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Early Application of Bevacizumab After Sclerocorneal Grafting for Patients With Severe Late-Stage Ocular Chemical Burns

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Purpose: To investigate whether subconjunctival bevacizumab help prevent corneal graft neovascularization and prolong the graft survival of patients with chemical burns.

Methods: We performed a prospective nonrandomized comparative case series study. Twenty-six eyes received subconjunctival bevacizumab (10 mg/0.4 mL) once and topical immunosuppressive agents after sclerocorneal lamellar keratoplasty as the treatment, and 13 eyes received a topical immunosuppressant alone and served as the control group. The main outcomes were a cumulative probability of graft survival, development of corneal neovascularization, and complications.

Results: The postoperative follow-up time was 14.3 months (range, 2–62 mo). The cumulative graft survival time was significantly longer in the treatment group than that in the control group (42.9 ± 5.9 vs. 4.8 ± 0.7 mo; log rank < 0.001). In the treatment group, 19 of the 26 grafts (73.1%) survived as transparent with a mean follow-up of 18.7 ± 3.0 months. At the end of the follow-up, 4 grafts remained free of neovascularization, 2 developed edema without neovascularization, and 15 remained transparent with a stable ocular surface and some neovascular vessels in the peripheral transplant interface. The other 5 grafts became opaque and neovascularized. In the control group, all grafts became opaque and neovascularized within the follow-up period (5.5 ± 0.7 mo). During the follow-up, a corneal epithelial defect developed in 9 eyes in the treatment group and 7 in the control group.

Conclusions: Early application of subconjunctival bevacizumab after sclerocorneal lamellar keratoplasty can significantly prevent corneal neovascularization and promote graft survival for severe late-stage ocular chemical burns.

Key Words: bevacizumab, corneal neovascularization, sclerocorneal grafting, ocular chemical burns

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In recent years, most burned cases have been reported in developing countries. In eyes with severe chemical burns, the consequences of chronic inflammation, symblepharon, corneal conjunctivization, and corneal neovascularization (CNV) are often significant.[1] Corneal grafts on eyes in this condition have a notorious prognosis because of a preexisting limbal stem cell failure and CNV.[2] A large-diameter corneal graft with a live limbus is usually needed to simultaneously restore the limbal function and central corneal clarity.[3] The postoperative prevention of high-risk corneal graft rejection is challenging in chemical burns. Moreover, no consensus on the efficacy of systemic immunosuppressive agents with variable success rates and unpredictable systemic side effects for high-risk keratoplasty has been reached.[4]

After keratoplasty for a burned cornea, the principal risk factor for graft failure is CNV.[5,6] An intense CNV outgrowth is a common phenomenon in this kind of condition.[7] Some reports have suggested that the application of antivascular endothelial growth factor (VEGF) agents may better prevent CNV in penetrating keratoplasty.[8,9] However, the neovascular vessels did not disappear for a few patients with burns after a bevacizumab treatment, a recombinant anti-VEGF monoclonal antibody.[10] Whether antiangiogenic therapy will inhibit the growth and invasion of neovascular vessels into a lamellar corneal graft is still unknown, thus preventing a lamellar graft rejection for patients with burns.

This comparative study aims to investigate whether the early application of subconjunctival bevacizumab combined with routine topical immunosuppressive agents and without

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systemic immunosuppression can prolong the sclerocorneal lamellar graft survival of patients with severe late-stage ocular chemical burns, thus improving the outcome and reducing the medical expense for low economic families.

METHODS

Patients

From May 2012 to July 2017, a total of 39 consecutive patients with severe late-stage ocular chemical injuries were enrolled in this prospective nonrandomized comparative study at the Zhongshan Ophthalmic Center. The study protocol conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. Informed consent was obtained from every patient before participation in the study.

The inclusion criteria were as follows: 1) diffusely neovascularized and opaque cornea due to chemical burns with complete limbal deficiency, 2) adult patients with a minimal interval of 6 months from the initial injury to surgery, and 3) a posterior cornea with at least 50 μm thickness with a normal reflectivity in an ultrasound biomicroscopy and a smooth endothelial surface in optical coherence tomography. The exclusion criteria were as follows: 1) a severe eyelid defect; 2) Schirmer test results of less than 2 mm; 3) no accurate light perception or intraocular pressure greater than 21 mm Hg or Tn+1 by finger perception; 4) a severity of symblepharon greater than grade 2, according to the Tseng classification[11]; 5) retinal detachment and/or dense vitreous clouds detected in a B-ultrasound imaging; 6) a history of any other treatment with anti-VEGF agents (topical or systemic); and 7) uncontrolled hypertension, pregnancy, or a history of thromboembolic events.

