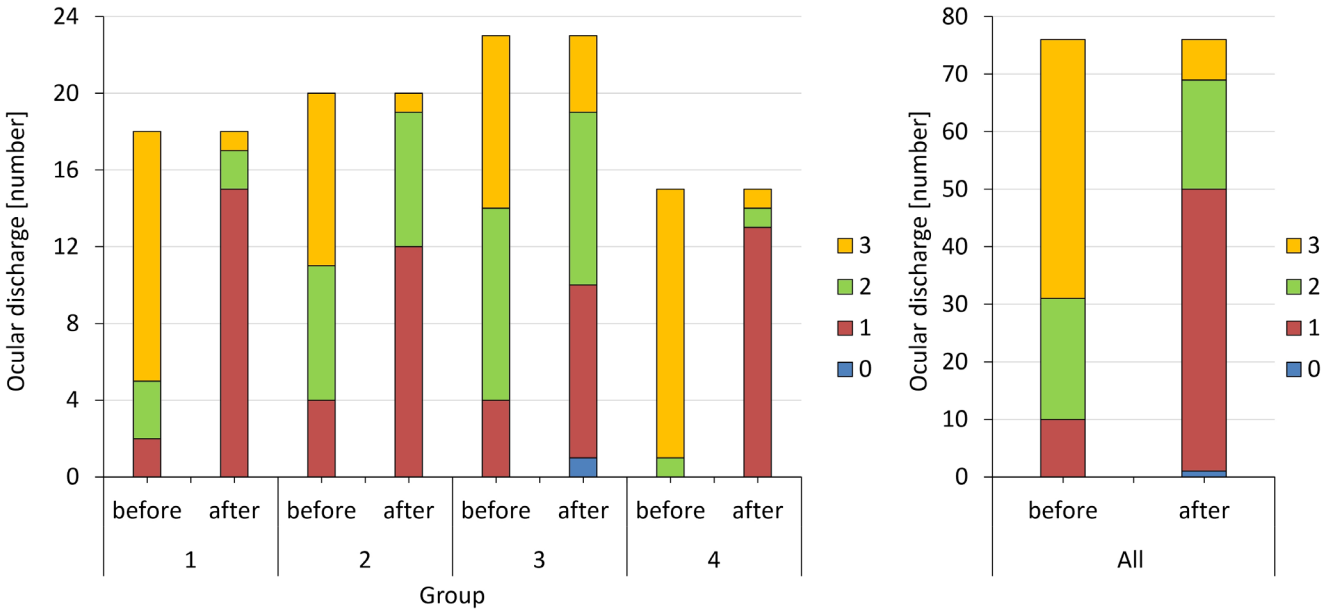

FIGURE 11 | Stacked bar charts illustrating the distribution of ocular discharge severity across all study groups before and after therapy. Grading of ocular discharge: Grade 1: Small amount of discharge present at the medial canthus or lower eyelid; Grade 2: Discharge spread across the cornea during blinking, leaving strands on the surface; Grade 3: Abundant discharge covering and adhering to the cornea.

of irritants delayed disease progression and led to a decrease in corneal pigment deposition and clinical signs of inflammation in the majority of treated Pugs during this long-term observational period. Notably, in two dogs, PK was entirely reversed by the therapy (MCP + TT).

With respect to therapeutic strategies, the diverse etiopathogenic factors associated with PK in Pugs [25] can be classified into the following categories:

1. Conformational and breed-related anatomical factors: ocular exposure resulting from brachycephalic exophthalmos, macroblepharon or macropalpebral fissure, and lagophthalmos [1, 2], as well as trichiasis due to medial entropion, distichiasis, or ectopic cilia [1, 2, 5, 9, 11, 16].

2. Qualitative tear film deficiency/evaporative dry eye disease (EDED) Tear film abnormalities have been reported in several brachycephalic breeds [26–33]. These include reduced tear film stability compared with non-brachycephalic dogs [34], often attributable to insufficient preocular mucins and/or lipids [35]. The tear film break-up time (TBUT) in brachycephalic breeds is approximately 18% shorter than

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer - Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

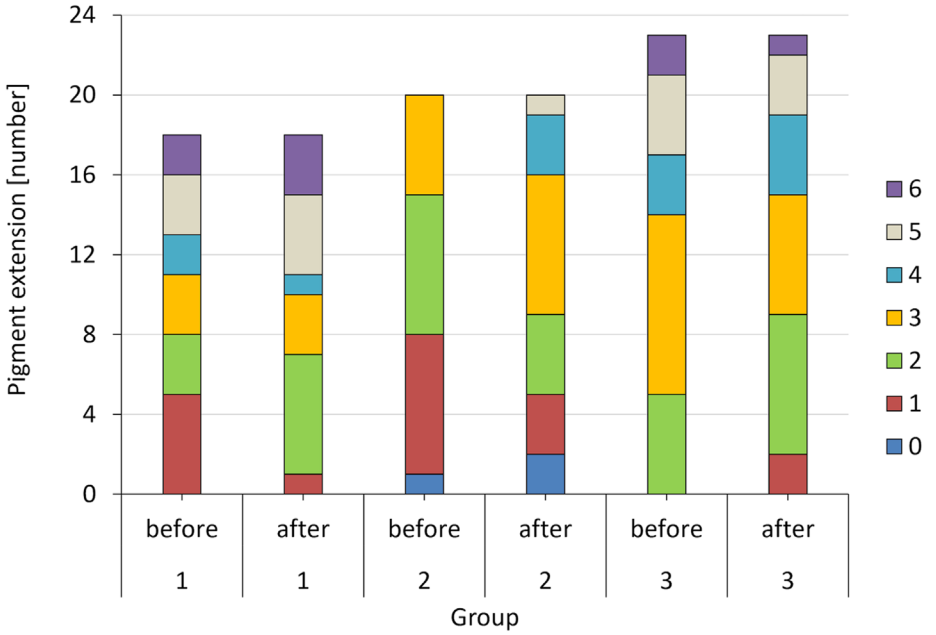

FIGURE 12 | Stacked bar charts comparing pigment extension across all participant groups pre- and post-therapy. Grading of pigment extension: Grade 1: Focal triangular pigmented area (< 3 mm) nasally adjacent to the limbus; Grade 2: Focal triangular pigmented area (≥ 3 mm) nasally adjacent to the limbus; Grade 3: Pigmented area extending from the nasal limbus to the center of the cornea; Grade 4: Pigmented area extending from the nasal limbus across the cornea; Grade 5: Pigmented area extending nasally over more than half of the corneal surface; Grade 6: Diffuse pigmentation involving the entire corneal surface.

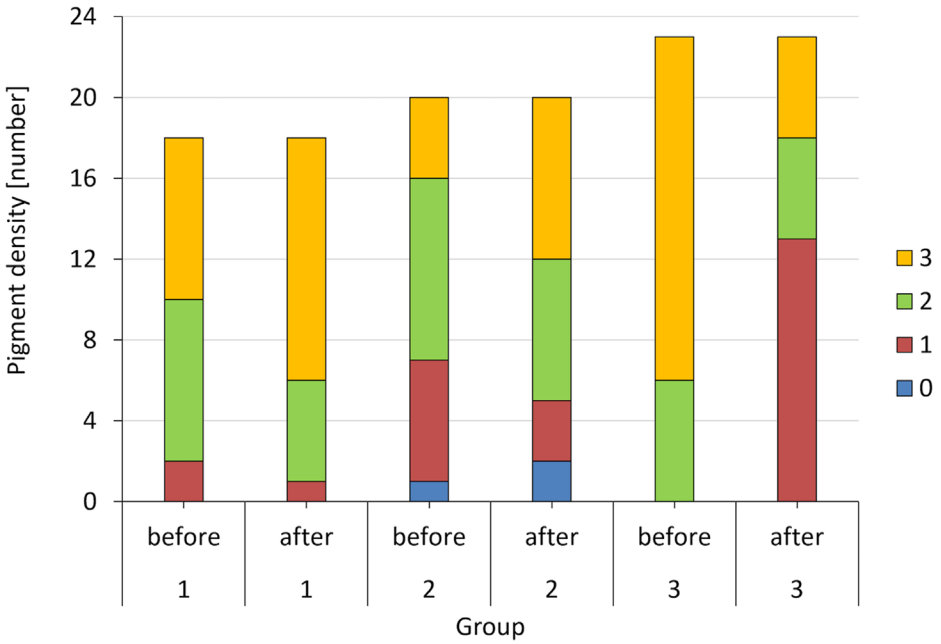

FIGURE 13 | Stacked bar charts depicting the distribution of pigment density in each group before and after therapy. Grading of pigment density: Grade 1: Pigment density allowed full visualization of the iris; Grade 2: Pigment partially obscured iris inspection; Grade 3: Pigment completely obscured the iris.

in non-brachycephalic breeds, and the lipid layer is significantly thinner [2, 26, 34, 36–39]. Importantly, there is no evidence of aqueous tear deficiency (ADED) [2, 8, 26], supporting the concept of a primarily qualitative tear film disorder consistent with EDED [26, 37–40]. In addition, Meibomian gland dysfunction (MGD) has been identified as a frequent underlying component of ocular surface disease in brachycephalic dogs [32]

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

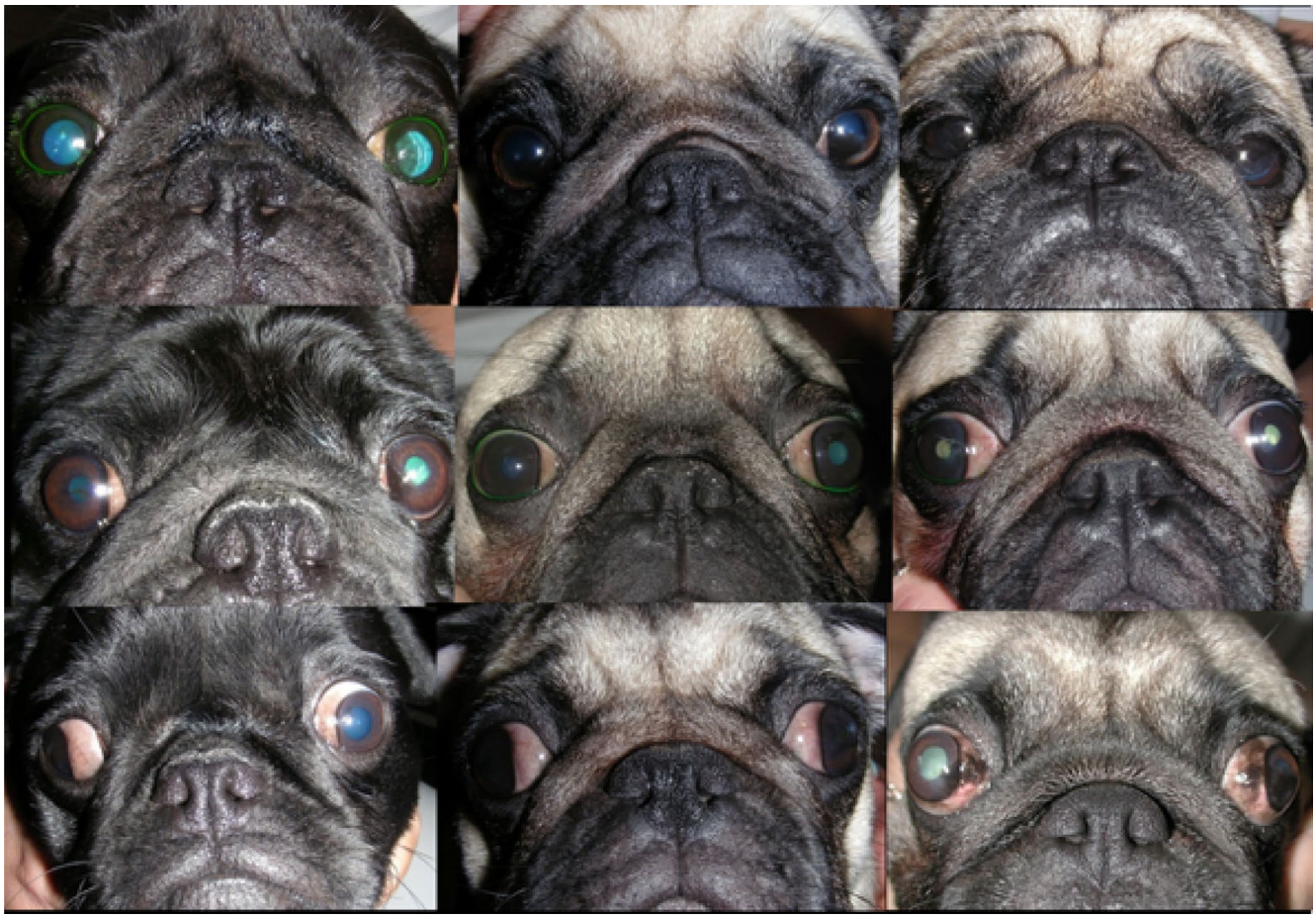

FIGURE 14 | Pugs with varying head and eyelid conformations and different PK severity. Head and eyelid conformation did not affect the severity of corneal pigmentation in this study.

