CORNEO-LIMBO-CONJUNCTIVAL TRANSPOSITION TO TREAT DEEP AND PERFORATING CORNEAL ULCERS IN DOGS: A REVIEW OF 418 EYES AND CORNEAL CLARITY SCORING IN 111 EYES.

Prado Cebrian[1], Natalia Escanilla[1], Robert C. Lowe[1], Charlotte Dawson[2], Rick F. Sanchez[3]

1 Optivet Referrals, Havant, UK

2 Royal Veterinary College, Hatfield, UK

3 Specialistische Dierenkliniek (Anicura) Utrecht, Utrecht, NL

Correspondence

Prado Cebrian, Optivet Referrals, 3 Downley Road, PO9 2NJ, Havant, UK

Telephone 00441243888091

Email: prado@optivet.com

ABSTRACT

Purpose: to report surgical and corneal clarity scores (CCSs) of corneo-limbo-conjunctival transpositions (CLCTs) in a large number of canine cases.

Methods: retrospective review of records that underwent CLCT to repair deep ulcers or perforations between 2002-2018. Signalment, concurrent eye disease, additional procedures, pathogenesis, medication, graft orientation, follow-up and CCSs were recorded.

Results: 418 eyes of 399 dogs were included. Brachycephalics were most commonly affected, comprising 325/418 (77.75%) of the eyes. The most commonly affected breeds were Pugs, Shih-Tzus, Cavalier King Charles Spaniels and French Bulldogs, with 116/418 (27.75%), 64/418 (15.31%), 34/418 (8.13%), and 34/418 (8.13%) ulcerated eyes, respectively. Mean age at surgery was 5.5 years (range 59 days – 17.7 years) and median follow-up time was 100 days (range 3 days - 7.64 years). The most common etiopathogenesis was spontaneous ulceration in 205/418 eyes (49.04%) of which 191 (93.17%) occurred in brachycephalics. Primary keratoconjunctivitis sicca affected 122/418 eyes (29.19%) and injury 39/418 eyes (9.33%). Mean ulcer width was 3.5 mm (0.5-10 mm). Success rate was 97.13% (406/418 eyes). Failure endpoints recorded included no menace response, secondary glaucoma and endophthalmitis. Pre-existing perforation was found in 101/418 (24.16%) of the eyes and significantly increased failure rate ($p<0.001$). The median CCS was G3 (G0-G4), which was lower for Pugs (G2). Graft orientation affected CCS, but did not reach statistical significance.

Conclusion: The high success rate and CCS for CLCT in dogs make it a good technique to treat deep ulcers but a less desirable outcome is anticipated when treating perforations and Pugs.

KEY WORDS: corneal grafting, canine, corneal transparency, corneal surgery, corneal reconstruction.

1. INTRODUCTION

Ulcerative keratitis is a common and widely varied ocular disease in the dog.[1–9] The facial characteristics of brachycephalics and some spaniels have long been suspected to play a role in the development of deep corneal ulcers associated with KCS.[4] Recently, a large population study reported that brachycephalic and spaniel-type dogs with prominent eyes have a general increased susceptibility to corneal ulceration.[10] Surgical repair of corneal ulcers is recommended in cases where corneal stromal loss is greater than 50% of corneal depth.[2] A common form of surgical repair is corneal reconstruction through grafting, which aims to preserve the eye and corneal clarity where possible.[1,2] Multiple corneal reconstructive techniques have been described utilizing conjunctiva, amniotic membrane, corneal tissue and synthetic or bioengineered materials.[3–21]. The use of lamellar corneal grafting techniques can better preserve corneal transparency when compared to conjunctival pedicle grafting. [18,22–26] Autologous grafts offer the advantage that they avoid immune mediated graft rejection and the need for corneal banking. [6,18,22] Lamellar corneo-scleral transplantation (CST) is an autologous graft technique that uses cornea and the adjacent sclera.[18] A modification of this technique is corneal-scleral-conjunctival transposition (CSCT), which uses a sliding graft of peripheral cornea, limbus and bulbar conjunctiva. [22,23] Other surgeons refer to this technique as corneo-conjunctival transposition (CCT)[24–26], while others refer to it as corneo-limbo-conjunctival transposition (CLCT)[27] to highlight the inclusion of the limbus. The authors of the present study have used the term CLCT.

Two separate reviews of CLCTs in cats reported surgical success rates of 100% and 94.8% in series of 17 and 97 patients respectively. [24,25] The studies in felines focused on the repair of corneas affected by corneal sequestrum and this is a disease that is extremely rare in dogs, as

suggested by the very low number of affected dogs in the veterinary literature.[28,29] Taking this into consideration in addition to potential interspecies differences that might affect corneal healing, studies that focus on CLCTs in dogs are warranted. Comparatively speaking, there have been very few reports on the use of CLCTs in canines including a preliminary report of canine cases with deep corneal ulcers that suggested the technique deserved further study in this species.[27] The first published series on the use of CLCT in dogs supported the use of this technique.[26] It also concluded that further evidence on the outcome of a larger number of cases and the use of an objective assessment of corneal clarity were needed.[26]

The main objectives of the present study are to report the surgical outcome of a large number of CLCTs in dogs and the resulting corneal clarity scores (CCSs) using a validated corneal clarity scoring scale [1] in a large proportion of these cases.

2. MATERIAL AND METHODS

2.1 Animals and inclusion criteria

The authors performed a retrospective review of the medical records of dogs that underwent a CLCT by a variety of surgeons to repair deep corneal ulcers (i.e. ≥50% depth) and perforations between 2002 and 2018 at the following centers: Optivet Referrals (Havant, UK), North Kent Referrals (Kent, UK), Royal Veterinary College (Hatfield, UK) and Specialistische Dierenkliniek Utrecht, (Utrecht, NL).

Inclusion criteria accepted animals with a complete ophthalmic examination prior to surgery that included Schirmer tear test I (Schering-Plough Animal Health Corporation, Union, NJ), menace response, palpebral, dazzle and pupillary light reflexes, slit lamp biomicroscopy (Kowa SL15 or SL17; Kowa Company, Ltd, Tokyo, Japan), rebound tonometry (TonoVet®; Tiolat, or Tono-pen Vet®; Icare, Finland), indirect ophthalmoscopy (Vantage Plus; Keeler or

Omega 500; Heine, Ettenheim, Germany) with a hand-held 30D lens (Volk Optical Inc, Ohio, USA) when not precluded by the corneal changes and fluorescein testing (Minims® Fluorescein sodium 1% w/v, Bausch & Lomb, UK).