All patients underwent lamellar keratoplasty with a large-diameter sclerocorneal graft that had been preserved within 5 days from death in a corneal preservative medium (Eurosol, Corneal Chamber; Alchimia, Ponte San Nicolo, Italy). Before and after the surgery, possible postoperative complications were explained in detail to all patients and they were advised to treat the grafted eye with an anti-VEGF agent. The treatment group patients volunteered to receive a subconjunctival injection of bevacizumab when their ocular surfaces were negative for fluorescein staining, whereas the control group patients opted for a routine treatment protocol.

Surgical Procedures

All surgeries were performed by the same surgeon (S.-y.Z.). In brief, a 360-degree perilimbal conjunctival incision was made under general and local anesthesia. After the release of symblepharon and excision of the scarring tenon tissues, the opaque corneal tissues were removed layer by layer with a self-made beard blade. During the surgery, the neovascular vessels and central opaque stroma in the recipient cornea were thoroughly removed under a surgical microscope. In cases of pre-Descemet stromal scarring, a combination of the techniques of big bubble,[12] stromal swelling,[13] fine forceps tearing,[14] and viscoelastic agent usage was used for exposing and separating Descemet membrane. A sclerocorneal graft without Descemet membrane was transplanted and fixed with interrupted 10-0 nylon suture bites. In cases with a bulbar conjunctival defect, an amniotic membrane graft was transplanted to reconstruct the conjunctival surface.

Routine Treatment and Follow-up After Lamellar Keratoplasty

Owing to the unaffordable cost and the various adverse events caused by systemic immunosuppressive agents, all patients in this study received no systemic immunosuppressive treatment, but they received a combination of topical immunosuppressive agents with topical dexamethasone 0.1% (TobraDex eye drops, Alcon Inc, Belgium), tacrolimus 0.05% eye drops (Zhongshan Ophthalmic Center Pharmaceuticals, China), and a night treatment with dexamethasone 0.1% eye ointment (TobraDex eye ointment; Alcon, Inc). The dexamethasone eye drops were given for 3 weeks and then tapered to twice daily for 5 months, followed by once daily for another 6 months. The administration of tacrolimus and lubricants eye drops was continued 4 times daily for half a year and then tapered to twice daily. During the follow-up period, the administration of dexamethasone and tacrolimus eye drops was discontinued if a persistent corneal epithelial defect was identified and usage resumed after a corneal reepithelialization. When the corneal graft was rejected, then the intensive administrations of dexamethasone (eye drops once per hour; 2.5 mg by subconjunctival injection and 10 mg by intramuscular injection) and tacrolimus eye drops (4 times daily) were given for at least 1 week.

Patient follow-ups were conducted every week for the first month, then every month for the first half year, and then every 3 months. In the cases with corneal epithelial defects, intensive care and follow-up were applied. If the entire corneal graft became neovascularized or opaque after an intensive antirejection treatment, then the follow-up would cease. At each check, visual acuity was routinely tested, intraocular pressure was measured, and slit-lamp microscopy was performed. The invasion of neovascular vessels into the interface between the recipient corneal bed and the corneal graft was documented. At each visit, the graft survival and CNV were monitored with slit-lamp photographs.

Injection Protocol for Subconjunctival Bevacizumab

The subconjunctival injection of bevacizumab was administered in the treatment group once the corneal graft had completely reepithelialized. The injection was performed in an outpatient clinic with a 25 G needle under topical anesthesia with proparacaine hydrochloride 0.5% eye drops (Alcaine eye drops; Alcon, Inc). Subconjunctival injections were provided in 4 quadrants at sites that were 2 mm away from the graft limbus, with each injection comprising 2.5 mg/0.1 mL of bevacizumab, resulting in a total of 10 mg of bevacizumab per patient.

