Treatment Failure after Lamellar Keratoplasty for Fungal Keratitis

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Purpose: To evaluate treatment failure after lamellar keratoplasty (LK) for fungal keratitis.

Design: Retrospective, interventional case series.

Participants: Two hundred eighteen patients (218 eyes) with fungal keratitis who failed to respond to medical therapy and underwent LK at the Shandong Eye Institute between January 1998 and July 2005.

Methods: Detailed medical history was obtained from each patient. Antifungal drugs were administered for at least 7 days before LK was performed. A trephine that was 0.5 mm larger in diameter than the fungal infection was used to create a lamellar incision to excise the ulcer. After treatment failure was observed after surgery, penetrating keratoplasty (PK) was performed. The distribution of risk factors for treatment failure was analyzed.

Main Outcome Measures: Clinical features and risk factors for treatment failure.

Results: Seventeen patients (7.8%) experienced treatment failure within 2 weeks after LK, including 15 patients within 1 week. All cases of treatment failure displayed increasing local irritation and hyphal infiltration in the recipient LK bed and subsequently were cured by PK. A higher rate of inadequate treatment with LK was found in the cases with *Aspergillus* species, in those to whom glucocorticoids or immunosuppressants were administered, and in those with hypopyon or endothelial plaque before LK.

Conclusions: Treatment failure after LK for fungal keratitis can be reduced significantly with adept intraoperative skills. *Aspergillus* species, use of glucocorticoids or immunosuppressants, and presence of hypopyon or endothelial plaque before LK should be noted as major risk factors. Prompt recognition and management of failed LK with PK can achieve successful outcomes.


Fungal keratitis is one of the most severe eye diseases worldwide that may lead to blindness, especially in the agricultural countries with temperate climates, such as China and India. Increased prevalence of fungal keratitis over the past 2 decades is believed to be related to the widespread use of corticosteroids. Primary treatment of fungal infection is the use of antifungal medications, including oral fluconazole combined with topical natamycin, amphotericin B, or fluconazole. This approach seems to be effective at the early stage of the disorder. For uncontrollable or further aggravated infection, surgical interventions are required. Some ophthalmologists prefer conjunctival flaps for small fungal ulcers, but almost one half of the cases failed because of the formation of abscess and perforation through the flap. Some believed that penetrating keratoplasty (PK) was the only way to control the fungal infection; however, many surgical complications were reported, including allograft rejection, refractive errors, and secondary cataract.

However, lamellar keratoplasty (LK) was considered a contraindication to the treatment of fungal keratitis because of its inadequacy for dissecting infected tissues. With the advancement of surgical techniques and increasing awareness of fungal keratitis, LK treatment has achieved good visual outcome. It seems to be effective in treating fungal infections that can not be cured by antifungal therapeutics, and few complications have been observed. Meanwhile, treatment failure after LK was focused and was believed to result from incomplete removal of infected tissues. In this study, we examined the clinical features, diagnosis, treatment, and risk factors of patients who experienced treatment failure after LK for fungal keratitis and evaluated approaches to reduce the failure.

Patients and Methods

Patients

From January 1998, through July 2005, 725 patients with fungal keratitis were hospitalized at the Shandong Eye Institute. Of these patients, 61 were cured with medical therapy, 417 required PK to save their eyes, 218 underwent LK, and 29 received ophthalmectomy or free-flap conjunctival autografting.

Of the patients undergoing LK, 76 were women and 142 were men. Their mean age was 37.8 years (range, 23–71 years). De-
Detailed medical history was recorded before surgery. One hundred nineteen patients reported a recent ocular trauma or sensation of foreign body in eyes, 23 had a history of using steroid drops for more than 3 months, 8 wore contact lenses, and 68 did not indicate any event that might have induced the infection. The interval between the day of onset and surgery ranged from 12 to 105 days (mean, 27.8 days).

Diagnostic Methods
The ocular surface was examined using confocal microscopy or culture of corneal scrapings as well as slit-lamp microscopy. A portion of each scraping was incubated with potassium hydroxide (KOH) and was examined as wet mounts. Another portion was subjected to fungal culture and strain identification. Fungal presence in the KOH preparations or confocal microscopic images, or positive culture results for fungal filaments, confirmed the diagnosis of fungal keratitis.

Clinical Features
The primary clinical feature of fungal keratitis in this study was that the ulcers involving the central cornea did not penetrate into the anterior chamber. Visual acuity was <20/200 in 181 eyes and between 20/200 and 20/100 in 37 eyes that had fungal ulcers at the peripheral area. The depth of the fungal lesions, determined using slit-lamp microscopy and Z-scanning confocal microscopy, was one half to three fourths of the corneal depth in 142 eyes. The diameter of fungal lesions was 9 mm or more in 21 eyes, 8 to 9 mm in 68 eyes, and 6 to 8 mm in 128 eyes. Hypopyon or endothelial plaque was observed in 47 patients.