Data collected included signalment, cause of the ulcer, depth, eye affected, concurrent eye diseases, surgeon, additional procedures performed, medication before and after surgery, graft orientation, follow up time, outcome, complications, and CCS, when available. Only CCSs that were performed on the patient, as opposed to images, were included.

Cases were excluded if patients underwent intraocular surgery (i.e. intracapsular lens extraction or phacoemulsification) at the same time of corneal repair.

2.2 Pre-operative preparation

Pre-medication was chosen for each patient based on their temperament and general health, including an opioid (methadone 0.1-0-3mg/kg IM/IV, Comfortan®, Dechra, UK) and/or acepromazine (0.005-0.1mg/kg IV/IM, Acepromazine, Elanco, UK) or dexmedetomidine (0.003-0.005mg/kg IV/IM, Dexdomitor®, Vetoquinol, UK). Following routine pre-oxygenation, anaesthesia was induced with propofol (PropoFlo®Plus, Zoetis UK Limited) or alfaxalone (Alfaxan®, Jurox, UK) to effect. General anesthesia was maintained at an appropriate level with Isofluorane (IsoFlo®, Zoetis UK Ltd.), or sevofluorane (SevoFlo®, Zoetis UK Ltd.) in 100% oxygen after endotracheal intubation. Patients received an intravenous antibiotic during general anesthesia if indicated (cefuroxime, Zinacef®, Glaxo Smith Kline, UK, at 15mg/kg of body weight) and an injectable nonsteroidal anti-inflammatory drug (carprofen, Rimadyl®, Zoetis UK limited, at 4mg/kg of body weight, or meloxicam, Metacam®, Boehringer Ingelheim Animal Health UK Ltd, at 0.1 mg/kg of body weight), unless it was considered contraindicated.

Patients were positioned under an operating microscope for the procedure, in dorsal or lateral recumbency, depending on the surgeon's preference, and an intravenous neuromuscular blocking agent was administered IV (atracurium, Tracrium®, GlaxoSmithKline, UK) alongside mechanical ventilation and train of four monitoring. A single drop of topical anesthesia (Minims® Proxymetacaine hydrochloride 0.5% w/v, Bausch & Lomb, UK) was applied 1 minute prior to first corneal incision, when available.

2.3 Surgical procedure

All cases included underwent a CLCT graft in a similar manner. The walls of the corneal ulcer were debrided with the aid of the disposable controlled depth blade (Sharpoint® 300-micron restricted depth knife, Pennsylvania, USA) and/or a diamond knife to remove any damaged stroma. Then, the ulcer was cut into a square in three of the four sides that make up this shape, purposely leaving the edge of the ulcer closest to the distal edge of corneal section of the graft to be cut later. Then, the path of the graft was cut into the tissue by extending the two parallel incisions on either side of the ulcer in the direction of the corneoscleral limbus until they went over it and into the adjacent conjunctiva. The surgeon made sure the sides of the graft diverged from one another so that the graft was approximately 0.5 to 1mm wider than the ulcer bed. Then, the corneal section of the graft was undermined at a depth of approximately half corneal thickness. This was achieved using a disposable lamellar dissection knife (Alcon® crescent knife angled bevel up, Texas, USA) starting at the side of the graft that offered the most comfortable position for the dominant hand of the surgeon. Care was taken to introduce the dissector knife at least 0.5mm under the entire limbal section of the graft to make sure the cutting edge of a scissor blade could be later introduced into it. When necessary and in order to completely free the corneal section of the graft, straight Vannas scissors were used to cut the incision line of the corneal section of the graft opposite

the one used as the entry point for the corneal lamellar dissector. Then, curved Westcott tenotomy scissors were used to undermine the conjunctival section of the graft. After this, one blade of the tenotomy scissor was introduced under the conjunctiva and the other under the limbus so the graft containing cornea, a section of limbus and conjunctiva could be freed from its limbal attachment. The entire graft was then lifted and transposed, which sometimes necessitated further subconjunctival dissection. The incomplete square cut around the ulcer permitted that a rim of ulcerated cornea remained attached to the distal end of the graft. This additional amount of corneal tissue served as a disposable grasping surface that helped manipulate the graft without risking traumatizing the rest of it. Once the graft was ready for suturing the rim of excess cornea was cut using the restricted depth blade, diamond knife and/or Vannas scissors. Some of the surgeons cut a complete square around the ulcer before extending the incision path in the direction of the corneoscleral limbus on either side of the ulcer. This meant there was no disposable grasping surface for graft handling later. The graft was secured to the recipient bed with monofilament 9-0 polyglactin 910 on a spatulated needle (Vicryl®, Johnson & Johnson Medical, UK) in all cases. All surgeons used a simple interrupted suture pattern while some surgeons also used a continuous suture pattern in parts of the graft or over the simple interrupted sutures. The first simple interrupted sutures were placed in the corneal section of the graft or in the limbal section of the graft followed by the corneal section of the graft. In either case, the conjunctival section was always sutured last. Depending on the surgeon, the first three sutures of the corneal section were placed either at the corners of the graft and then between them, or at the center between the corners, and then also at the corners. At the end, some surgeons rotated the simple interrupted suture knots into the suture tracks to bury the knots.