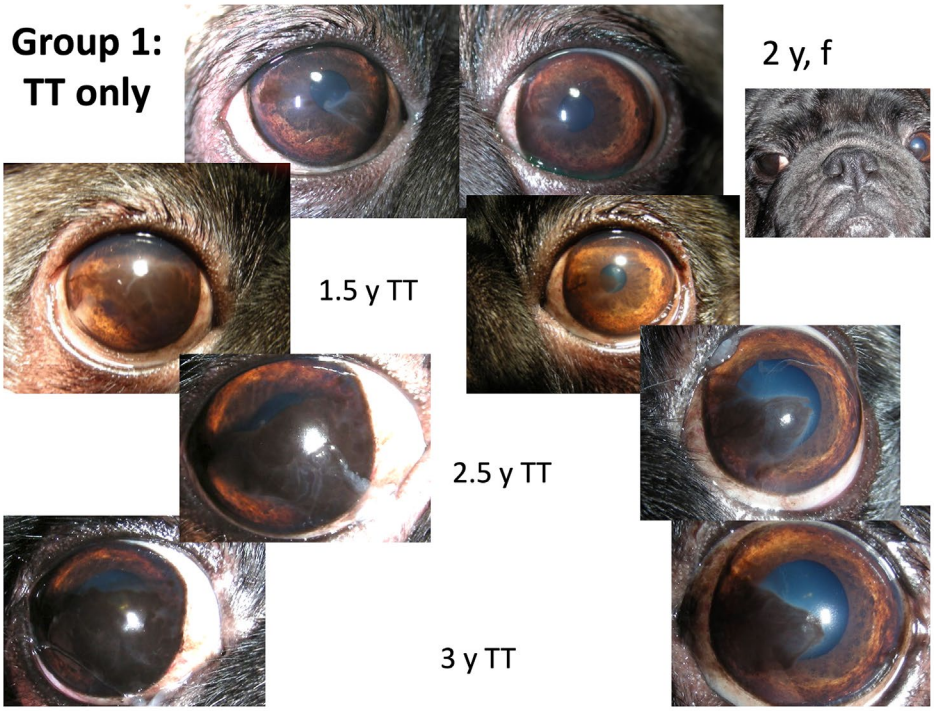

FIGURE 15 | Female Pug, 2 years old, Group 1 (TT only). Despite topical therapy, pigmentation progressed bilaterally over 3 years.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

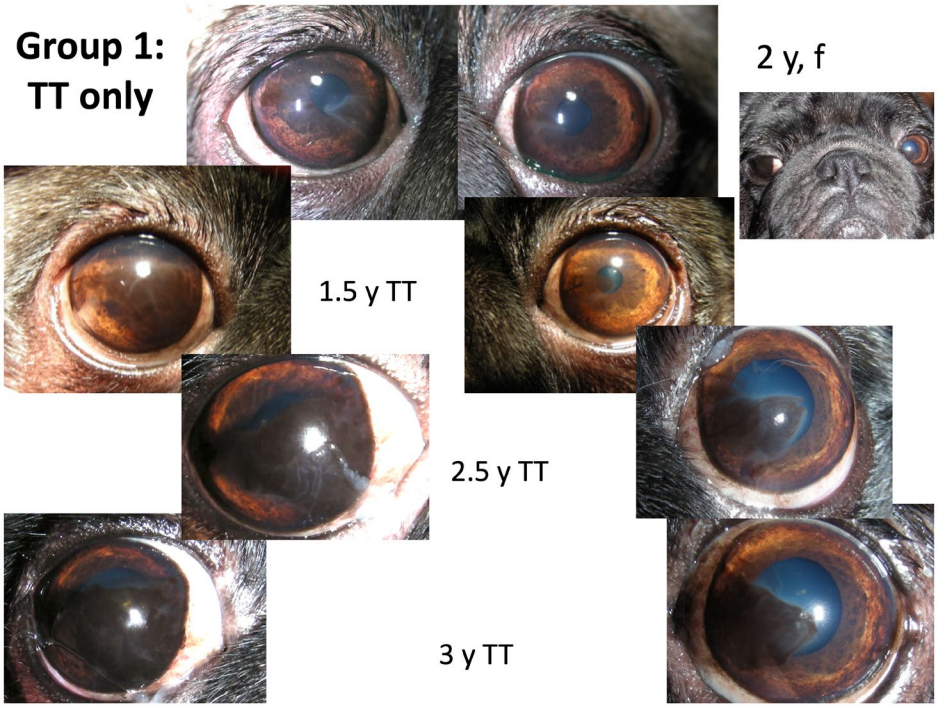

FIGURE 16 | Female Pug, 6 months old, Group 2 (MCP + TT). Complete regression of corneal pigmentation over 2.5 years.

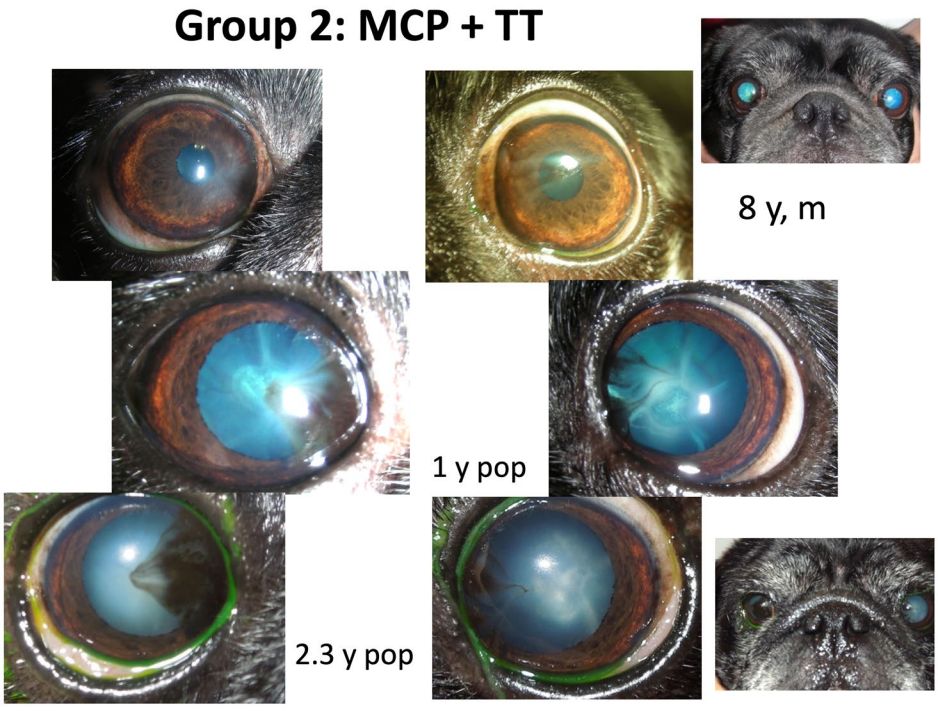

FIGURE 17 | Male Pug, 8 years old, Group 2 (MCP + TT). Right eye: Pigmentation progressed over 2.3 years despite treatment.

4.1 | Influence of Tear-Film on PK

Although the tear film in Pugs has not yet been studied comprehensively, several investigations have demonstrated altered tear quality in Pugs affected by PK [3, 5, 9, 12, 13], including a high prevalence of reduced TBUT [3, 5, 9, 12, 36, 37, 41]. A significant association between TBUT and PK was demonstrated, with higher mean TBUT in unaffected dogs [12]. Furthermore, TBUT decreased with increasing PK severity, whereas Schirmer tear test (STT) values were comparable to those reported in other breeds [12]. Pugs with advanced PK exhibited reduced tear production [6] and lower TBUT [3, 5]. These findings suggest that diminished tear production and shortened TBUT may represent consequences of chronic corneal disease rather than

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

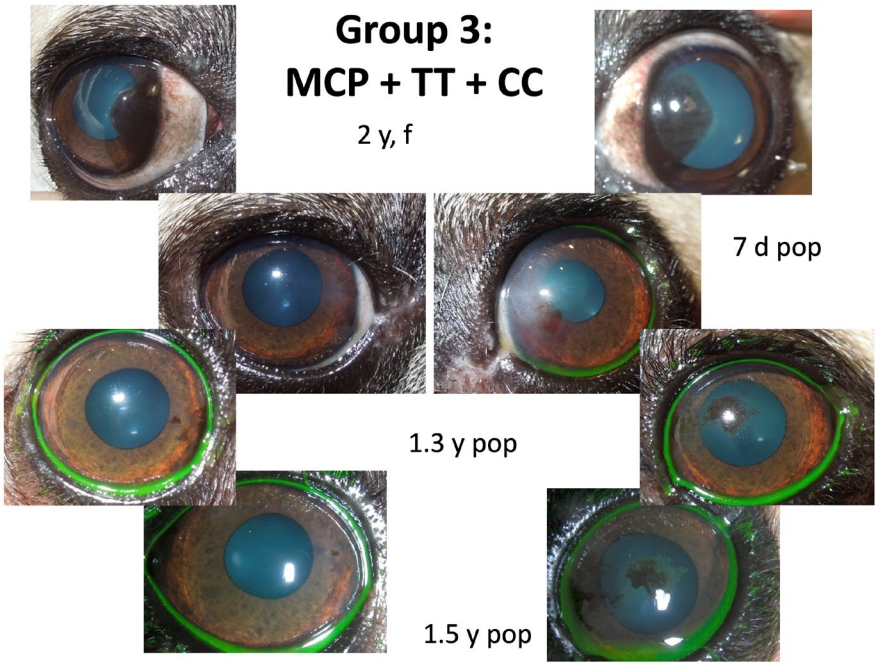

FIGURE 18 | Female Pug, 2 years old, Group 3 (NCP + CC bilaterally + TT). Symmetrical regression of pigmentation over 1.5 years, with mild residual pigment in the palpebral fissure region (left > right).