Statistical Analysis

All statistical analyses were performed by Professor Futian Luo with SPSS software (version 16.0, SPSS, Inc, Chicago, MI) for Microsoft Windows (Microsoft Inc, Redmond, WA). Comparisons were made between treatment and control groups. A χ^2 test compared the gender, distribution of eyelid disorder, and preexistence of CNV before the treatment, and an independent samples t test compared the age, uncorrected visual acuity, intervals from burns to surgery, timing of the injections, and follow-up time. The Kaplan–Meier method, Tarone–Ware test, and log-rank test compared the rates of graft survival. Graft survival was defined as a transparent corneal graft, whereas graft failure was defined as an opaque corneal graft and the development or regrowth of CNV. A P value of less than 0.05 was considered statistically significant.

RESULTS

This study enrolled 39 eligible patients. A treatment group with 26 patients received a postoperative treatment with subconjunctival bevacizumab, and a control group with 13 patients did not. The postoperative follow-up time varied from 2 to 62 months (mean, 14.3 ± 2.2) up to February 2018. Patients' information before and after the treatment is listed in Table 1. No significant differences were found between treatment and control groups in age, gender, preoperative visual acuity, intervals from a chemical injury to surgery, existence of CNV on the first day after the surgery, and number and area of combined amniotic membrane grafts.

TABLE 1. The Characteristics of Included Patients With Chemical Burns

	Bevazicumab	Control	*P*
Gender, no. (%)			
Men	21 (81)	12 (92)	0.36
Women	5 (81)	1 (8)	
Age, mean (SD), (range), yrs			
Years	27.2 (10.7), (16–46)	33.7 (14.2), (16–60)	0.12
Chemical property, No. (%)			
Alkaline	23 (88)	10 (77)	0.38
Acidity	3 (12)	3 (23)	
UCVA			
Preoper., mean (SD)	2.86 (0.23)	2.73 (0.65)	0.51
Postoper., (LogMAR)	0.93 (0.58)	1.86 (0.78)	<0.001
With eyelid disorder	5 (88)	6 (46)	0.27
Combined AMT	10 (36)	5 (38)	>0.99
CNV residual	6 (23)	2 (15)	0.69
Intervals, mean (SD), (range), mo	40.1 (62.2), (6–240)	58.2 (75.3), (4–193)	0.16
Timing of injection, mean (SD), (range), d	11.2 (0.6), (6–14)	NA	
Follow-up, mean (SD), (range), mo	18.7 (3.0), (3–62)	5.5 (0.7), (2–9)	0.003

AMT, amniotic membrane transplantation; CNV, corneal neovascular vessels; CNV Residual, corneal neovascular vessels were noted in the recipient corneal stroma at the first day postoperatively; Intervals, the time from injury to surgery; LogMAR, logarithm of the minimum angle of resolution; NA, nonavailable; Postoper., postoperative; Preoper., preoperative; UCVA, uncorrected visual acuity.

In the treatment group, 19 of 26 grafts (73.1%) successfully survived after the bevacizumab treatment to the end of the follow-up period (mean, 18.7 mo). On the first postoperative day, in the treatment group, 20 eyes did not have any residual recipient corneal vessels, whereas 6 eyes had peripheral CNV. After giving the bevacizumab treatment, 9 of 20 eyes developed new vessels. The time from the surgery to injection varied from 6 to 14 days (mean, 11.2 ± 0.6). During the first half-year of follow-up for the patients treated with bevacizumab, a new peripheral CNV formed in another 5 eyes and residual CNV narrowed in 6 eyes. Among the 5 eyes with a newly formed CNV, 1 eye had acute graft rejection and diffuse CNV at the first month after injection and 1 eye was chronically rejected and became an opaque failure. At the end of the follow-up period, 4 grafts remained free of CNV (Fig. 1), 2 grafts developed edema without CNV because of endothelial dysfunction secondary to endotheliitis, and 15 grafts remained transparent with a stable ocular surface and some CNV in the peripheral transplant interface (see Supplemental Figure 1, Supplemental Digital Content 1, http://links.lww.com/ICO/A973). The other 5 grafts became opaque and neovascularized, among which 2 eyes experienced acute graft rejection at the 9th and 21st months, respectively. The limbal area remained intact in 6 grafts, and superficial CNV or fibroconnective pannus locally encroached onto 20 eyes within 7.8 ± 1.4 months (range, 0.5–23 mo). Acute limbal graft rejection was noted after 1 postoperative year in 1 patient who had received bevacizumab. Nonetheless, the patient was successfully treated.