Medical Treatment before Surgery
Antifungal medications were administered for 7 to 46 days (mean, 14 days), including treatment days before patients were referred to the authors’ institution. At the Shandong Eye Institute, the 218 patients received hourly 0.5% fluconazole combined with 0.25% amphotericin B or hourly 0.5% fluconazole combined with 5% natamycin so that 1 drop was received every 30 minutes while awake. Antifungal ointments of 1% fluconazole and 1% amphotericin were used at night. The patients also were treated with 200 mg oral fluconazole daily. Those with hypopyon received an intravenous injection of fluconazole (100 mg) twice daily and atropine drops once daily. If the infection was not controlled with the intensive antifungal therapy and did not penetrate into the anterior chamber, LK was recommended.

Surgical Procedure
A Hessburg-Barron trephine that was 0.5 mm larger in diameter than the fungal infection was used. The depth of the trephine incision, 350 to 400 μm, was deeper than the actual penetration of the fungal ulcer. After the infected lamellae were excised, the recipient bed was washed with 0.2% fluconazole. If there was gray residual infiltration in the lamellar corneal bed, excision was continued until the clear portion was visible. When it was difficult to distinguish hyphal infiltration from inflammatory reaction, dissected tissues were processed with KOH staining to search for any hyphae. If the surgeon suspected that hyphae had penetrated the corneal endothelium, PK was performed rather than LK to ensure that infected tissues were removed completely. A donor lamellar corneal graft, 0.25 mm larger in diameter than the recipient site, was secured in place with 12 to 16 interrupted 10-0 nylon sutures.

Postoperative Treatment and Evaluation
After surgery, fluconazole was given subconjunctivally once daily for 3 days. Topical 0.5% fluconazole, 0.25% amphotericin B or 5% natamycin, antibiotic drops, and nonsteroidal antiinflammatory drops were used 4 times daily. Administration of oral fluconazole began from the day before surgery and lasted for 21 days. In addition, fluconazole or amphotericin B ointment was administered before bedtime. Hepatic function was monitored by serum liver enzyme analysis. Antifungal chemotherapeutic treatment was continued for 2 weeks and was tapered thereafter. Corticosteroids were not used within the first week after LK; for the cases with hypopyon or endothelial plaque, it was administered from the second week.

All patients were evaluated daily in the first postoperative week, once every other day in the second week, and weekly thereafter for at least 8 months. Follow-up ranged from 8 to 48 months. Each evaluation included graft clarity, late fungal recurrence, visual acuity, and intraocular pressure assessments.

Treatment failure was considered to have occurred when the operated eye demonstrated increasing local irritation, inflammatory reaction, and gray hyphal infiltration in the recipient LK bed. Antiinflammatory and antifungal treatment was reinforced. If poor response was achieved after 3 days, PK was performed.

Histopathologic Examination
The diseased corneal specimens were bisected. One was subjected for histologic examination by periodic acid–Schiff staining. The other was cultured for microbiologic evaluation.

Statistical Analysis
SPSS software version 10.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Distribution of risk factors in the cases with LK treatment failure was compared by chi-square analysis. A P value <0.05 was considered statistically significant.

Results
Among the 218 cases treated with LK, fungal hyphae were revealed in 157 (72.0%) on examination of corneal scrapings and in 203 (93.1%) by confocal microscopy. One hundred ninety-four specimens (89.0%) had positive culture results for fungi, with 206 (94.5%) exhibiting hyphal elements on pathologic examination. One hundred forty-two pathogens were identified as Fusarium species, 26 were Aspergillus species, 9 were Alternaria species, 8 were Candida species, and 5 were Penicillium species. There were also 28 unidentified species.

Cases Successfully Treated with Lamellar Keratoplasty
The LK treatment was successful in 201 patients. Mean follow-up was 20 months (range, 8–48 months). All grafts were transparent. Best-corrected visual acuity ranged from 20/63 to 20/20. There was no recurrence of infection.

Cases Not Successfully Treated with Lamellar Keratoplasty
After surgery, 39 cases demonstrated local irritation and gray infiltration in the recipient LK bed. Some of them experienced...
After 3 days of antiinflammatory and antifungal treatment, the status of 22 cases did not deteriorate, whereas the other 17 cases required PK because of the aggravated symptoms and decreased visual acuity. All specimens obtained during PK had positive culture results for fungal filaments, confirming the incomplete removal of the infected tissues by LK. The clinical data of the 17 cases in which treatment failed are shown in Table 1. The interval between LK and the onset of the treatment failure (failure time) varied from 2 to 9 days (mean, 4.5 days), which was 4.9 days in eyes with *Fusarium* species, 4.2 days in eyes with *Aspergillus* species; 3.2 days in those treated with corticosteroids before surgery, and 5.1 days in those with no preoperative corticosteroid treatment.