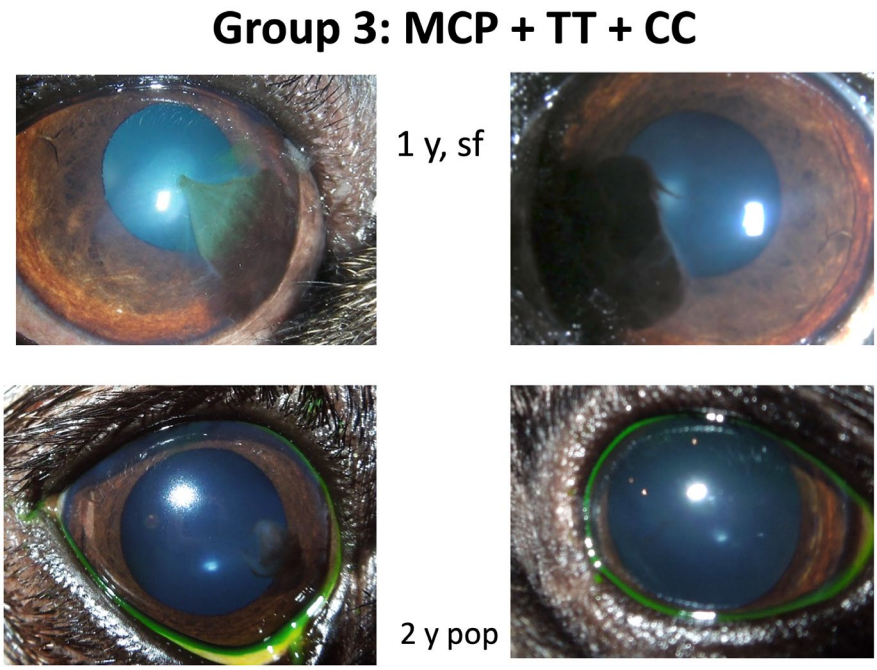

FIGURE 19 | Spayed female Pug, 1 year old, Group 3 (MCP + CC bilaterally + TT). Complete resolution of corneal pigment in the left eye, residual pigment in the right eye at the palpebral fissure region over 2 years.

primary causative factors [12]. Conversely, the presence of low TBUT despite normal STT values supports the notion that PK in Pugs is not solely attributable to brachycephalic conformational syndrome, but may also involve intrinsic alterations in tear film composition [5, 8]. Furthermore, one form of dry eye may predispose patients to the other. In animals with evaporative dry eye disease (EDED), chronic surface damage resulting from reduced tear film stability can lead to diminished corneal sensitivity, thereby weakening the neural stimulus for tear secretion and ultimately causing secondary aqueous tear deficiency (ADED) [42, 43]. Similarly, several canine studies have demonstrated reduced Schirmer tear test (STT) values in animals with Meibomian gland dysfunction (MGD), consistent with mixed dry eye disease [3, 5, 44].

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

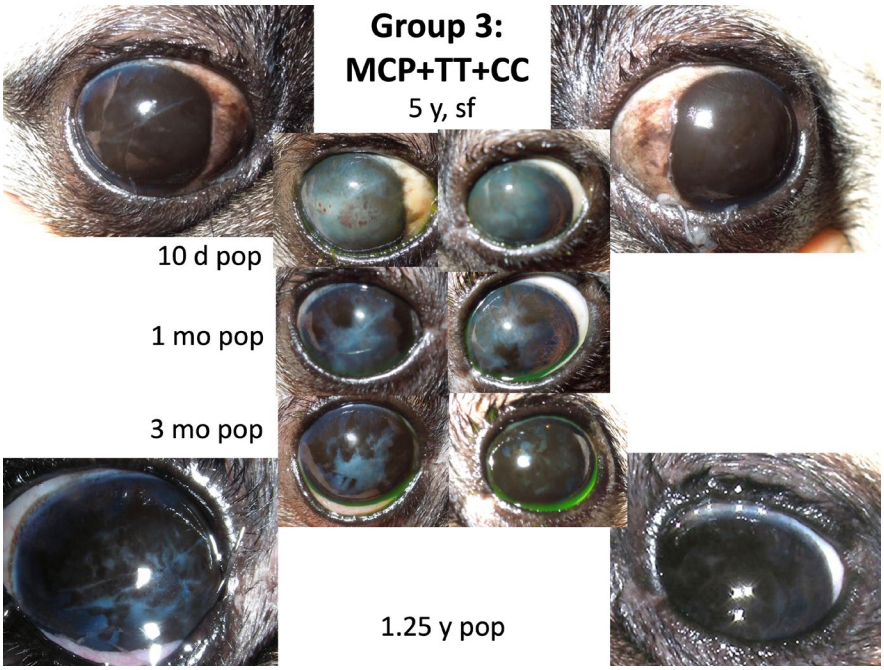

FIGURE 20 | Female Pug, 2 years old, Group 3 (MCP +CC bilaterally +TT). Pigment extension was Grade 3 in both eyes at baseline; post-therapy pigment nearly disappeared but partially recurred over 1.5 years.

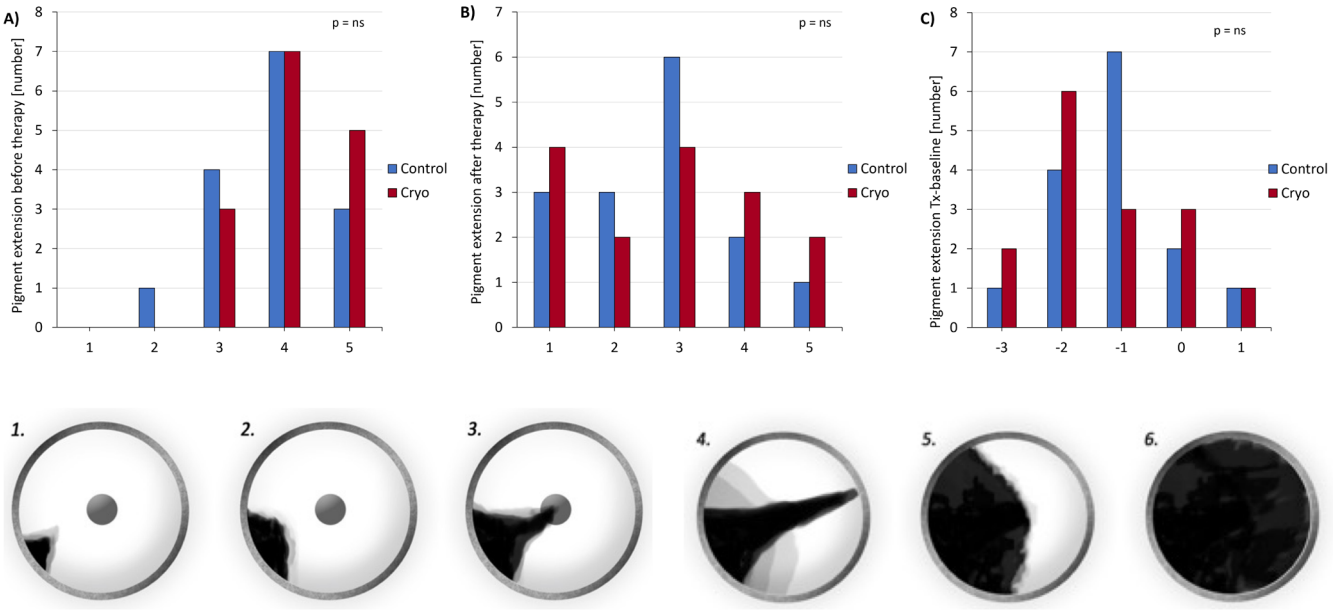

FIGURE 21 | Group 4: Comparison of pigment extension in eyes treated with MCP + TT only (control—blue) versus MCP + CC + TT (red). Top: Baseline (A), post-treatment (B), difference from baseline (C), signed-rank test. Bottom: Graphical illustration of pigment extension Grades 1–6.

Histological examination of tissue samples from the medial canthus of Pugs, compared with eyelid tissue from other brachycephalic breeds, revealed a higher density of inflammatory infiltrates around the Meibomian glands, within the conjunctiva, and in the dermis. Whether these infiltrates represent primary lesions or secondary chronic changes remains unclear [8].

Other potential risk factors for severe PK include previous ocular disease and increasing age [12]. Age-related impairment of ocular surface homeostasis has been documented in both dogs and humans [2, 10, 30, 45], with older dogs exhibiting a higher risk of PK [9, 12–14]. This predisposition may reflect progressive MGD, reduced conjunctival goblet cell density, and altered blink physiology in dogs [2], all of which promote microtrauma and chronic corneal epithelial irritation. Each additional year of life increases the risk of keratoconjunctivitis sicca (KCS) or corneal pigmentation by approximately 10% [10], while the risk of MGD increases by 20% [32]. In dogs older than 10 years, loss of Meibomian glands, thinning of the tear film lipid layer, and

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

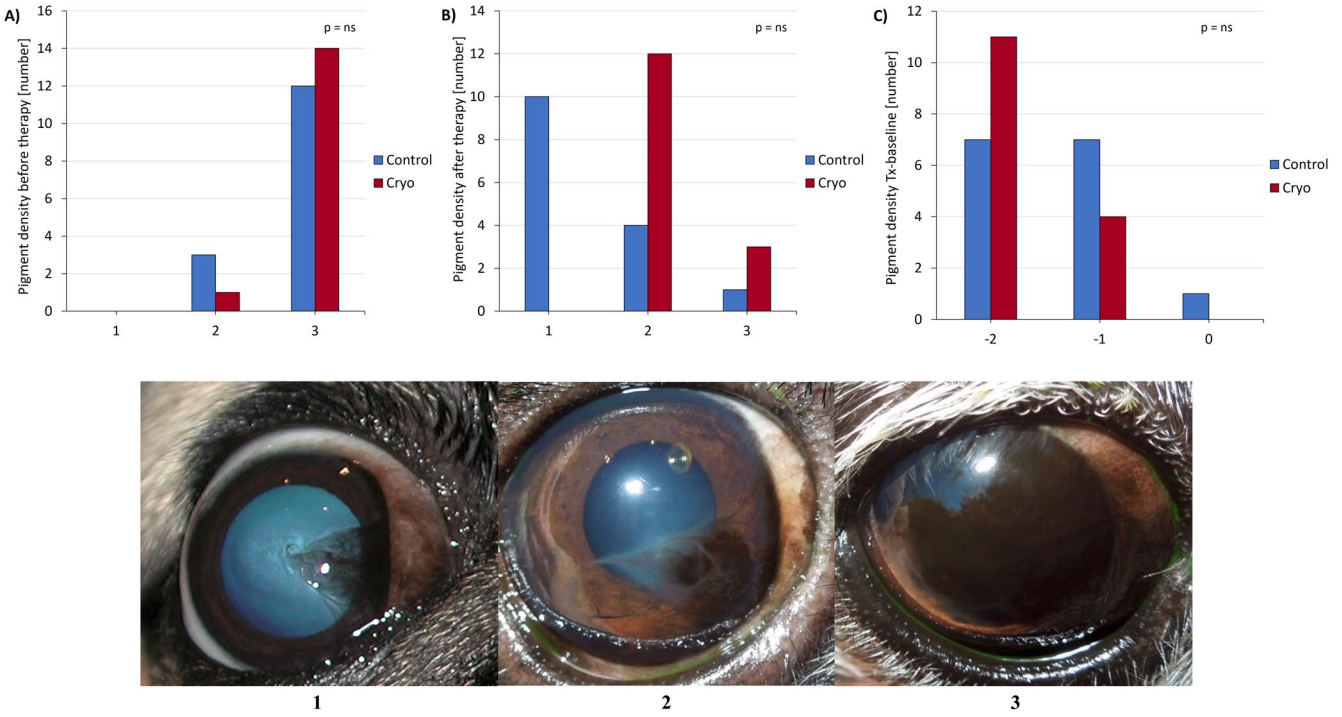

FIGURE 22 | Group 4: Comparison of pigment density between MCP + TT only (control—blue) and MCP + CC + TT (red). Top: Baseline (A), post-treatment (B), difference from baseline (C), signed-rank test. Bottom: Photographic examples of pigment density Grades 1–3.