In the control group, all grafts became opaque and neovascularized failures, with a mean follow-up of 5.5 months (range, 2–9 mo). On the half-year visit, 4 of 13 grafts remained clear at the center and had some peripheral CNV. At the end of the follow-up period, all grafts had become irreversibly opaque. Neovascularization could be seen in all control group patients. Clinical episodes included a rapid neovascular invasion of the transplant interface and into the graft, which, in some instances, occurred as early as the first postoperative week (Fig. 2).

A Kaplan–Meier graft survival curve shows that the cumulative graft survival time in the treatment group was significantly longer than that in the control group (42.9 ± 5.9 vs. 4.8 ± 0.7 mo; $P < 0.001$) (Fig. 3). The postoperative uncorrected visual acuity at the end of the follow-up period was better in the treatment group than that in the control group (logarithm of the minimum angle of resolution, 0.96 ± 0.57 vs. 1.63 ± 0.78; $P = 0.031$).

In the treatment group, 9 of 26 eyes developed a corneal epithelial defect after the bevacizumab injection at 15 to 100 days (mean, 40.9 ± 29.9 d). The defect was cured in 5 to 32 days (mean, 14.5 ± 8.0 d) after treatment with artificial tears and a corneal bandage contact lens (see Supplemental Figure 2, Supplemental Digital Content 2, http://links.lww.com/ICO/A974). Newly formed neovascular vessels developed in 9 eyes, and the existing interface of neovascular vessels