2.4 Post-operative treatment

All the animals were prescribed a broad-spectrum antibiotic such as triple topical antibiotic combination drop containing neomycin sulphate 1700 IU/ml, polymixin B sulphate 5000 IU/ml and gramycidin 25 IU/ml (Neosporin®, Dominion Pharma, England, UK), or chloramphenicol (Chloramphenicol 0.5% eye drops, Martindale Pharma, UK) or ofloxacin eye drops (Exocin® 0.3% eye drops, Allergan, Ireland) 3 to 4 times daily for approximately 2 weeks. Depending on the degree of reflex uveitis and presence of concurrent KCS, a course of topical atropine sulfate 1% (Atropine Eye Drops, Martindale, England, UK or Minims® atropine sulfate 1% w/v, Bausch & Lomb, UK) or cyclopentolate (Minims® cyclopentolate hydrochloride 1% w/v , Bausch & Lomb, UK) was also prescribed for 1 to 10 days. A course of oral nonsteroidal anti-inflammatory (Carprofen, Rimadyl®, Zoetis UK limited, at 4mg/kg of body weight, or meloxicam, Metacam®, Boehringer Ingelheim Animal Health UK Ltd, at 0.1 mg/kg of body weight) was prescribed once daily for 5-7 days in every case unless there was a contraindication. A one-week course of oral antibiotic (cephalosporine, Rilexine®, Virbac limited, at 15 mg/kg of body weight or amoxicillin-clavulanic acid, Synulox®, Zoetis UK limited, at 12.5 mg/kg of body weight) was prescribed in patients undergoing concurrent eyelid surgery and/or in perforated globes. All animals were fitted with a protective collar for 1 to 2 weeks. Animals with KCS were prescribed cyclosporine 0.2% ointment (Optimmune®, Scherring Plough Animal Health, England, UK) once to twice daily, and a tear supplement, such as a carbomer 2mg/g containing viscous gel (Viscotears®, Novartis Pharmaceuticals, UK Ltd) or sodium hyaluronate based (Clinitas®, sodium hyaluronate 0.4%, Altacor, UK). Depending on surgeons´ preference, a 2 to 4-week course of topical steroid vs. non-steroidal eye drops and/or topical tacrolimus was dispensed 7-10 days after surgery in cases that showed a prominent vascular reaction and/or progressing corneal pigmentation.

2.5 Follow-up

Re-examinations were scheduled at approximately 1, 4, 8 and 12 or 16 weeks postoperatively with some cases having further visits if recommended for a variety of reasons such as re-evaluation of KCS.

2.6 End points:

These included severe corneal opacification leading to a negative menace response, graft failure that despite surgical or medical treatment led to loss of vision or loss of the eye, and other postoperative events that led to development of potentially blinding diseases such as glaucoma or endophthalmitis. These were considered failures. Graft problems that were amenable to medical or surgical therapy and led to a visual and comfortable eye were counted as complications but not as failures.

2.7 Corneal clarity scoring:

Corneal clarity scoring was assessed at last scheduled visit following the validated CCS scale (e.g. a scale shown to have good inter- and intra-observer reproducibility when used to score the central corneas of small animals therefore making it apt to assess the outcome of surgically managed corneal diseases and to compare outcomes of surgical techniques between authors) described by Sanchez *et al* [1], as summarized below:

G0: no fundus reflection is visible on retroillumination using a head-mounted indirect ophthalmoscope.

G1: a fundus reflection is visible with retroillumination as described in G0.

G2: a 0.1 -mm diameter light beam from a handheld slit lamp is visible with the naked eye on the anterior surface of the iris and/or lens.

G3: gross fundic features are visible when viewed with indirect ophthalmoscopy using a head-mounted indirect ophthalmoscope and a handheld 30D lens, although fine details are not clear.

G4: fine details of the fundic features are clearly visible with indirect ophthalmoscopy as described in G3.

2.8 Statistical analysis

Tests of associations between pre-existing conditions and surgical failure were calculated with a Pearson's Chi Square test of association and a P-value ≤ 0.05 was considered as significant. Correlations between CCS, breed, pre-existing perforation and concurrent medial canthoplasty surgery were assessed by Mann-Whitney U Test, and a P-value ≤ 0.05 was considered as significant. Comparison of graft position (dorsal, dorsolateral, lateral, ventrolateral, ventral, ventromedial, medial and dorsomedial), and comparison of effects of medication either as individual medications or grouped as steroidal, non-steroidal anti-inflammatory or other immunosuppressive were also performed with Mann-Whitney U Test, and a P-value ≤ 0.05 was considered as significant. These comparisons were made across the entire cohort and also between Pug and non-Pug breeds.

Wizard 1.9.39 was used for statistical analysis.

3. RESULTS

3.1 Animals

The search yielded 399 medical records comprising a total of 418 surgeries that complied with the inclusion criteria. There were 203 right eyes and 213 left eyes and in two cases the side of the affected eye was not specified. There were 19 bilateral cases. Brachycephalic breeds were most commonly affected accounting for 325/418 (77.75 %) of the eyes affected.

Non-brachycephalic breeds accounted for 93/418 (22.25%) of the eyes affected. The most common breeds affected were Pugs (116/418, 27.75%), Shih-Tzus (64/418, 15.31%), Cavalier King Charles Spaniels (34/418, 8.14%) and French Bulldogs (34/418, 8.14%). (Graph 1). There were 249 males (59.57%) and 169 females (40.43%). Mean age at surgery was 5.5 years (range 59 days – 17.7 years), with a median of 4.8 years.

3.2 Pathogenesis, ulcer description and additional procedures

The cause of the ulceration was not determined in 205/418 cases, of which 191 (93.17%) were brachycephalics. KCS was found in 122/418 eyes (29.19%), injury in 39/418 eyes (9.33%) and calcium keratopathy in 19/418 eyes (4.55%). Other causes, such as ectopic cilium and entropion, accounted for 33/418 of the eyes (7.89%).

All the ulcers were centrally or pericentrally located (i.e. affected the visual axis). Mean ulcer width was 3.5 mm (0.5-10 mm). Small perforations that were pre-existing or that occurred intra-operatively were identified in 101/418 cases (24.16%). Concurrent surgeries were performed in 172/418 (41.15%) of the cases, including medial canthoplasty (135/418, 32.3%), distichia removal (16/418, 3.83%) (i.e. wedge excision, cryotherapy or electrolysis), entropion correction (10/418, 2.39%), eyelid mass resection (8/418, 1.91%), subconjunctival cyclosporine implantation (5/418, 1.2%) and ectopic cilium excision (5/418, 1.2%).