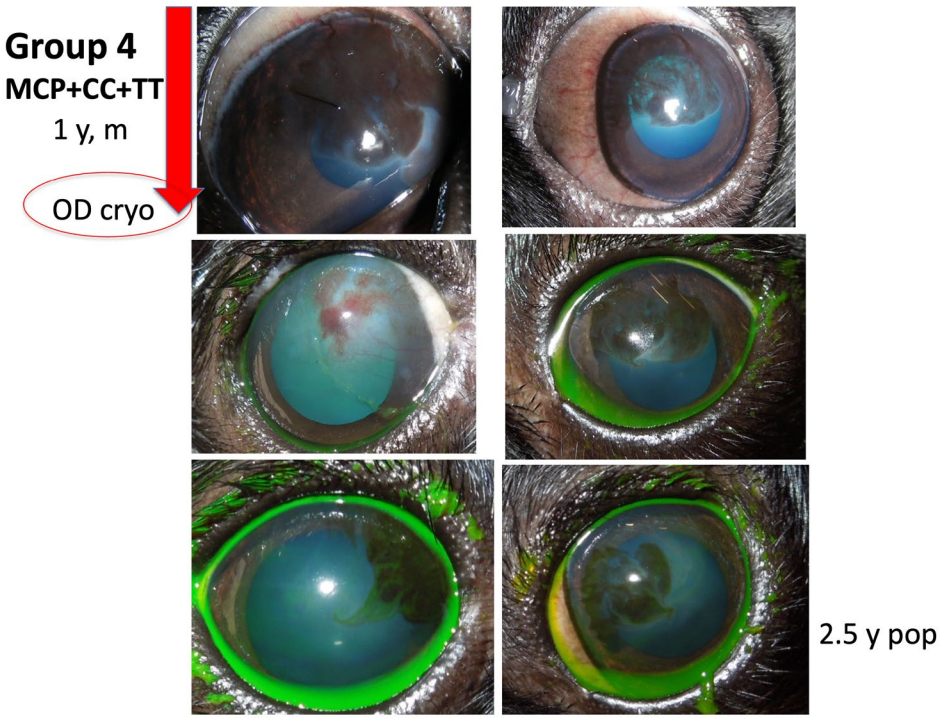

FIGURE 23 | Male Pug, 1 year old, Group 4 (MCP + CC right eye + TT). The right eye (red arrow) underwent cryotherapy. Pigment cleared post-cryotherapy but partially returned over 2.5 years; the left eye showed minimal change. Vascularization is visible where pigment has cleared after cryotherapy.

reduced STT values were significantly more pronounced than in dogs under 4 years of age [45]. Conversely, chronic ocular surface inflammation itself promotes MGD [31, 46], perpetuating a self-sustaining cycle. Preserved aqueous tear secretion despite advancing age may represent a compensatory mechanism [34] that nevertheless contributes to qualitative tear film deficiency (EDED).

Brachycephalic breeds, irrespective of the presence of ocular surface disease (OSD), are predisposed to structural and

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

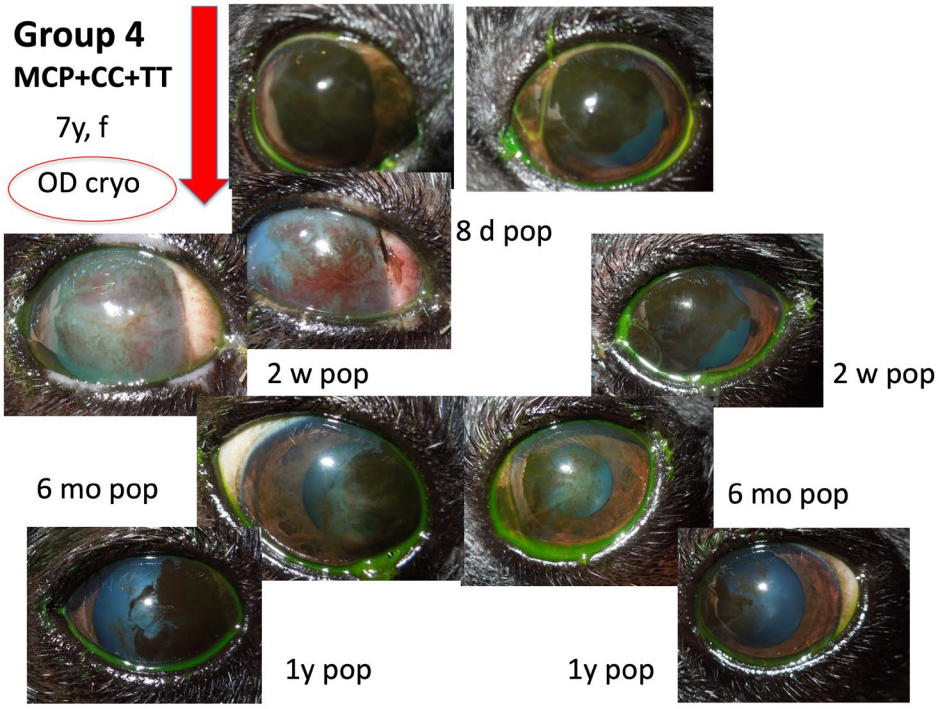

FIGURE 24 | Female Pug, 7 years old, Group 4 (MCP + CC right eye + TT). Both eyes were initially densely pigmented; the right eye (red arrow) was treated with cryotherapy. Post-depigmentation, the right cornea re-pigmented to a lesser extent over 1 year; the left eye (no CC) showed decreased pigmentation and increased transparency. Extensive vascularization is visible in the previously pigmented right eye after cryotherapy.

functional abnormalities of the Meibomian glands. These changes impair tear film distribution and accelerate evaporation, further destabilizing ocular surface homeostasis [2]. Breed-typical conformational features, such as exophthalmos and lateral strabismus due to shallow orbits and macroblepharon, predispose to lagophthalmos and insufficient ocular surface protection. Additional factors, including reduced corneal nerve fiber density [47], diminished corneal sensitivity [48], decreased blink frequency [49], incomplete closure [2], and inadequate tear film quality, further increase susceptibility to chronic epithelial microtrauma, epithelial defects, and ulceration [15, 32, 34]. Pugs have a 20-fold higher risk of corneal ulcers, and even a 10% increase in relative palpebral fissure width has been shown to triple the risk [15].

4.2 | Influence of Entropion on PK

While French Bulldogs have a relatively low prevalence of medial entropion despite the development of PK [11, 50], a significant correlation between the severity of PK and the extent of medial entropion has been found in Pugs: the more pronounced the entropion, the more severe the PK [9].

In Pugs, the initial site of corneal pigmentation typically coincides with the area of most pronounced trichiasis caused by stiff medial entropion hairs, combined with ocular exposure (Figure 1). In contrast, Shih Tzus showed medial entropion and caruncular trichiasis in 100% of cases, but PK in only 27% of eyes [26]. One explanation may be the softer hair texture of Shih Tzus compared to the coarse hair of Pugs, resulting in less corneal friction and irritation. Consequently, epiphora is the predominant clinical sign in Shih Tzus, rather than PK [21]. Notably, in our cohort, the degree of exophthalmos and scleral exposure did not influence PK development (Figure 25).

4.3 | Corneal Pigmentation in PK

Corneal pigmentation in PK is histopathologically characterized by chronic epithelial inflammation [7]. In brachycephalic breeds, superficial pigmentation appears as centripetal migration of limbal microanatomical elements (pigment, vessels, leukocytes) towards the axial cornea, rather than as a stem cell deficiency [7]. The pigment likely reaches the superficial corneal epithelium via physiological epithelial turnover [51]. This migration may represent an adaptive response that can be excessively activated by even mild inflammatory triggers [7]. Corneal melanosis itself is a non-specific protective response to chronic irritation caused by exposure, friction, tear film instability, or immunological stimulation [52]. The degree of limbal pigmentation correlates with the likelihood and density of corneal melanosis, and in Pugs, this association has been confirmed [9, 12]. As expected, all eyes in this study exhibited dense limbal pigmentation, most prominently on the nasal side.

Comparable mechanisms exist in humans. Individuals with heavily pigmented limbus may develop "striate melanokeratosis," characterized by centripetal migration of pigmented epithelial cells, often triggered by chronic irritation [53, 54]. The corneal limbus is characterized by a band of melanoblasts with pigments of variable density, depending on the individual. In eyes with marked perilimbal pigmentation, benign migration beneath an intact epithelium has been documented, resulting in localized, permanent opacification [55]. *Striate*

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

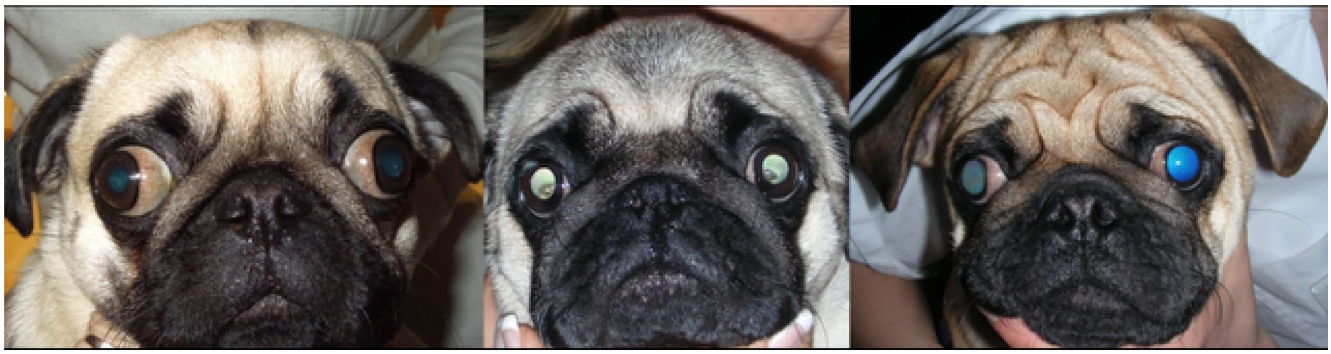

FIGURE 25 | Three one-year-old Pugs with differing skull shapes, scleral visibility, exophthalmos, and PK. The middle Pug, with minimal exophthalmos and scleral visibility, exhibits the most severe PK.

melanokeratosis, by contrast, may develop secondary to epithelial injury or chronic corneal inflammation. Melanin may be transferred from melanocytes to neighboring epithelial cells, or melanocytes themselves may migrate centrally, resulting in pigmented subepithelial lines as part of an adaptive healing response [55]. Another condition, "hurricane keratopathy," describes the vortex pattern of epithelial cell migration that occurs under conditions of increased epithelial turnover, resolving once the stimulus is removed [56]. In veterinary patients, removing the irritative stimulus typically prevents or slows melanosis progression but rarely reverses it [52]. Severe or chronic irritation is associated with additional corneal epithelial changes, including thickening, metaplasia, vascularization, and keratinization [52] (Figure 3c).

Taken together, PK in Pugs does not represent a distinct disease entity but rather a non-specific clinical manifestation of chronic corneal irritation with multiple etiologies [1].