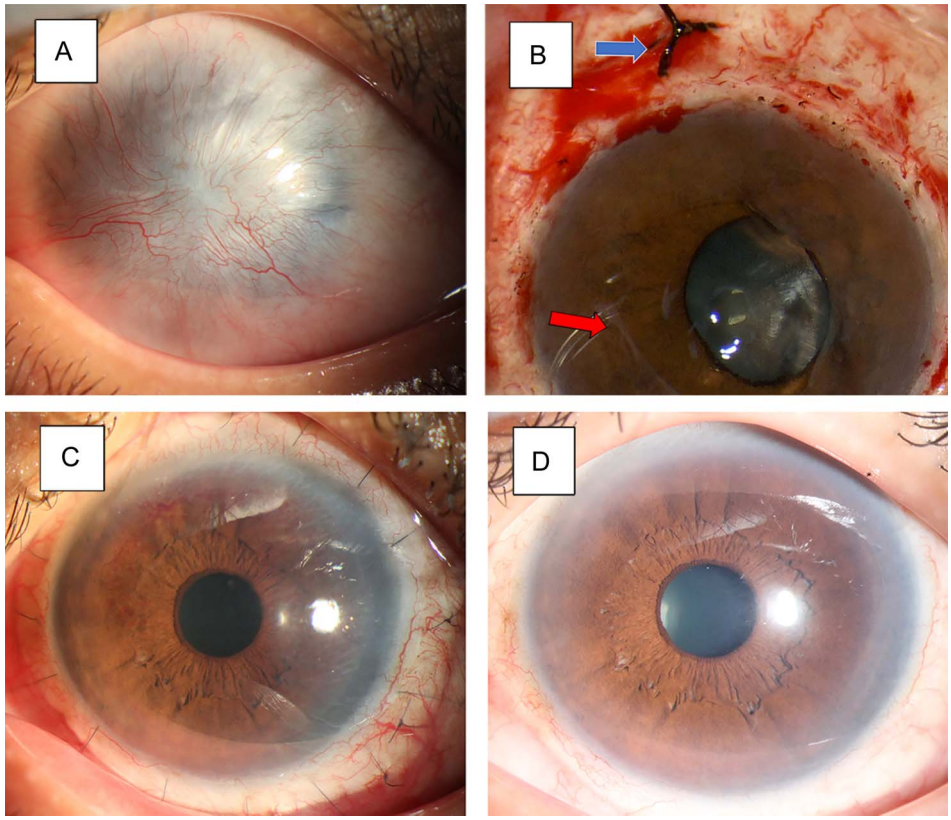

FIGURE 1. Graft remained free of CNV after surgery in the treatment group. A, A 27-year-old man with a diffusely neovascularized and opaque cornea due to quicklime burn. B, Scleral traction suture provides exposure (blue arrow). Neovascular vessels were thoroughly removed and the central Descemet membrane exposed, covered by viscoelastic agents and surrounded by a peripheral planting bed (red arrow). C, Ten days after surgery; when the subconjunctival bevacizumab was injected, the entire lamellar corneal graft was clear, the corneal surface was integrated, and mild blood was present at the transplant interface. D, Nineteen months after the bevacizumab treatment, the corneal graft remained clear without any neovascular vessels.

progressed in 5 eyes. In the control group, 7 eyes developed corneal epithelial defects during the follow-up period, which were cured with the development of corneal neovascular vessels. In those patients with corneal graft epithelial defects, repeated trichiasis, entropion, or lagophthalmos was noted in 3 eyes in the treatment group and in 6 eyes in the control group. These eyelid disorders were treated during the follow-up. No significant difference was observed in the occurrence of the corneal epithelial defects ($P = 0.25$) between treatment and control groups and in the eyelid disorders in the patients with epithelial defects ($P = 0.27$).

Interface hemorrhage was noted in 9 eyes in the treatment group and 5 eyes in the control group at the first postoperative day. Each hemorrhage was flushed in the operation room and completely resolved within 1 month. After resolution of the interface hemorrhage, a regrowth of the neovascular vessels into the peripheral transplant interface was noted in 6 of 9 eyes in the treatment group and in all 5 eyes in the control group. Subconjunctival hemorrhaging was noted in 4 eyes after the bevacizumab injection and was resolved within 2 weeks.

DISCUSSION

The prognosis of the corneal graft for severe corneal burns is very poor. Systemic cyclosporine A has been shown to have no positive effect for suppressing rejection in high-risk corneal transplantation but a relatively high incidence of systemic side effects.[15] Without systematic administration, topical immunosuppressive agents combined with antiangiogenic therapy are considered an efficient option for improving the prognosis of high-risk keratoplasty.[16–18] The sclerocorneal lamellar grafts of 73.1% survived as transparent in 18.7 ± 2.9 months with the single use of subconjunctival bevacizumab and topical tacrolimus and corticosteroid agents alone in this study. By contrast, all the corneal grafts were rejected within half a year in the control cases with a routine topical immunosuppression.

For chemically burned eyes, CNV is the principal risk factor for corneal graft failure, which can cause lamellar interface scarring and graft rejection like in this report.[2,5] Preoperative fine-needle vessel coagulation of CNV combined with bevacizumab is almost infeasible for our patients because of the deep corneal vessels and opaque cornea despite its efficiency for occluding vessels.[19] Thus, a complete removal of the CNV until the exposure of Descemet membrane is important during surgery for patients with chemical burns. Even with great effort during surgery, some residual peripheral CNV resumed at the first postoperative day. The false impression of vessel removal under surgical microscopy may be attributed to the discontinuation of blood perfusion in the deep located peripheral CNV.

This study demonstrated that an early bevacizumab treatment significantly improved the corneal graft survival. In the treatment group, 19 of 26 (73.1%) grafts remained clear, with a mean follow-up of 18.7 months. By contrast, all the corneal grafts in the control group became neovascularized and opaque. The effect of subconjunctival bevacizumab on high-risk lamellar keratoplasty was similar to that of a previous study on penetrating keratoplasty.[20] A meta-analysis