3.3 Follow up and outcome

Median follow up time was 100 days (range 3 days – 7.64 years). Success rate was 97.13% (406/418). Failure endpoints recorded included absence of menace response or loss of the globe (i.e. enucleation due to severe complications such as endophthalmitis or secondary glaucoma). Severe complications that led to the loss of the eye accounted for the short follow up times. There were 12/418 (2.87%) failures, leading to enucleation in 11/12 (91.66%) cases

and blindness in one eye (1/12 (8.33%). A total of 5/12 (41.67%) were right eyes and 7/12 (58.33%) were left eyes. Males and females were equally represented, and brachycephalic breeds accounted for 7/12 (58.33%). A total of 8/12 (66.67%) eyes had a pre-existing perforation at the site of the ulcer, 2/12 (16.67%) had a descemetocele and 2/12 (16.67%) had a deep stromal ulcer. A total of 9/12 (75%) of the failures were enucleated due to secondary glaucoma or endophthalmitis at a mean time to failure of 24.5 days. The diagnosis of endophthalmitis were clinical and later confirmed with histopathology. A total of 2/12 (16.67%) eyes developed a corneal perforation post-operatively and were enucleated. Neither of the 2 eyes presented with a perforation prior to surgery. Lastly, 1/12 (8.33%) eyes lost all functional vision after developing graft retraction and excessive scarring.

There were a total of 32/418 (7.65%) additional post-operative complications that did not fit the criteria to be considered failures, bringing the total of complications to 44/418 (10.52%). There were four cases that developed corneal perforation post-grafting bringing the total count of post-operative perforations to 6/418 (1.43%). These additional 4 cases were treated successfully with a second graft. Of these, 3 presented with a perforation prior to surgery. Graft retraction occurred in 6/418 (1.43%) cases but all healed successfully, with 5 requiring only medical therapy and 1 requiring re-suturing of the graft. A total of 22/418 (5.3%) cases developed epithelial inclusion cysts and 2/418 (0.5%) cases developed post-operative superficial stromal mineral deposition in the periphery of the corneal graft (i.e. corneal degeneration). Two of the 22 (9.09%) cases with epithelial inclusion cyst development were considered for surgical removal of the cysts because the cysts were large. One case underwent epithelial inclusion cyst excision a year after the initial graft surgery and it resolved without problems. The owner of the other patient decided against surgery because there was no obvious ocular discomfort and the dog was aggressive and geriatric.

Pre-existing perforation, which was found in 24.16% (101/418) of the eyes, significantly increased failure rate (i.e. 8 failures compared to 4 for non-perforated eyes) ($p<0.001$). No significant association was found between risk of corneal perforation and age of the patient or size of the corneal ulcer at presentation.

3.4 Corneal clarity score

Corneal clarity was scored in a total of 111/418 (26.55%) eyes. Brachycephalic breeds were also most common in this part of the study as they accounted for 96/111 (86.49%). A total of 33/111 (29.73%) were Pugs, which made up 33/96 (34.37%) of the brachycephalic patients. The overall median CCS was G3 (range G0 to G4) (Fig. 1). Pugs had a median CCS of G2, and they were statistically more likely to have a poor CCS compared to all other breeds ($p=0.002$) (Graph 2). Corneal clarity in Pugs was affected by the development pigmentary keratitis. There was no statistically significant difference on median CCS between brachycephalic and non-brachycephalic groups ($p=0.321$). The median CCS was lower in perforated eyes (G2, $p=0.015$) compared to non-perforated eyes (G3) (Graph 3).

The median post-operative follow-up at the time of corneal clarity scoring was 1 year (range 3 months – 5.8 years). Performing a medial canthoplasty in brachycephalics at the time of CLCT did not have an effect on CCS (Graph 4).

Comparison of the effects of medication (either as individual medications or grouped as steroidal, non-steroidal anti-inflammatory or other immunosuppressive) on CCS were made across the entire cohort and between Pug and non-Pug breeds, and did not show significant correlations (i.e. median CCS of each group was not affected, overall G3 and G2 in Pugs). Comparison of graft position (dorsal, dorsolateral, lateral, ventrolateral, ventral, ventromedial, medial and dorsomedial) across the entire cohort and between Pug and non-Pug breeds showed trends that affected CCS without reaching statistical significance. These included a worse CSS in non-Pug breeds on CLCTs that were harvested from the dorsal cornea (median

dorsal graft CCS of 3 compared to 4 for other orientations (p=0.06). In Pugs there was less of a difference between dorsal and other orientations (median dorsal graft CCS of 1.5 compared to 2 for other orientations. (p=0.131)).

4. DISCUSSION

There is a relatively low number of clinical studies in the veterinary literature that describe the use of autologous lamellar grafting in dogs.[6,16–18,26] The first published series on the use of CLCT in dogs suggested that further evidence on the outcome of a larger number of cases and the use of an objective assessment of corneal clarity were needed.[26] The objectives of the present study were to report the surgical outcome of a large number of CLCTs in dogs and the CCS of a proportion of these cases using the validated scoring system proposed by Sanchez *et al*.[1] The results of the present study showed that the CLCT technique is associated with a high success rate in dogs and a median CCS that is in the high end of the scale used.

Some authors recommend autologous corneal lamellar transposition for defects smaller than 25–30% of the corneal diameter to avoid potentially deficient tectonic support if used to reconstruct larger defects and due to the fact there is a limited availability of healthy cornea that can be used to treat larger defects.[22] In the current study, most CLCTs fell inside this size category, though some were larger. Larger CLCTs did not develop more problems than smaller ones. However, large CLCTs may mobilize a non-desirable amount of conjunctiva into the visual axis, which can negatively affect quality of vision even in cases with a positive menace response and an acceptable CCS in the corneal section of the graft. Graft dehiscence can occur due to corneal melting, inappropriate graft bed preparation (i.e. insufficient conjunctival dissection, unhealthy bed), aqueous humor leakage, excessive tension on the graft or sutures, inappropriate suture placement and/or infection of the graft.[13] The retrospective nature of this study meant that it was not easy to determine the exact cause of

each graft failure. Failure was seen in a relatively low number of cases, but it meant enucleation could not be completely avoided. In the study by Gogova *et al* there were no graft failures.[26] Comparatively speaking, the existence of failures in the present study, though low, highlights the importance of analyzing as high a number of cases as possible when studying the results of a surgical technique. All of the graft complications reported in this study healed following intensive medical management, graft re-suturing or use of a second graft. This is in agreement with the Gogova *et al* study.[26] However, the Gogova *et al* study had a total complication rate of 21%[26], which was approximately twice as high as the one reported in the present study. The study of Gogova *et al* did not report further on the possible factors that might have been associated with the failures observed. However, the higher number of cases in the present study might account for a part of this difference. Breed, gender, size of the graft and cause of the ulcer did not appear to have a statistically significant association with graft failure in the present study, but the existence of a pre-existing perforation did.