4.4 | Therapies of PK

Despite its high prevalence, few peer-reviewed studies have addressed therapeutic interventions specifically for PK in Pugs [20]. Most therapeutic studies have included multiple brachycephalic breeds and have not focused on Pug-specific PK [11, 17, 21, 57, 58]. Long-term comparative data on treatment efficacy in Pugs are lacking, with only preliminary reports available [22].

In our study, therapeutic strategies were directed at the underlying etiopathogenic factors. Tear film deficiencies were managed with topical therapy. Given the central role of inflammation in the pathogenesis of dry eye disease [59, 60], a combination of topical immunomodulatory and anti-inflammatory agents (ciclosporin, tacrolimus, and dexamethasone) was administered. All dogs received long-term topical ciclosporin and tacrolimus therapy. Both agents are established immunomodulators in OSD and DED management [61–63], acting by enhancing aqueous tear secretion in ADED [64–66], reducing ocular surface inflammation [67], and supporting corneal nerve regeneration [40, 68]. We used several topical anti-inflammatory drugs (ciclosporin, tacrolimus, and dexamethasone) simultaneously to combat the various factors that cause EDED, as each of these drugs has a different effect on the meibomian glands, the conjunctiva, the CGCs, the tear film, and the cornea.

The therapeutic mechanism of action of ciclosporin in DED through its immunomodulatory effect, regulation of the adaptive immune response, and the numerous benefits at all stages of DED is well described in the published literature [69, 70]. Topical ciclosporin has a direct effect on mucin production by CGCs, independent of any direct effect on tear-secreting cells. It thus contributes to the overall effect of therapy on tear film disorders [71].

Ciclosporin not only enhances mucin production and increases goblet cell density but also exerts anti-apoptotic effects [70–73], contributing to ocular surface health. Clinically, it improves Meibomian gland expressibility and tear film stability [74, 75], while modestly reducing corneal neovascularization compared with dexamethasone [76], highlighting its therapeutic value in managing ocular surface disorders. Tacrolimus, 10–100 times more potent than ciclosporin [77], improves goblet cell density [78], reduces ocular surface inflammation, enhances Meibomian secretion and tear stability [79–81]. In canine EDED, tacrolimus significantly improved TBUT and ocular surface scores within 6 weeks [40] and was shown to halt pigment progression more effectively than ciclosporin in KCS [82]. In this study, dexamethasone was added in cases of severe inflammation and post-cryotherapy. Corticosteroids effectively suppress inflammatory neovascularization and acute inflammatory responses, although they are less effective against mature vessels [83–85]. Topical corticosteroids are most effective when administered in the early stages of corneal neovascularization. Among the agents studied, dexamethasone has consistently demonstrated greater efficacy than fluorometholone and prednisolone [85, 86]. Moreover, dexamethasone has been reported to exert a beneficial effect on conjunctival goblet cell density in patients with Sjögren's syndrome [87, 88].

In this study, conformational risk factors, particularly medial entropion, were addressed surgically using a modified medial canthoplasty (MCP). Correction of medial entropion is essential, as it represents a major trigger for PK progression [9]. MCP may reduce postoperative ocular irritation and corneal ulceration, thereby contributing to an improved quality of life in affected dogs [17]. The specific MCP technique employed

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

in that study, however, was not disclosed. Notably, two dogs required a subsequent entropion correction, and only 69.2% of the treated dogs (72/104) demonstrated a satisfactory eyelid position at the initial postoperative evaluation [17]. The simplified MCP technique employed in this study simultaneously reduces macroblepharon and corrects medial trichiasis as well as medial entropion [23].

In cases of severe PK, corneal cryotherapy (CC) was performed in addition to MCP and topical therapy. We applied the cryotherapy technique as previously described for corneal melanosis [24]. However, that study did not include Pugs. In another study, seven Pugs with severe PK underwent corneal cryotherapy followed by long-term TT (tacrolimus 0.03% and twice-daily tear replacement), without concurrent eyelid correction. After 4 weeks, 30%–60% of the patients demonstrated re-pigmentation, and after 6 months of follow-up, all patients exhibited partial re-pigmentation [20]. That underscores the importance of surgical lid correction for a successful long-term result. In the present study, cryotherapy was performed exclusively in combination with MCP, followed by long-term TT.

4.5 | Evaluation of PK in This Study

To assess pigment distribution, we employed a classification scheme adapted to the characteristic PK pattern in Pugs (Figure 4). Corneal epithelial integrity was evaluated by fluorescein staining, as tear film break-up time (TBUT) testing has significant methodological variability [34, 89–92]. The reliability of the TFBUT test has been reported as poor to moderate [93], and its results should therefore be interpreted with caution [43]. A study conducted in brachycephalic dogs (excluding Pugs) demonstrated that non-invasive TBUT (NITBUT), measured using the OSA-Vet (SBM Sistemi, Italy), showed poor correlation with conventional standardized tests [37]. Further investigations are required to validate ocular surface analyzers and to establish breed-specific reference values [37]. Although several novel approaches for evaluating NITBUT [94] and the ocular surface [95] have been proposed, none of these methods has yet undergone formal validation.

Fluorescein staining is a well-established method for assessing ocular surface integrity by analyzing the staining patterns following its application [43]. It represents the most widely employed diagnostic procedure for evaluating tear film disorders in human clinical practice [96]. Multifocal punctate staining is observed when fluorescein dye adheres to areas of the cornea that emerge as epithelial cells are desquamated [97, 98]. Fluorescein dot-staining is a hallmark of EDED in humans, arising when tear film instability disrupts the ocular surface. The corneal staining score is widely accepted as an objective endpoint for assessing therapeutic efficacy in DED [99, 100]. Analogous punctate staining has been reported in canine ADDE [101] and is equally applicable to epithelial damage in EDED. Several grading systems for fluorescein dot-staining in dogs have been proposed [26, 92, 101, 102]. Here, a scheme adapted from the SPOTS framework [102] was employed, comprising four grades: 1 (no staining), 2 (mild punctate staining in a circumscribed corneal area), 3 (confluent punctate staining), and 4 (fluorescein-positive epithelial defect) (Figure 6). The additional SPOTS grade for combined epithelial and stromal loss was omitted as irrelevant to the present objectives.

ADED and EDED were differentiated using Schirmer test-1 (STT-1) combined with a 3-point grading of mucus discharge (Figure 5). The elastic mucous discharge likely represents a CGC-mediated compensatory response to tear film evaporation, leading to epithelial irritation. CGCs secrete soluble mucins into the tear film, supporting lubrication and surface integrity [103]. Goblet cell density and function decline in multiple ocular surface diseases (OSDs), including DED of diverse etiologies [103, 104]. Goblet cell secretion is controlled by neural and inflammatory signals. Neural reflex arcs link afferent sensory nerves with efferent parasympathetic and sympathetic fibers to trigger mucin release. In early DED and allergic conjunctivitis in humans, epithelial injury and nerve activation enhance reflex-driven goblet cell secretion, particularly the large gel-forming mucin MUC5AC [46]. The release of mucin is triggered by inflammatory mediators such as histamine, leukotrienes, cytokines, and prostaglandins [103, 105]. The number and functional activity of CGCs are tightly regulated in response to external stimuli [103]. Moreover, their abundance generally correlates negatively with the degree of inflammation [103, 104]. Alterations in the quantity, morphology, and function of CGCs are therefore regarded as both a cause and a consequence of dry eye disease (DED). Although CGCs are clearly involved in the onset and progression of DED in humans, their precise role in the underlying pathophysiology remains poorly understood [46]. Consequently, the role and function of goblet cells in brachycephalic breeds in general—and in the pathophysiology of the Pug in particular—warrant further detailed investigation.

In this study, fluorescein staining scores decreased over time across all treatment groups, paralleling reductions in mucous discharge and improvements in tear film parameters. At the initiation of therapy, the majority of dogs exhibited Grade 2 or 3 discharge, which progressively decreased in all treatment groups. This finding is consistent with the expected pharmacological effect, as ciclosporin, tacrolimus, and dexamethasone were administered topically and are known to enhance CGC and MG function through immunomodulatory mechanisms. A reduction in fluorescein staining was also noted, as were decreases in STT-I values, the latter returning to physiological levels. These improvements were observed across all four groups and reached statistical significance.

4.6 | Discussion of the Results

In Group 1 (TT only), a significant reduction in the extent of corneal pigmentation was observed following treatment; however, pigment density remained largely unchanged. In contrast, Group 2 exhibited a more pronounced therapeutic benefit, with complete regression of corneal pigmentation documented in two dogs (Figure 18). This superior response is likely attributable to the correction of both exposure and trichiasis, as well as the earlier timing of intervention—implemented when PK was still in its initial stages and therefore more amenable to modification.

Across both groups, more favorable outcomes were consistently associated with lower baseline pigmentation, a finding that

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

aligns with the known etiopathogenesis of PK and its association with chronic inflammatory OSD. Although the clinical improvements in Group 2 (MCP + TT) appeared superior to those observed in Group 1, this difference did not achieve statistical significance, most likely due to the limited sample size and resultant constraints on statistical power.

In severe PK cases, CC was added to MCP and TT (bilaterally in Group 3, unilaterally in Group 4) to selectively target melanocytes through cryolysis [106, 107]. In CC-treated eyes, corneal pigmentation regressed within days after application of CC. After clearance, underlying vascularization became visible, revealing chronic stromal keratitis (Figures 23 and 24) and supporting the classification of PK as a non-specific chronic inflammatory corneal disease. Re-pigmentation in Groups 3 and 4 remained below baseline, yet even partial re-pigmentation showed more pronounced regression than in cryo-untreated eyes.

Higher improvement scores in Groups 3 and 4 should be interpreted cautiously, as more extensive baseline pigmentation made improvements appear greater than in milder cases. This should be considered when comparing Group 1 with Groups 2 and 3. In Group 4, intra-individual comparisons suggested a clinical benefit of adjuvant cryotherapy, though it was not statistically significant.

In this study, MCP was performed in Pugs as early as 6 months of age. Given that both time and age represent critical factors in the development and progression of PK and the associated tear film deficiencies in this breed, initiation of combined therapy is recommended as soon as alterations of the ocular surface are detected.

4.7 | Limitations of the Study

The retrospective design constitutes the major limitation of this study, as it is associated with subjective documentation and heterogeneous follow-up intervals. The clinical nature of the study population may have introduced bias, resulting in variable baseline characteristics and PK assessments.

Additionally, the limited sample size restricts the extent to which these findings can be generalized. This small cohort is largely attributable to the need for consistent owner compliance with the prescribed treatment regimen, attendance at scheduled recheck appointments, and follow-up duration.

The inclusion of additional diagnostic measures, such as non-invasive tear break-up time (NITBUT), would have enabled a more accurate assessment of changes in tear film quality during the observation period. Despite NIBUT correlating poorly with conventional standardized tests in one study (measured with the OSA-Vet; SBM Sistemi, Italy) [37], its reproducibility across visits has been reported [108].

Further research is warranted to clarify the underlying mechanisms of PK in Pugs, with particular attention to tear film deficiency (EDED), conjunctival goblet cell function, and the role of limbal stem cells.

5 | Conclusions and Clinical Relevance

The results of this study support the superiority of combined simplified MCP and topical therapy for tear film deficiency over topical therapy alone in the management of PK in Pugs. Clinical efficacy seems to be highly dependent on simultaneous entropion correction during MCP. In advanced cases with marked pigmentation at presentation, cryotherapy provides additional therapeutic benefit when combined with MCP and topical treatment, although partial recurrence of pigmentation may occur over time.