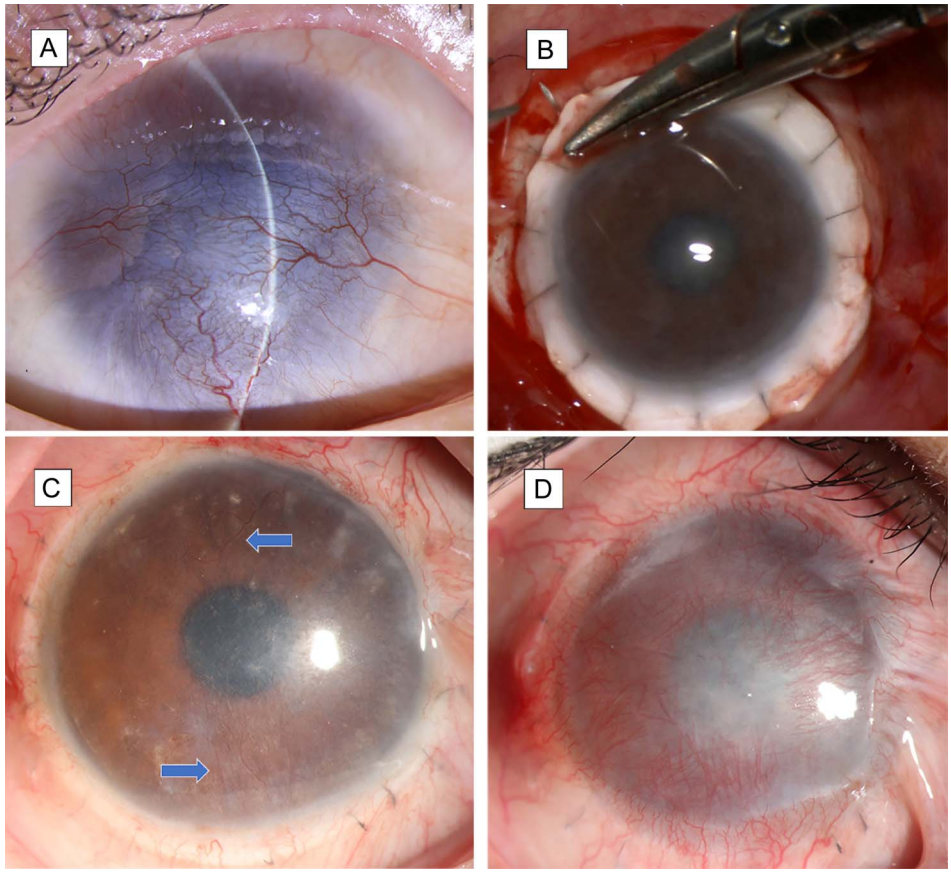

FIGURE 2. Result of the sclerocorneal lamellar grafting in the control group. A, Preoperative slit-lamp appearance. B, Fixation of the sclerocorneal graft during surgery. C, After sclerocorneal lamellar keratoplasty combined with an amniotic membrane transplantation, the corneal graft was clear at week 1 with slight residual vascular vessels in the recipient cornea (blue arrow). D, Four months after the surgery, the transplant interface was full of vascular vessels (red arrow), which invaded the on-lay sclerocorneal graft with a symblepharon formation.

demonstrated that a larger number of quadrants of CNV increase the risk of graft failure.[2] A high amount of VEGF-A detected in the recipient corneal stroma[18] may suggest that anti-VEGF treatment possibly prevents corneal stromal rejection. The suppression of CNV is also beneficial in preventing lipid deposition, which may worsen visual acuity.

In this study, bevacizumab prevented the development of CNV in 6 of 11 eyes and inhibited the progression of residual and newly formed corneal vessels. However, old and new corneal vessels did not disappear after the bevacizumab treatment. Reports have suggested that bevacizumab treatment may be more effective on recent-onset CNV than on mature vessels in animal[21,22] and human.[23,24] Some authors have suggested that preconditioning and/or early bevacizumab application can be beneficial for high-risk keratoplasty.[9,25,26] In this study, the timing of the bevacizumab injection depended on the patients' decision to join the treatment group. Based on our results, subconjunctival bevacizumab should be given immediately after surgery. If so, then using a fresh donor cornea with an intact epithelium would be essential because a prolonged epithelial defect and corneal erosion may occur after the application of anti-VEGF agents.[25,27]

Despite special precaution to use short-term preserved donor cornea with intact epithelium, 9 of 26 eyes developed new corneal epithelial defects within 3 months of the bevacizumab injection. However, whether the occurrence of the corneal epithelial defects was related to the application of the anti-VEGF agents is still unclear because some patients had repeated trichiasis or entropion. Bevacizumab has been reported to be safe at a dosage of 2.5 mg/0.1 mL per quadrant and up to 12.5 mg/0.5 mL, as reported in previous animal[28,29] and clinical studies.[9,25]