The use of autologous lamellar corneal transposition procedures in dogs have previously been described to repair deep corneal ulcers and small corneal perforations.[18,20,26,27] However, it stands to reason that since there is no endothelium in the transposed section of cornea, progressive corneal edema and possible graft dehiscence could result if a graft were used to repair a corneal perforation that had also damaged the endothelial layer in that spot. The study of Brightman *et al* (1989) reported finding clear corneas after autologous grafting of full thickness defects using a transposition of lamellar cornea that lacked an endothelial cell layer.[6] The authors described finding endothelial cells with features of polymorphism and polymegathism under the graft over time, suggesting an attempt by the endothelium to res-establish itself.[6] In the Gogova *et al* study just over half the cases were perforations and the study did not mention if any of the complications encountered were associated with the presence of perforation prior to surgery.[26] There were almost twice as many perforations in

the present study, which found that CLCT grafting of perforated ulcers had a higher risk of graft failure. Therefore, the authors recommend caution when choosing to perform a CLCT to treat a perforated ulcer, even if it is a perforation with a small diameter.

A proportion of failures in the present study were due to the development of endophthalmitis and/or secondary glaucoma. Endophthalmitis requires the entrance and settlement of bacterial into the eye,[30] and it is an obvious potential complication of perforated ulcers. However, clinicians need to also consider secondary glaucoma as a potential complication after corneal reconstruction with CLCT even in eyes with no obvious pre-existing predisposition to glaucoma. Secondary glaucoma is caused through a number of complex pathways including chronic inflammation, infection and/or damage to the iridocorneal angle[31–33] and, therefore, it could be difficult to avoid in some of the patients undergoing CLCT.

Other complications observed in this study included corneal scarring, corneal degeneration and development of epithelial inclusion cysts. However, none of these complications altered the subjective appreciation of ocular comfort in any of the patients and did not appear to alter the visual outcome in the cases where the problem developed after the cornea had healed.

It is difficult to pinpoint the exact cause of an epithelial inclusion cyst, but generally speaking these cysts are theorized to occur through the forceful introduction of epithelial cells into the tissue that lies under the epithelial cover, where the cyst is formed.[34] Surgical trauma during tissue handling and/or needle trauma during suturing are the most likely causes of the epithelial inclusion cysts seen in this study.

Corneal pigmentation and corneal scarring affect corneal clarity and maintenance of corneal clarity should be a primary goal of corneal reconstruction techniques that affect the central cornea. Corneal transparency following corneal grafting has previously been evaluated in other studies.[13,24,26,35–37] However, whenever possible it is advisable that a validated corneal clarity scoring system (i.e. one that has been shown to have a high intra- and inter-user

variability) is utilized to evaluate corneal clarity, as this tool supports the objective analysis of the surgical results and the comparison between techniques and authors.[1] The overall median corneal clarity score in the present study was high. This appears to be in agreement with the results of the Gogova *et al* study[26], although a more balanced comparison of clarity between the two studies is not possible.

It is interesting to note that Pugs in this study had a lower CCS than other breeds. Corneal pigmentation is a concern in the breed, with studies showing pigmentary keratitis in the Pug affecting anywhere from 70% to up to 91.1% of animals in a variety of populations from the US and the EU.[38–41] A study published by O'Neil *et al* in 2017 provided evidence of a strong predisposition to corneal ulcerative disease in brachycephalic dogs using an exceptionally large, non-referral population.[42] The O'Neil *et al* study also showed that compared to crossbred dogs, the Pug, Boxer and Shih Tzu were specifically at risk.[42] Not surprisingly, brachycephalics, including Pugs, were the most common breeds in the current study. Interestingly, though the median CCS in brachycephalics in the present study was high, it was significantly lower for Pugs. The reduced corneal clarity in Pugs in the present study was associated with the development of pigmentary keratitis. This supports the finding of the Gogova *et al* study that also found pigmentary keratitis to negatively affect corneal clarity.[26]

A study that performed in vivo confocal microscopy in affected and unaffected Pugs, reported the presence of inflammatory changes in the corneas of Pugs with pigmentary keratitis.[40] Another study found, for the first time, an association between the presence and severity of medial entropion of the lower eyelid and the presence of pigmentary keratitis in Pugs.[41] This supported the theory that the irritation caused by the entropion leads to the pigmentation response typically seen in pigmentary keratitis in this breed.[41] The study by Gogova *et al* found that patients that had pigmentary keratitis prior to surgery had a more opaque cornea than patients that did not have corneal pigmentation.[26] Due to the retrospective nature of the

present study it was not possible to know with certainty how many patients had pigmentary keratitis prior to surgery. The Gogova *et al* study also found that medial canthoplasty had no effect on the degree of corneal opacification in the 8 cases included in that group.[26] This is in agreement with the findings of the present study that included 10 cases in this category. It is possible the low number of animals in this category in each of the studies might have affected the results. It is also possible that other factors besides the presence of medial lower eyelid entropion played a role in the progression of pigmentary keratitis in Pugs after CLCT. Pigmentary keratitis in Pugs has been shown to progress with increasing age.[41] However, in the comparatively shorter time of the post-operative period it seems likely that the development of pigmentary keratitis could partly be due to the irritation caused by the surgery and/or the presence of corneal sutures. All the surgeries reported here were performed with the same type of absorbable suture material (polyglactin 910, 9-0), which has been shown to elicit a foreign body response in the corneas of dogs.[43] Absorption of polyglactin is usually complete within six weeks[43,44] and CCSs in the present study deteriorated rapidly in some cases, and especially in Pugs, reaching the worst CCS at the time the sutures had completely disappeared (Fig. 2). A study that compared the effects of nylon and Polyglactin 910 on perilimbal corneal wound healing in dogs reported that both materials were associated with a similar foreign body (e.g. granulomatous) response.[43] However, polyglactin sutures used in conjunctival surgery of humans resulted in slightly more conjunctival reaction in the early postoperative period compared to nylon sutures.[45] Interestingly, a study on autologous lamellar corneal grafting in dogs determined that although non-absorbable sutures produced less corneal vascularization initially, absorbable sutures were associated with less vascularization in the longer term because they disappeared once they dissolved compared to non-absorbable sutures that were left in place for more than six weeks.[6] It is possible that the same factors that appear to predispose Pugs to pigmentary keratitis in association with lower

medial eyelid entropion,[41] and that at least in part appear to be inflammatory,[40] could also play a role in the reaction to corneal inflammation caused by surgery and absorbable sutures compared to other breeds less likely to develop corneal pigmentation. Further studies that investigate the type of suture used in CLCT surgeries are needed to improve the CCS of dog breeds with predictably lower CCS after surgery, such as Pugs. These studies will need to take into account the pre-operative presence or absence of pigmentary keratitis and entropion of the medial lower eyelid of the animals included.