Most importantly, the findings underscore the clinical relevance of early intervention: initiation of combined treatment (simplified MCP with lifelong topical therapy for DED) at the earliest stage of ocular surface alteration is associated with markedly improved long-term outcomes and might be considered the standard of care in affected Pugs. Furthermore, this study confirms that PK in the Pug is a non-specific chronic inflammatory corneal disease triggered by multiple irritating factors, which can be reversed when treated effectively in its initial stages.

Author Contributions

Ingrid Allgoewer: conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, writing – review and editing, project administration, data curation, supervision, resources, formal analysis.

Disclosure

Artificial Intelligence Statement: The author has not used AI to generate the manuscript.

Ethics Statement

The study was performed according to GERVO guidelines and was approved by the responsible authority, Berlin State Office for Health and Social Affairs (Landesamt für Gesundheit und Soziales, Berlin, Germany; approval no. StN. 22/25).

Conflicts of Interest

The author declares no conflicts 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.

References

1. D. J. Maggs, *Slatter's Fundamentals of Veterinary Ophthalmology*, 5th ed., ed. D. Maggs, P. E. Miller, and R. Ofri (Elsevier Saunders, 2013).

2. L. Sebbag and R. F. Sanchez, "The Pandemic of Ocular Surface Disease in Brachycephalic Dogs: The Brachycephalic Ocular Syndrome," *Veterinary Ophthalmology* 26, no. Suppl 1 (2023): 31–46, https://doi.org/10.1111/vop.13054.

3. A. L. Labelle, C. B. Dresser, R. E. Hamor, M. C. Allender, and J. L. Disney, "Characteristics of, Prevalence of, and Risk Factors for Corneal Pigmentation (Pigmentary Keratopathy) in Pugs," *Journal of the American Veterinary Medical Association* 243, no. 5 (2013): 667–674, https://doi.org/10.2460/javma.243.5.667.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

4. D. G. O'Neill, E. C. Darwent, D. B. Church, and D. C. Brodbelt, "Demography and Health of Pugs Under Primary Veterinary Care in England," *Canine Genetics and Epidemiology* 3 (2016): 5, https://doi.org/10.1186/s40575-016-0035-z.

5. M. Krecny, A. Tichy, J. Rushton, and B. Nell, "A Retrospective Survey of Ocular Abnormalities in Pugs: 130 Cases," *Journal of Small Animal Practice* 56, no. 2 (2014): 96–102, https://doi.org/10.1111/jsap.12291.

6. S. Anoop, C. B. Devanand, K. V. Syam, et al., "Pigmentary Keratitis in Dogs—A Study on Incidence in 83 Corneas," *Malaysian Journal of Veterinary Research* 7, no. 1 (2016): 15–20.

7. L. V. Vallone, A. M. Enders, H. O. Mohammed, and E. C. Ledbetter, "In Vivo Confocal Microscopy of Brachycephalic Dogs With and Without Superficial Corneal Pigment," *Veterinary Ophthalmology* 20, no. 4 (2017): 294–303, https://doi.org/10.1111/vop.12416.

8. M. Krecny, K. Witter, A. Tichy, and B. Nell, "Untersuchung des Tränenfilms und Histologische Untersuchung der Augenlider von Möpsen," *Tierärztliche Praxis. Ausgabe K, Kleintiere/Heimtiere* 46, no. 6 (2018): 380–385, https://doi.org/10.1055/s-0038-1677390.

9. S. Maini, R. Everson, C. Dawson, Y. M. Chang, C. Hartley, and R. F. Sanchez, "Pigmentary Keratitis in Pugs in the United Kingdom: Prevalence and Associated Features," *BMC Veterinary Research* 15, no. 1 (2019): 384, https://doi.org/10.1186/s12917-019-2127-y.

10. S. V. Palmer, F. E. Gomes, and J. A. A. McArt, "Ophthalmic Disorders in a Referral Population of Seven Breeds of Brachycephalic Dogs: 970 Cases (2008–2017)," *Journal of the American Veterinary Medical Association* 259 (2021): 1318–1324, https://doi.org/10.2460/javma.20.07.0388.

11. J. Costa, A. Steinmetz, and E. Delgado, "Clinical Signs of Brachycephalic Ocular Syndrome in 93 Dogs," *Irish Veterinary Journal* 74, no. 1 (2021): 3, https://doi.org/10.1186/s13620-021-00183-5.

12. D. Sarmiento Quintana, I. Morales Fariña, J. González Pérez, J. R. Jaber, and J. A. Corbera, "Ocular Surface Characteristics in Pugs With Pigmentary Keratitis in the Canary Islands, Spain," *Animals* 14, no. 4 (2024): 580, https://doi.org/10.3390/ani14040580.

13. W. I. Lau and R. M. Taylor, "The Prevalence of Corneal Disorders in Pugs Attending Primary Care Veterinary Practices in Australia," *Animals* 15, no. 4 (2025): 531, https://doi.org/10.3390/ani15040531.

14. R. T. Manikumar, S. K. Mahajan, N. U. Devi, J. S. Khosa, and A. Anand, "Evaluation of Pigmentary Keratitis in Pugs: A Study on Prevalence and Corneal Pigmentation Patterns," *Archives of Current Research International* 25, no. 7 (2025): 726–734, https://doi.org/10.9734/acri/2025/v25i71373.

15. R. M. A. Packer, A. Hendricks, and C. C. Burn, "Impact of Facial Conformation on Canine Health: Corneal Ulceration," *PLoS One* 10 (2015): e0123827, https://doi.org/10.1371/journal.pone.0123827.

16. H. Iwashita, S. Wakaiki, Y. Kazama, and A. Saito, "Breed Prevalence of Canine Ulcerative Keratitis According to Depth of Corneal Involvement," *Veterinary Ophthalmology* 23 (2020): 849–855, https://doi.org/10.1111/vop.12808.

17. A. L. M. M. Andrews, K. L. Youngman, R. M. A. Packer, D. G. O'Neill, and C. Kafarnik, "A Review of Clinical Outcomes, Owner Understanding and Satisfaction Following Medial Canthoplasty in Brachycephalic Dogs in a UK Referral Setting (2016–2021)," *Animals* 13, no. 12 (2023): 2032, https://doi.org/10.3390/ani13122032.

18. H. Appelboam, "Pug Appeal: Brachycephalic Ocular Health," *Companion Animal* 21, no. 1 (2016): 29–36.

19. I. Allgoewer and S. Sahr, "Preliminary Results of the Evaluation of the Long-Term Effect of Different Therapies for Pigmentary Keratitis in the Pug. Abstracts: Annual Scientific Meeting of the European College of Veterinary Ophthalmologists, London, UK, May 15–18, 2014," *Veterinary Ophthalmology* 17, no. 6 (2014): E16–E30, https://doi.org/10.1111/vop.12191.

20. E. Ghiggi, P. S. Huenning, M. C. C. Andrade, G. P. Valdetaro, T. Martinez, and R. K. Carneiro, "Cryosurgery Using Dimethyl Ether in the Treatment of Pigmentary Keratitis in Pug Dogs," *Ciência Animal* 32, no. 3 (2022): 200–208.

21. N. Y. Yi, S. A. Park, M. B. Jeong, et al., "Medial Canthoplasty for Epiphora in Dogs: A Retrospective Study of 23 Cases," *Journal of the American Animal Hospital Association* 42, no. 6 (2006): 435–439, https://doi.org/10.5326/0420435.

22. I. Allgoewer, S. Sahr, and K. Neumann, "Abstracts: American College of Veterinary Oph-Thalmologists, Monterey, CA October 26–29, 2016 Abstract 75: Results of the Evaluation of the Long-Term Effect of Different Therapies on Pigmentary Keratitis (PK) of the Pug," *Veterinary Ophthalmology* 19, no. 6 (2016): E21–E43.

23. I. Allgoewer, "Simplified Medial Canthoplasty: Technique and Early Postoperative Complications in 601 Dogs (1180 Eyes)," *Veterinary Ophthalmology* 28, no. 2 (2025): 497–505, https://doi.org/10.1111/vop.13266.

24. T. Azoulay, "Adjunctive Cryotherapy for Pigmentary Keratitis in Dogs: A Study of 16 Corneas," *Veterinary Ophthalmology* 17, no. 4 (2014): 241–249.

25. A. Labelle and P. Labelle, "Diagnosing Corneal Pigmentation in Small Animals," *Veterinary Clinics of North America. Small Animal Practice* 53, no. 2 (2023): 339–352, https://doi.org/10.1016/j.cvsm.2022.11.001.

26. L. Sebbag, A. P. S. M. Silva, Á. P. B. Santos, A. C. S. Raposo, and A. P. Oriá, "An Eye on the Shih Tzu Dog: Ophthalmic Examination Findings and Ocular Surface Diagnostics," *Veterinary Ophthalmology* 26, no. Suppl 1 (2023): 59–71, https://doi.org/10.1111/vop.13022.

27. H. Bolzanni, A. P. Oriá, A. C. S. Raposo, and L. Sebbag, "Aqueous Tear Assessment in Dogs: Impact of Cephalic Conformation, Inter-Test Correlations, and Test-Retest Repeatability," *Veterinary Ophthalmology* 23, no. 3 (2020): 534–543, https://doi.org/10.1111/vop.12751.

28. S. Carrington, P. Bedford, J. Guillon, and E. Woodward, "Biomicroscopy of the Tear Film: The Tear Film of the Pekingese Dog," *Veterinary Record* 124, no. 13 (1989): 323–328, https://doi.org/10.1136/vr.124.13.323.

29. R. F. Sanchez, G. Innocent, J. Mould, and F. M. Billson, "Canine Keratoconjunctivitis Sicca: Disease Trends in a Review of 229 Cases," *Journal of Small Animal Practice* 48, no. 4 (2007): 211–217, https://doi.org/10.1111/j.1748-5827.2006.00185.x.

30. Y. Kitamura, A. Saito, and S. Maehara, "Observation of Canine Meibomian Gland With Noncontact-Type Meibography," *Journal of the Japan Veterinary Medical Association* 67, no. 11 (2014): 857–861, https://doi.org/10.12935/jvma.67.857.

31. Y. Kitamura, S. Maehara, T. Nakade, et al., "Assessment of Meibomian Gland Morphology by Noncontact Infrared Meibography in Shih Tzu Dogs With or Without Keratoconjunctivitis Sicca," *Veterinary Ophthalmology* 22, no. 6 (2019): 744–750, https://doi.org/10.1111/vop.12645.

32. M. Vinas, F. Maggio, N. D'Anna, R. Rabozzi, and C. Peruccio, "Meibomian Gland Dysfunction (MGD), as Diagnosed by Non-Contact Infrared Meibography, in Dogs With Ocular Surface Disorders (OSD): A Retrospective Study," *BMC Veterinary Research* 15, no. 1 (2019): 443, https://doi.org/10.1186/s12917-019-2203-3.

33. Y. Kitamura, R. Arita, Y. Miwa, H. Iwashita, and A. Saito, "Histopathologic Changes Associated With Meibomian Gland Dropout in a Dog," *Veterinary Ophthalmology* 23, no. 3 (2020): 575–578, https://doi.org/10.1111/vop.12754.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

34. H. Faghihi and S. M. Rajaei, "Tear Film Breakup Time and Schirmer Tear Test in Normal Dogs: Effects of Age, Sex, Reproductive Status, Skull Type, and Nasolacrimal Duct Patency," *Veterinary Ophthalmology* 26, no. Suppl 1 (2023): 72–80, https://doi.org/10.1111/vop.13021.

35. C. P. Moore, "Qualitative Tear Film Disease," *Veterinary Clinics of North America: Small Animal Practice* 20, no. 3 (1990): 565–581, https://doi.org/10.1016/s0195-5616(90)50071-6.