The effect of bevacizumab on the fate of limbal allografts is interesting to note. After the first introduction by Kenyon and Tseng,[30] the long-term survival of limbal grafts remains a challenge.[31] Antiangiogenic therapy has been demonstrated to improve the long-term survival of limbal allograft.[32] In our study, 6 sclerocorneal grafts with a heterogeneous limbus remained intact during the entire follow-up period, and 15 of 20 grafts with locally encroached superficial CNV or fibrovascular pannus. However, in 1 patient without interface CNV, an acute limbal rejection occurred after 1 postoperative year. These results support that subconjunctival bevacizumab may prolong the survival of limbal allografts while inhibiting the invasion of neovascular vessels into the cornea, but it may not prevent chronic dysfunction and acute graft rejection. Limbal graft rejection may be independent of CNV because of the limbal's abundant antigen-presenting cells, such as Langerhans cells.[33]

The main drawbacks of this study are the limited sample size, short follow-up period, and nonrandomization of the patients' enrolment. A randomized multicenter clinical trial should be conducted to determine the optimal method,

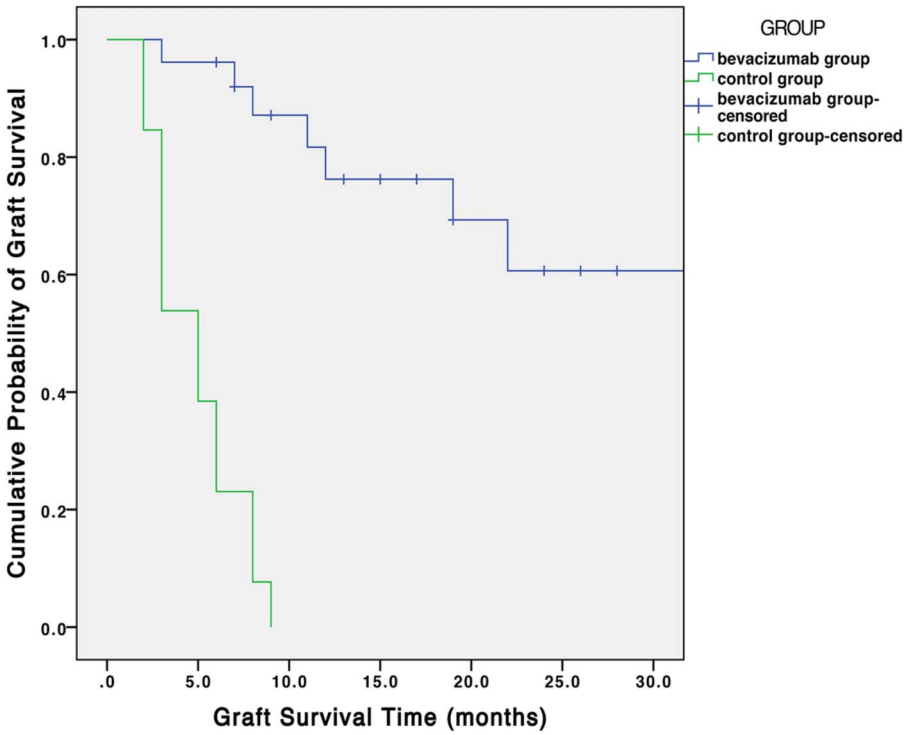

FIGURE 3. Kaplan–Meier survival analysis of sclerocorneal lamellar grafts. The results show that the corneal graft failed significantly earlier in the control group than that in the bevacizumab treatment group (42.9 ± 5.9 vs. 4.8 ± 0.7 mo; *log rank* < 0.001, Tarone–Ware < 0.001). The symbol + denotes the censored data, which included participants without graft failure up to the last follow-up.

timing, and dosage of bevacizumab usage in keratoplasty after acquiring chemical burns.

In conclusion, this study demonstrated that the early application of an anti-VEGF agent with a topical immunosuppressant alone can significantly improve the lamellar corneal graft survival for patients with CNV due to chemical burns. A thorough removal of the corneal vessels combined with an early application of anti-VEGF agents after surgery is critical for preventing the growth and development of postoperative CNV. In addition, a fresh sclerocorneal graft with an intact corneal epithelium is preferred. After the application of anti-VEGF agents, an intense follow-up and monitoring of the ocular surface epithelial defects are required, especially for patients with an abnormal eyelid function.

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