The authors of the present study attempted to identify whether graft orientation had an effect on CCS. A trend showing that dorsally harvested CLCTs might have worse CCS was not statistically significant. The Gogova *et al* study found no statistical correlation between degree of corneal opacity and graft orientation. Graft orientation could also be part of future investigative efforts, though it is possible that an even higher number of cases than in the present study might be necessary to further our understanding of this specific aspect of CLCTs.

All the dogs in this study that were diagnosed with KCS were treated with an immune-modulator/lacrimostimulant drug since before or immediately after surgery and for the long-term. Overall, cases affected with KCS did not show a significantly different CCS compared to the rest of the animals in the study. This is in agreement with the findings of the study by Gogova *et al* that included 40 cases diagnosed with KCS.[26] The large number of cases with KCS in the present study would have allowed to have non-treated controls. However, no untreated cases were included, as their inclusion in a clinical paper could have easily had a negative impact on individual patient welfare.

The limitations in this study are those of a retrospective multicenter study, such as the different medical management used pre- and postoperatively in some cases, and the fact that re-examination periods were performed at different times due to differences in personal

preference and scheduling factors. For instance, this accounted for a wide time range of the follow up when the corneal clarity score was granted. Due to the retrospective nature of the study it was not possible to know if the length of the follow up time had a positive or negative effect on the corneal clarity scoring. A prospective study design could help determine this. Moreover, the retrospective nature of the study did not allow the authors to examine the effects of pre-existing tissue pigmentation, such as conjunctival, corneal, and/or limbal pigmentation, on the postoperative development of pigmentation of the graft. Lastly, there were multiple surgeons with different degrees of experience, although every surgeon followed the same technique with very small variations.

5. CONCLUSIONS

Corneo-limbo-conjunctival transposition is an effective surgical treatment for deep, central and paracentral corneal ulcers, as demonstrated through the large number of treated eyes included in this study. Although the technique may also be used in small perforations the success rate is lower than for non-perforations. In addition, the visual outcome, evaluated for the first time using a validated CCS, was good, but it was reduced in Pugs and in cases with pre-existing corneal perforation.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

REFERENCES

1. Sanchez RF, Dawson C, Matas Riera M, Escanilla N. Preliminary results of a prospective study of inter- and intra-user variability of the Royal Veterinary College corneal clarity score (RVC-CCS) for use in veterinary practice. *Vet Ophthalmol*. 2016;19(4):313-318.

2. Sanchez RF. The cornea. In: *BSAVA Manual of Canine and Feline Ophthalmology*. British Veterinary Small Animal Association; 2014:200-231.

3. Lavignette A. Lamellar keratoplasty in the dog. *Small Anim Clin*. 1962;2:183-187.

4. Mueller FO. Short-term experiments on grafting fresh and frozen corneal tissue in dogs. *Br J Ophthalmol*. 1968;52(10):752.

5. Blogg, J. R., Dutton, A. G., & Stanley RG. Use of third eyelid grafts to repair full-thickness defects in the cornea and sclera. *J Am Anim Hosp Assoc*. 1989.

6. Brightman, A. H., McLaughlin, S. A., & Brogdon JD. Autogenous lamellar corneal grafting in dogs. *J Am Vet Med Assoc*. 1989;195(4):469-475.

7. Hacker D V. Frozen corneal grafts in dogs and cats: a report on 19 cases. *J Am Anim Hosp Assoc*. 1991.

8. Barros, P. S., Garcia, J. A., Laus, J. L., Ferreira, A. L., & Salles Gomes TL. The use of xenologous amniotic membrane to repair canine corneal perforation created by penetrating keratectomy. *Vet Ophthalmol*. 1998;1(2-3):119-123.

9. Hansen, P. A., & Guandalini A. A retrospective study of 30 cases of frozen lamellar

corneal graft in dogs and cats. *Vet Ophthalmol*. 1999;2(4):233-241.

10. Lewin GA. Repair of a full thickness corneoscleral defect in a German shepherd dog using porcine small intestinal submucosa. *J Small Anim Pract*. 1999;40(7):340-342.

11. Barros, P. S., & Safatle AM. Congenital scleral staphyloma in a dog repaired with preserved homologous peritoneum. *Vet Ophthalmol*. 2000;3(1):27-29.

12. Barros, P. S., Safatle, A. M., Godoy, C. A., Souza, M. S., Barros, L. F., & Brooks DE. Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. *Vet Ophthalmol*. 2005;8(3):189-192.

13. Chow DWY, Westermeyer HD. Retrospective evaluation of corneal reconstruction using ACell VetTM alone in dogs and cats: 82 cases. *Vet Ophthalmol*. 2016;19(5):357-366.

14. Tanner Jr, J. C., Smith, J. P., Bradley, W. H., & Rife CC. Lamellar keratoplasty: use of a collagen graft for corneal replacement. *Eye Ear Nose Throat Mon*. 1968;47(8):368.

15. Mueller, F. O., & Formston C. Keratoplasty in the dog. *Res Vet Sci*. 1969;10(2):168-179.

16. Dice, P. F., Severin, G. A., & Lumb W V. Experimental autogenous and homologous corneal transplantation in the dog. *Anim Hosp*. 1973.

17. Keller WF. The clinical application of homologous corneal and scleral grafts in the dog. *Vet Surg*. 1976;5(1):32-34.

18. Parshall CJ. Lamellar corneal–scleral transplantation. *J Am Anim Hosp Assoc*. 1973;9:220–277.

19. Kuhns EL. Conjunctival patch grafts for treatment of corneal lesions in dogs. *Mod Vet Pract*. 1979.

20. Hakanson NE MR. Conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. *J Am Anim Hosp Assoc*. 1987;23:641.

21. Scagliotti RH. Tarsoconjunctival island graft for the treatment of deep corneal ulcers, desmetoceles, and perforations in 35 dogs and 6 cats. *Semin Vet Med Surg (Small Anim)*. 1988;1:69–76.