36. T. S. Arnold, L. A. Wittenburg, and C. C. Powell, "Effect of Topical Naltrexone 0.3% on Corneal Sensitivity and Tear Parameters in Normal Brachycephalic Dogs," *Veterinary Ophthalmology* 17 (2014): 328–333, https://doi.org/10.1111/vop.12079.

37. J. N. Voitena, F. L. C. Brito, T. O. C. Marinho, et al., "Application of OSA-VET and Qualiquantitative Tear Tests in Brachycephalic Dogs With and Without Keratoconjunctivitis Sicca," *Veterinary Research Communications* 49, no. 1 (2024): 40, https://doi.org/10.1007/s11259-024-10610-x.

38. R. C. Vitor, J. B. de Carvalho Teixeira, K. C. Dos Santos, et al., "Shih-Tzu Dogs Show Alterations in Ocular Surface Homeostasis Despite Adequate Aqueous Tear Production," *Acta Veterinaria Scandinavica* 66, no. 1 (2024): 3, https://doi.org/10.1186/s13028-024-00724-2.

39. J. Spornberger, P. Soukup, A. Meyer-Lindenberg, and I. Allgoewer, "Tear Film Characteristics in French Bulldogs vs. Non-Brachycephalic Dogs," *Frontiers in Veterinary Science* 12 (2025): 1732237, https://doi.org/10.3389/fvets.2025.1732237.

40. L. Sebbag, S. F. Barbosa, and A. P. Oriá, "Tacrolimus and Hyaluronate Therapy Enhance Tear Film Stability in Canine Evaporative Dry Eye Disease," *Veterinary Ophthalmology* 29, no. 2 (2025): e70042, https://doi.org/10.1111/vop.70042.

41. J. A. M. Tapping, "Comparison of Tear Film Break Up Time (TFBUT) Test in Healthy Brachycephalic and Mesocephalic Dogs," Diploma thesis. University of Veterinary Medicine and Pharmacy in Košice (2020), https://www.researchgate.net/publication/341460249.

42. A. J. Bron, C. S. de Paiva, S. K. Chauhan, et al., "TFOS DEWS II Pathophysiology Report," *Ocular Surface* 15, no. 3 (2017): 438–510, https://doi.org/10.1016/j.jtos.2017.05.011. Erratum in *Ocular Surface* 17, no. 4 (2019): 842, https://doi.org/10.1016/j.jtos.2019.08.007.

43. E. A. Hisey, A. Galor, and B. C. Leonard, "A Comparative Review of Evaporative Dry Eye Disease and Meibomian Gland Dysfunction in Dogs and Humans," *Veterinary Ophthalmology* 26, no. Suppl 1 (2023): 16–30, https://doi.org/10.1111/vop.13066.

44. D. Jeong, S. Kang, J. Shim, E. Lee, Y. Jeong, and K. Seo, "Evaluation of Ocular Surface Parameters in Dogs With and Without Meibomian Gland Dysfunction," *Veterinary Record* 121, no. 2 (2022): e1682.

45. A. Cirla and L. Ventura, "Age-Related Changes of Meibomian Glands in Healthy Brachycephalic Dogs Assessed by Infrared Meibography and Tear Film Interferometry. Abstracts: Annual Scientific Meeting of the European College of Veterinary Ophthalmologists, Salzburg, Austria, June 2–5, 2022," *Veterinary Ophthalmology* 26, no. 4 (2023): e1–e36, https://doi.org/10.1111/vop.13056.

46. Y. Liu, Z. Duan, J. Yuan, and P. Xiao, "Imaging Assessment of Conjunctival Goblet Cells in Dry Eye Disease," *Clinical & Experimental Ophthalmology* 52, no. 5 (2024): 576–588, https://doi.org/10.1111/ceo.14379.

47. C. Kafarnik, J. Fritsche, and S. Reese, "Corneal Innervation in Mesocephalic and Brachycephalic Dogs and Cats: Assessment Using In Vivo Confocal Microscopy," *Veterinary Ophthalmology* 11, no. 6 (2008): 363–367, https://doi.org/10.1111/j.1463-5224.2008.00659.x.

48. P. M. Barrett, R. H. Scagliotti, R. E. Merideth, and P. A. Jackson, "Absolute Corneal Sensitivity and Corneal Trigeminal Nerve Anatomy in Normal Dogs," *Veterinary and Comparative Ophthalmology* 1 (1991): 245–254.

49. M. Collins, R. Seeto, L. Campbell, and M. Ross, "Blinking and Corneal Sensitivity," *Acta Ophthalmologica* 67, no. 5 (1989): 525–531.

50. I. Allgoewer, "Ocular Disorders of 809 French Bulldogs. Abstracts: Annual Scientific Meeting of the European College of Veterinary Ophthalmologists, Antwerp, Belgium May 23–26, 2019," *Veterinary Ophthalmology* 22, no. 5 (2019): E1–E27, https://doi.org/10.1111/vop.12702.

51. R. A. Thoft and J. Friend, "The X, Y, Z Hypothesis of Corneal Epithelial Maintenance," *Investigative Ophthalmology & Visual Science* 24, no. 10 (1983): 1442–1443.

52. D. J. Maggs, *Slatter's Fundamentals of Veterinary Ophthalmology*, 6th ed., ed. D. Maggs, P. E. Miller, and R. Ofri (Elsevier Saunders, 2017).

53. T. H. Cowan, "Striate Melanokeratosis in Negroes," *American Journal of Ophthalmology* 57 (1964): 443–449, https://doi.org/10.1016/0002-9394(64)92661-3.

54. A. Boto de los Bueis, D. Diaz-Valle, S. Llorente-Gonzales, N. Pastora-Salvador, and M. Patron-Romaero, "Post-Traumatic Striate Melanokeratosis in a Caucasian Woman," *Archivos de la Sociedad Española de Oftalmología* 84, no. 4 (2009): 213–215.

55. K. N. Ma and W. M. Munir, "Striate Melanokeratosis," in *Encyclopedia of Ophthalmology*, ed. U. Schmidt-Erfurth and T. Kohnen (Springer, 2018), https://doi.org/10.1007/978-3-540-69000-9_807.

56. H. S. Dua and J. A. Gomes, "Clinical Course of Hurricane Keratopathy," *British Journal of Ophthalmology* 84, no. 3 (2000): 285–288, https://doi.org/10.1136/bjo.84.3.285.

57. A. R. Costa, T. A. Ribeiro, D. M. C. Silva, et al., "Effects of Canthoplasty in Preventing Secondary Ocular Surface Lesions in Brachycephalic Dogs," *Veterinary Sciences* 12, no. 9 (2025): 889, https://doi.org/10.3390/vetsci12090889.

58. B. D. Reynolds, C. J. Whittaker, K. A. Caruso, W. M. Irving, P. M. McCarthy, and S. J. Smith, "Treating Evaporative Dry Eye Disease in Dogs Based Upon Tear Film Lipid-Layer Thickness: A Retrospective Cohort Study," *Veterinary Ophthalmology* 29 (2025): 1–9, https://doi.org/10.1111/vop.70079.

59. J. P. Craig, K. K. Nichols, E. K. Akpek, et al., "TFOS DEWS II Definition and Classification Report," *Ocular Surface* 15, no. 3 (2017): 276–283, https://doi.org/10.1016/j.jtos.2017.05.008.

60. M. I. Golden, J. J. Meyer, M. Zeppieri, and B. C. Patel, "Dry Eye Syndrome," in *StatPearls* (StatPearls Publishing, 2019).

61. T. Reinhard, A. Reis, S. Mayweg, H. Oberhuber, G. Mathis, and R. Sundmacher, "Topical Fk506 in Inflammatory Corneal and Conjunctival Diseases. A Pilot Study," *Klinische Monatsblätter für Augenheilkunde* 219, no. 3 (2002): 125–131.

62. B. H. Grahn and E. S. Storey, "Lacrimostimulants and Lacrimomimetics," *Veterinary Clinics of North America. Small Animal Practice* 34, no. 3 (2004): 739–753, https://doi.org/10.1016/j.cvsm.2003.12.009.

63. F. Maggio, "Ocular Surface Disease in Dogs Part 2: Diagnosis and Treatment," *Companion Animal* 24, no. 6 (2019): 319–328, https://doi.org/10.12968/coan.2019.24.6.319.

64. A. Berdoulay, R. V. English, and B. Nadelstein, "Effect of Topical 0.02% Tacrolimus Aqueous Suspension on Tear Production in Dogs With Keratoconjunctivitis Sicca," *Veterinary Ophthalmology* 8, no. 4 (2005): 225–232, https://doi.org/10.1111/j.1463-5224.2005.00390.x.

65. D. V. Hendrix, E. A. Adkins, D. A. Ward, J. Stuffle, and B. Skorobohach, "An Investigation Comparing the Efficacy of Topical Ocular Application of Tacrolimus and Cyclosporine in Dogs," *Veterinary Medicine International* 2011 (2011): 487592, https://doi.org/10.4061/2011/487592.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

66. K. Radziejewski and I. Balicki, "Comparative Clinical Evaluation of Tacrolimus and Cyclosporine Eye Drops for the Treatment of Canine Keratoconjunctivitis Sicca," *Acta Veterinaria Hungarica* 64, no. 3 (2016): 313–329, https://doi.org/10.1556/004.2016.030.

67. A. Kumar, A. Gopinathan, K. Singh, R. Sasikala, and C. R. Swapana, "Effect of Tacrolimus Treatment on Tear Fluid Cytokines of Keratoconjunctivitis Sicca Affected Dogs," *International Journal of Current Microbiology and Applied Sciences* 9, no. 9 (2020): 3347–3356.

68. E. Toker and E. Asfuroğlu, "Corneal and Conjunctival Sensitivity in Patients With Dry Eye: The Effect of Topical Cyclosporine Therapy," *Cornea* 29, no. 2 (2010): 133–140, https://doi.org/10.1097/ICO.0b013e3181acf68d.

69. S. Tatlipinar and E. K. Akpek, "Topical Ciclosporin in the Treatment of Ocular Surface Disorders," *British Journal of Ophthalmology* 89 (2005): 1363–1367, https://doi.org/10.1136/bjo.2005.070888.

70. L. M. Periman, F. S. Mah, and P. M. Karpecki, "A Review of the Mechanism of Action of Cyclosporine A: The Role of Cyclosporine A in Dry Eye Disease and Recent Formulation Developments," *Clinical Ophthalmology* 14 (2020): 4187–4200, https://doi.org/10.2147/OPTH.S279051.

71. C. P. Moore, J. B. McHugh, J. G. Thorne, and T. E. Phillips, "Effect of Cyclosporine on Conjunctival Mucin in a Canine Keratoconjunctivitis Sicca Model," *Investigative Ophthalmology & Visual Science* 42, no. 3 (2001): 653–659.

72. K. S. Kunert, A. S. Tisdale, and I. K. Gipson, "Goblet Cell Numbers and Epithelial Proliferation in the Conjunctiva of Patients With Dry Eye Syndrome Treated With Cyclosporine," *Archives of Ophthalmology* 120 (2002): 330–337, https://doi.org/10.1001/archopht.120.3.330.

73. J. W. Moon, H. J. Lee, K. C. Shin, W. R. Wee, J. H. Lee, and M. K. Kim, "Short Term Effects of Topical Cyclosporine and Viscoelastic on the Ocular Surfaces in Patients With Dry Eye," *Korean Journal of Ophthalmology* 21, no. 4 (2007): 189–194, https://doi.org/10.3341/kjo.2007.21.4.189.