22. Wilkie DA, Whittaker C. Surgery of the cornea. *Vet Clin North Am - Small Anim Pract*. 1997;27(5):1067-1107.

23. Gelatt KN, Brooks DE. Surgery of the cornea and sclera. In: *Veterinary Ophthalmic Surgery*. Elsevier Ltd; 2011:191-236.

24. Andrew SE, Tou S, Brooks DE. Corneoconjunctival transposition for the treatment of feline corneal sequestra: A retrospective study of 17 cases (1990-1998). *Vet Ophthalmol*. 2001;4(2):107-111.

25. Graham KL, White JD, Billson FM. Feline corneal sequestra: outcome of corneoconjunctival transposition in 97 cats (109 eyes). *J Feline Med Surg*. 2017;19(6):710-716.

26. Gogova S, Leiva M, Ortillés Á, et al. Corneoconjunctival transposition for the treatment of deep stromal to full-thickness corneal defects in dogs: A multicentric retrospective study of 100 cases (2012-2018). *Vet Ophthalmol*. January 2020:vop.12740.

27. Sanchez RF. Repair of deep corneal ulcers associated with primary keratoconjunctivitis sicca (KCS) in dogs: technique, follow up and outcome of 3 cases treated with corneolimbalconjunctival transposition (CLCT) and cyclosporine 0.2% (Optimmune®, Scherring Slough, Eng. In: *ECVO Meeting Proceedings*. ; 2007.

28. Pizzirani S, Beamer G. Corneal sequestrum in a dog with chronic unilateral

keratoconjunctivitis sicca. *Artic J Am Vet Med Assoc*. 2013.

29. Bouhanna L, Liscoët LB, Raymond-Letron I. *Corneal Stromal Sequestration in a Dog*. Vol 11.; 2008.

30. Bell CM, Pot SA, Dubielzig RR. Septic implantation syndrome in dogs and cats: A distinct pattern of endophthalmitis with lenticular abscess. *Vet Ophthalmol*. 2013;16(3):180-185.

31. Gelatt KN, MacKay EO. Secondary glaucomas in the dog in North America. *Vet Ophthalmol*. 2004;7(4):245-259.

32. Johnsen, D. A., Maggs, D. J., & Kass PH. Evaluation of risk factors for development of secondary glaucoma in dogs: 156 cases (1999–2004). *J Am Vet Med Assoc*. 2006;229(8):1270-1274.

33. Strom AR, Hässig M, Iburg TM, Spiess BM. Epidemiology of canine glaucoma presented to University of Zurich from 1995 to 2009. Part 2: secondary glaucoma (217 cases). *Vet Ophthalmol*. 2011;14(2):127-132.

34. Dubielzig R, Ketring K, McLellan G, Albert D, Davis FA. Veterinary Ocular Pathology. In: *Veterinary Ocular Pathology*. Elsevier Inc.; 2010.

35. Goulle F. Use of porcine small intestinal submucosa for corneal reconstruction in dogs and cats: 106 cases. *J Small Anim Pract*. 2012;53(1):34-43.

36. Laguna F, Leiva M, Costa D, Lacerda R, Peña Gimenez T. Corneal grafting for the treatment of feline corneal sequestrum: a retrospective study of 18 eyes (13 cats). *Vet Ophthalmol*. 2015;18(4):291-296.

37. Lacerda RP, Peña Gimenez MT, Laguna F, Costa D, Ríos J, Leiva M. Corneal grafting for the treatment of full-thickness corneal defects in dogs: a review of 50 cases. *Vet*

Ophthalmol. 2017;20(3):222-231.

38. Labelle AL, Dresser CB, Hamor RE, Allender MC, Disney JL. Characteristics of, prevalence of, and risk factors for corneal pigmentation (pigmentary keratopathy) in Pugs. *J Am Vet Med Assoc*. 2013;243(5):667-674.

39. Krecny M, Tichy A, Rushton J, Nell B. A retrospective survey of ocular abnormalities in pugs: 130 cases. *J Small Anim Pract*. 2015;56(2):96-102.

40. Vallone L V., Enders AM, Mohammed HO, Ledbetter EC. In vivo confocal microscopy of brachycephalic dogs with and without superficial corneal pigment. *Vet Ophthalmol*. 2017.

41. Maini S, Everson R, Dawson C, Chang YM, Hartley C, Sanchez RF. Pigmentary keratitis in pugs in the United Kingdom: prevalence and associated features. *BMC Vet Res*. 2019;15(1):384.

42. O'Neill DG, Lee MM, Brodbelt DC, Church DB, Sanchez RF. Corneal ulcerative disease in dogs under primary veterinary care in England: epidemiology and clinical management. *Canine Genet Epidemiol*. 2017;4(1):1-12.

43. van EE RT, NASISSE MP, HELMAN G, SANDERS W. Effects of Nylon and Polyglactin 910 Suture Material on Perilimbal Corneal Wound Healing in the Dog. *Vet Surg*. 1986;15(6):435-440.

44. Cowden JW. The clinical evaluation of 9-0 and 10-0 monofilament Polyglactin 910 absorbable suture for corneal surgery. *Ophthalmic Surg*. 1979;10(1):50-52.

45. Wong VWY, Rao SK, Lam DDC. Polyglactin sutures versus nylon sutures for suturing of conjunctival autograft in pterygium surgery: A randomized, controlled trial. *Acta Ophthalmol Scand*. 2007;85(6):658-661.

Graph 1. Distribution of breeds included in the study.

Graph 2. Comparison between CCS in Pugs (n = 116) versus other breeds (n = 302), showing that Pugs have a lower CCS with a median of G2, compared to G3 in the rest of the population (Results of Mann-Whitney U Test between “CCS" and Pug vs. others, p value: 0.002)

Graph 3. Comparison between CCS in perforated (n = 101) vs. non-perforated eyes (n = 317), showing perforated eyes had a lower CCS (Results of Mann-Whitney U Test between CCS and perforation vs. non-perforation, p value: 0.015)

Graph 4. Comparative CCS between brachycephalics having medial canthoplasty surgery (n = 135) or not (n = 190), showing no statistically significant differences (Results of Mann-Whitney U Test between CCS and MCP, p value: 0.545)

FIGURE 1. Examples of different surgical outcomes and the CCS assigned to each.