74. H. Y. Kim, J. E. Lee, H. N. Oh, J. W. Song, S. Y. Han, and J. S. Lee, "Clinical Efficacy of Combined Topical 0.05% Cyclosporine A and 0.1% Sodium Hyaluronate in the Dry Eyes With Meibomian Gland Dysfunction," *International Journal of Ophthalmology* 11, no. 4 (2018): 593–600, https://doi.org/10.18240/ijo.2018.04.09.

75. D. Jee, S. Y. Han, H. S. Kim, and E. C. Kim, "Comparison of the Effect of and Compliance With Cyclosporine 0.1% After Various Pretreatments in Dry Eye Disease," *Journal of Ophthalmology* 2025 (2025): 6744482, https://doi.org/10.1155/joph/6744482.

76. Y. Y. Bucak, M. Erdurmus, E. H. Terzi, A. Kükner, and S. Çelebi, "Inhibitory Effects of Topical Cyclosporine A 0.05% on Immune-Mediated Corneal Neovascularization in Rabbits," *Graefe's Archive for Clinical and Experimental Ophthalmology* 251 (2013): 2555–2561.

77. T. Kino, H. Hatanaka, S. Miyata, et al., "FK-506, a Novel Immunosuppressant Isolated From a Streptomyces. II. Immunosuppressive Effect of FK-506 In Vitro," *Journal of Antibiotics* 40 (1987): 1256–1265.

78. M. Golmohammadi, M. S. Ivraghi, S. A. Jasim, et al., "A Comprehensive Review of the Effects of Tacrolimus (FK-506) on Dry Eye Disease (DED): Focus on Inflammation and Biochemical Processes," *Authorea* (2024), https://doi.org/10.22541/au.171653157.78342929/v1.

79. F. E. Sakassegawa-Naves, H. M. M. Ricci, B. K. Moscovici, et al., "Tacrolimus Ointment for Refractory Posterior Blepharitis," *Current Eye Research* 42, no. 11 (2017): 1440–1444, https://doi.org/10.1080/02713683.2017.1339805.

80. B. Khan, A. Ahmad, J. Rasul, M. Farhan, and H. Rehman, "Efficacy of Tacrolimus 0.03% Ointment in Resistant Meibomian Gland DysfunctionEfficacy of Tacrolimus 0.03% Ointment in Resistant Meibomian Gland Dysfunction," *JCCO* 2, no. 1 (2024): 2–6.

81. G. Katonis, A. Tzamalis, I. Tsinopoulos, and N. Ziakas, "Topical Use of Tacrolimus in Corneal and Ocular Surface Pathologies: A Systematic Review," *Journal of Clinical Medicine* 14, no. 15 (2025): 5347, https://doi.org/10.3390/jcm14155347.

82. C. John, A. Gopinathan, K. Singh, et al., "Clinical Evaluation of Topical Tacrolimus Ointment Usage in Different Stages of Keratoconjunctivitis Sicca in Dogs," *Turkish Journal of Veterinary and Animal Sciences* 42 (2018): 259–268, https://doi.org/10.3906/vet-1801-1.

83. S. Nakao, Y. Hata, M. Miura, et al., "Dexamethasone Inhibits Interleukin-1beta-Induced Corneal Neovascularization: Role of Nuclear Factor-kappaB-Activated Stromal Cells in Inflammatory Angiogenesis," *American Journal of Pathology* 171, no. 3 (2007): 1058–1065, https://doi.org/10.2353/ajpath.2007.070172.

84. P. Mirabelli, B. B. Peebo, M. Xeroudaki, M. Koulikovska, and N. Lagali, "Early Effects of Dexamethasone and Anti-VEGF Therapy in an Inflammatory Corneal Neovascularization Model," *Experimental Eye Research* 125 (2014): 118–127, https://doi.org/10.1016/j.exer.2014.06.006.

85. D. Wu, K. E. Chan, B. X. H. Lim, et al., "Management of Corneal Neovascularization: Current and Emerging Therapeutic Approaches," *Indian Journal of Ophthalmology* 72, no. Suppl 3 (2024): S354–S371, https://doi.org/10.4103/IJO.IJO_3043_23.

86. D. Hos, D. R. Saban, F. Bock, et al., "Suppression of Inflammatory Corneal Lymphangiogenesis by Application of Topical Corticosteroids," *Archives of Ophthalmology* 129, no. 4 (2011): 445–452, https://doi.org/10.1001/archophthalmol.2011.42.

87. L. Contreras-Ruiz, F. A. Mir, B. Turpie, A. H. Krauss, and S. Masli, "Sjögren's Syndrome Associated Dry Eye in a Mouse Model Is Ameliorated by Topical Application of Integrin α4 Antagonist GW559090," *Experimental Eye Research* 143 (2016): 1–8, https://doi.org/10.1016/j.exer.2015.10.008.

88. J. Franz, T. F. Ng, S. Gupta, M. R. Wilson, M. E. Fini, and S. Masli, "Clusterin Reverses Epitheliopathy, Reduces Inflammation, and Restores Goblet Cells and Corneal Nerves in a Mouse Model of Autoimmune Dry Eye," *bioRxiv*: 2025.06.05.658110, https://doi.org/10.1101/2025.06.05.658110.

89. D. Welch, G. W. Ousler, and M. B. Abelson, "An Approach to a More Standardized Method of Evaluating Tear Film Break-Up Time," ARVO Annual Meeting Abstract (2003).

90. E. Moeller, "Tearfilm Deficiencies: Quality vs. Quantity," VetBloom Blog (2018), http://blog.vetbloom.com/ophthalmology/tear-film-deficiencies.

91. S. B. Han, Y. C. Liu, K. Mohamed-Noriega, L. Tong, and J. S. Mehta, "Objective Imaging Diagnostics for Dry Eye Disease," *Journal of Ophthalmology* 2020 (2020): 3509064, https://doi.org/10.1155/2020/3509064.

92. H. Iwashita, L. Sebbag, B. C. Leonard, and A. Saito, "A Review of Diagnostic Tests for Qualitative and Quantitative Tearfilm Deficiency in Dogs," *Veterinary Ophthalmology* 26, no. Suppl 1 (2023): 5–15, https://doi.org/10.1111/vop.13044.

93. L. D. Seyer, R. W. Wills, and C. M. Betbeze, "Investigation of Fluorescein Stain-Based Tearfilm Breakup Time Test Reliability in Dogs in a Clinical Setting," *American Journal of Veterinary Research* 82, no. 12 (2021): 1019–1025, https://doi.org/10.2460/ajvr.21.01.0002.

94. S. J. Lee, M. G. Han, S. J. Yang, Y. S. Choi, and J. Y. Kim, "A Novel Method of Evaluating the Non-Invasive Tear Film Break-Up Time and Progression of Corneal Opacification in Dogs Using Imaging Video," *Frontiers in Veterinary Science* 11 (2024): 1298467, https://doi.org/10.3389/fvets.2024.1298467.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

95. J. Y. Kim, M. G. Han, J. H. Chun, E. A. Huh, and S. J. Lee, "Developing a Diagnosis Model for Dry Eye Disease in Dogs Using Object Detection," *Scientific Reports* 12, no. 1 (2022): 21351, https://doi.org/10.1038/s41598-022-25867-y.

96. J. S. Wolffsohn, R. Arita, R. Chalmers, et al., "TFOS DEWS II Diagnostic Methodology Report," *Ocular Surface* 15, no. 3 (2017): 539–574, https://doi.org/10.1016/j.jtos.2017.05.001.

97. A. J. Bron, P. Argüeso, M. Irkec, and F. V. Bright, "Clinical Staining of the Ocular Surface: Mechanisms and Interpretations," *Progress in Retinal and Eye Research* 44 (2015): 36–61, https://doi.org/10.1016/jpreteyeres.2014.10.001.

98. S. C. Pflugfelder and C. S. de Paiva, "The Pathophysiology of Dry Eye Disease: What we Know and Future Directions for Research," *Ophthalmology* 124, no. 11S (2017): S4–S13, https://doi.org/10.1016/j.ophtha.2017.07.010.

99. K. J. Hirabayashi, E. K. Akpek, and S. Ahmad, "Outcome Measures to Assess Dry Eye Severity: A Review," *Ocular Immunology and Inflammation* 30, no. 2 (2022): 282–289, https://doi.org/10.1080/09273948.2022.2027461.

100. E. M. Messmer, S. Ahmad, J. M. Benitez Del Castillo, et al., "Management of Inflammation in Dry Eye Disease: Recommendations From a European Panel of Experts," *European Journal of Ophthalmology* 33, no. 3 (2023): 1294–1307, https://doi.org/10.1177/11206721221141481.

101. A. Saito, H. Iwashita, Y. Kitamura, Y. Miwa, and R. Arita, "Punctate Fluorescein Staining Scores in Dogs With or Without Aqueous Tear Deficiency," *Veterinary Ophthalmology* 24, no. 1 (2021): 28–36, https://doi.org/10.1111/vop.12829. Erratum in *Veterinary Ophthalmology* 26, no. 3 (2023): 275, https://doi.org/10.1111/vop.13106.

102. J. S. Eaton, P. E. Miller, E. Bentley, S. M. Thomasy, and C. J. Murphy, "The SPOTS System: An Ocular Scoring System Optimized for Use in Modern Preclinical Drug Development and Toxicology," *Journal of Ocular Pharmacology and Therapeutics* 33, no. 10 (2017): 718–734, https://doi.org/10.1089/jop.2017.0108.

103. X. Li, M. Wang, C. Chen, and N. Dong, "Advances in Understanding Conjunctival Goblet Cell Traits and Regenerative Processes," *Eye Science* 2, no. 1 (2025): 85–96.

104. S. K. Swamynathan and A. Wells, "Conjunctival Goblet Cells: Ocular Surface Functions, Disorders That Affect Them, and the Potential for Their Regeneration," *Ocular Surface* 18, no. 1 (2020): 19–26, https://doi.org/10.1016/j.jtos.2019.11.005. Erratum in *Ocular Surface* 23 (2022): 224, https://doi.org/10.1016/j.jtos.2020.02.010.

105. D. A. Dartt, R. R. Hodges, D. Li, M. A. Shatos, K. Lashkari, and C. N. Serhan, "Conjunctival Goblet Cell Secretion Stimulated by Leukotrienes Is Reduced by Resolvins D1 and E1 to Promote Resolution of Inflammation," *Journal of Immunology* 186, no. 7 (2011): 4455–4466, https://doi.org/10.4049/jimmunol.1000833.

106. A. A. Gage, M. A. Meenaghan, J. R. Natiella, and G. W. Greene, Jr., "Sensitivity of Pigmented Mucosa and Skin to Freezing Injury," *Cryobiology* 16, no. 4 (1979): 348–361, https://doi.org/10.1016/0011-2240(79)90048-8.

107. G. S. Chuang, W. Farinelli, and R. R. Anderson, "Selective Cryolysis of Melanocytes: Critical Temperature and Exposure Time to Induce Selective Pigmentary Loss in Yucatan Pig Skin," *Lasers in Surgery and Medicine* 53, no. 7 (2021): 978–985, https://doi.org/10.1002/lsm.23372.

108. S. M. Cox, K. K. Nichols, and J. J. Nichols, "Agreement Between Automated and Traditional Measures of Tear Film Breakup," *Optometry and Vision Science* 92, no. 9 (2015): e257–e263, https://doi.org/10.1097/OPX.0000000000000648.

14635224, 2026, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/vop.70163 by Ingrid Allgoewer , Wiley Online Library on [07/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License