FIGURE 2. Example of progression of pigment in a case with no pre-existing pigmentary keratitis. Same eye at 1, 6 and 15 weeks post operatively (concurrent subconjunctival CsA implant)

Figure 1

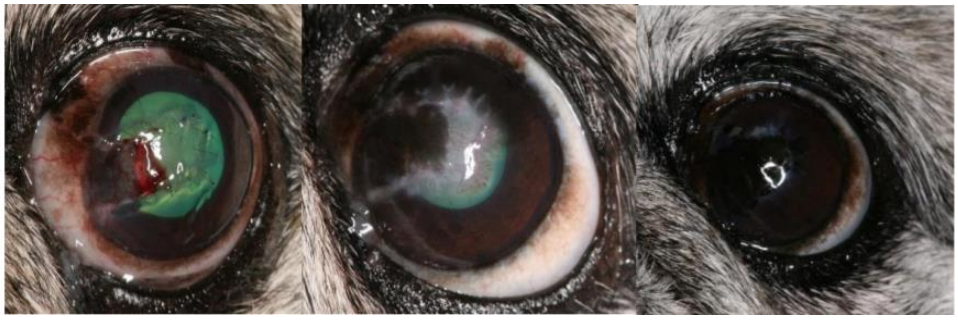
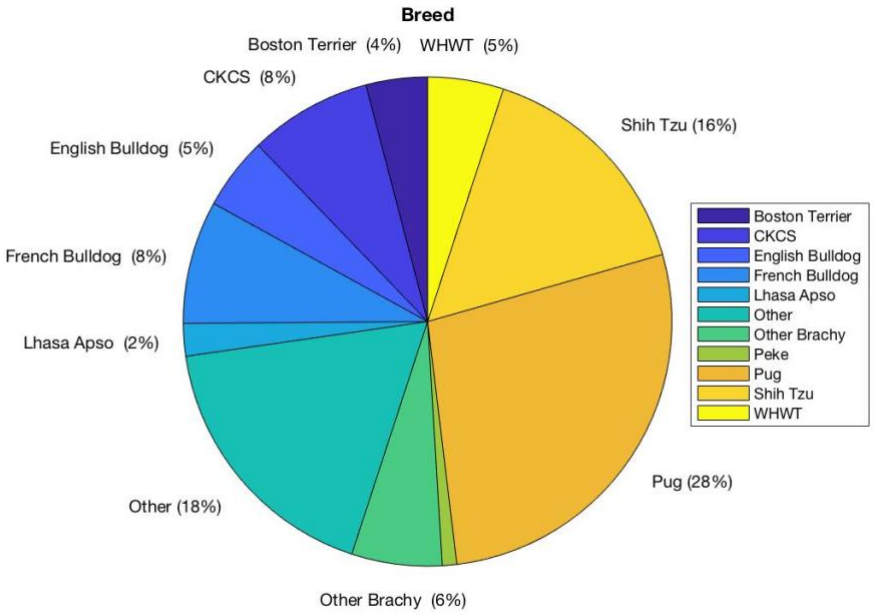
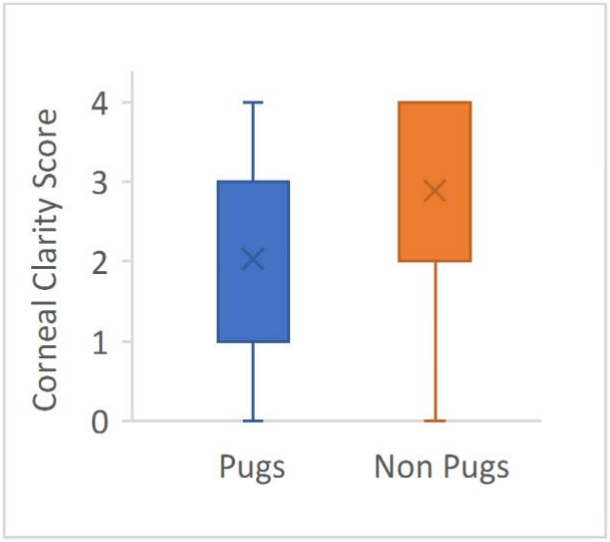


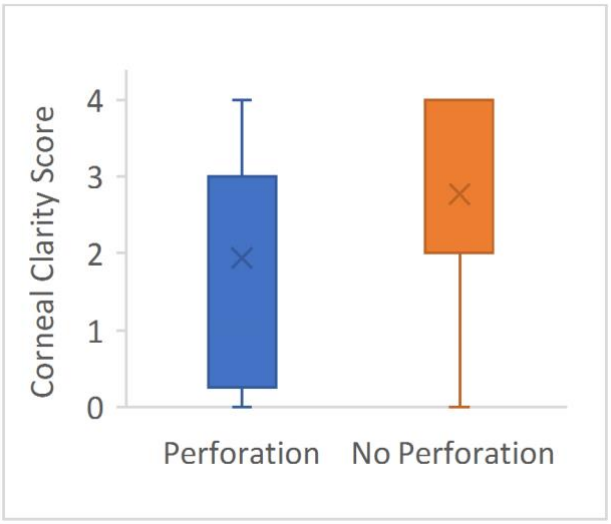
Figure 2



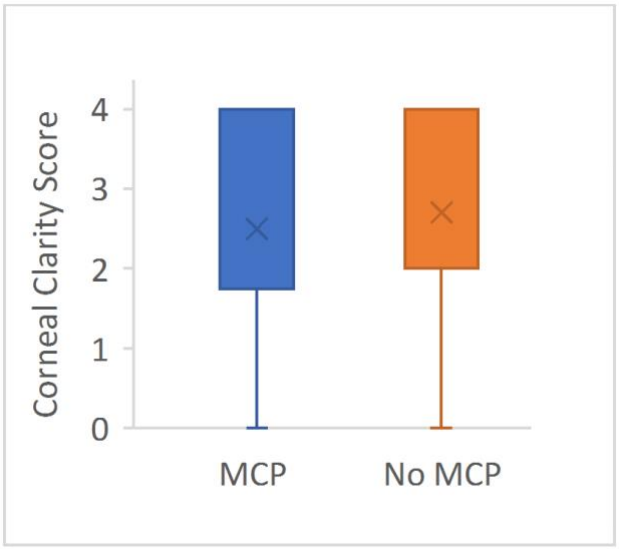
Graph1



Graph2



Graph3



Graph4