All the 17 cases with LK treatment failure were cured by PK. The grafts remained clear with no recurrence of the infection during the follow-up.

### Risk Factors

Treatment failure of LK occurred in 7.8% (17/218) of the patients. In eyes with *Fusarium* keratitis, 5.6% (8/142) experienced failure, significantly lower than those with *Aspergillus* (19.2%) within 1 week. The failure time in eyes with *Fusarium* species was similar to that in eyes with *Aspergillus* infection. The failure occurred earlier in eyes with preoperative corticosteroid treatment than in those without it. The first 2 weeks after surgery is a critical period for treatment outcomes of LK for fungal keratitis. Antifungal therapy is advised to continue. However, corticosteroids should be prohibited in the first postoperative week when the risk is high for treatment failure.

In the current study, 17 cases of treatment failure occurred at 2 to 9 days after surgery, including 15 failures (88.2%) within 1 week. The failure time in eyes with *Fusarium* species was similar to that in eyes with *Aspergillus* infection. The failure occurred earlier in eyes with preoperative corticosteroid treatment than in those without it. The first 2 weeks after surgery is a critical period for treatment outcomes of LK for fungal keratitis. Antifungal therapy is advised to continue. However, corticosteroids should be prohibited in the first postoperative week when the risk is high for treatment failure.

### Discussion

In the treatment of fungal keratitis, the first step is to use systemic and topical antifungal medications for 1 week, before considering surgical interventions. With fewer surgical complications and more easily obtained donor corneas than PK, therapeutic LK seems more effective for fungal keratitis uncontrollable with medical treatment. However, LK was reported to be inadequate in removing the infected tissues, resulting in treatment failure. In a study by Zhang et al., the rate of LK failure in the treatment of fungal keratitis was 14.3% (2/14). The 2 eyes that failed treatment experienced mycotic endophthalmitis and finally underwent ophthalmectomy. In our study, the failure rate was 7.8% (17/218), but all failed cases were cured by PK. We conclude that the rate of treatment failure was related to fungal species, glucocorticoid or immunosuppressant treatment, the presence of hypopyon or endothelial plaque before surgery, surgical skill, and continuous rational medications after surgery.

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Sometimes it is not easy to distinguish treatment failure from local inflammation after LK for fungal keratitis. The corneal scraping smears and confocal microscopic examination may not be helpful. According to the authors, cases of treatment failure usually show the following characteristics: (1) patients report increasing irritation and decreasing vision of the operated eyes; (2) exudation in the anterior chamber aggravates, and hypopyon develops in some cases;
(3) the focus of infection demonstrates gray infiltration and expands rapidly in the transplanted cornea; (4) antifungal medications are ineffective. After diagnosis is determined, PK is required to resolve the treatment failure.

Treatment failure may be caused when LK dissections are not complete. Surgical skills include two aspects. First, a trephine with a diameter 0.5 mm larger than the area of fungal infection may ensure adequate peripheral excision. Second, the infected tissue should be incised as deeply as possible in the corneal stroma. Washing the recipient LK bed may help to distinguish the infected residua with gray and coarse infiltration; sometimes intraoperative KOH staining and microscopic checking of the corneal sections can be helpful in accomplishing this. If the hyphae are found approaching Descemet’s membrane, PK should be performed instead.

We investigated the morphologic features of fungal growth in the corneal stroma and observed different patterns in different fungal species.14,15 Hyphae of Aspergillus fumigatus and Candida albicans traversed the cornea perpendicularly to the stroma, whereas those of Fusarium solani lay parallel to the corneal lamellae in rabbits.15 The growth patterns of fungi in human corneas are still under investigation. In this study, the higher rate of treatment failure in eyes with Aspergillus species may be related to the perpendicular growth of fungal filaments that made the infection penetrate deep into the corneal layers in a short time such that the tissue could not be excised thoroughly with LK. Therefore, identification of fungal species before surgery may provide the surgeon with valuable information.

It was reported previously that immune status played a role in fungal infection, and immunosuppressants may increase disease severity and may delay fungal clearance.16,17 Kiryu et al17 found the hyphae that were surrounded by neutrophils showed double or triple cell wall formation or sometimes a hypha-in-hypha structure in the dexamethasone-treated corneal lesions. This special structure was regarded as a protective device for the survival of F. solani. In this study, the failure rate in eyes that had received preoperative corticosteroid treatment was much higher than in those that were not so treated. This may be the result of the change of corticosteroid-treated hyphae in growth velocity and pattern. Wide invasion of fungal hyphae increased the difficulty in removing the infected tissue completely when LK was performed.

Lamellar keratoplasty may be effective for fungal keratitis with hypopyon or endothelial plaque.12 However, there was a much higher rate of treatment failure in this study, although most hypopyon was sterile. Because it may be a sign of the hyphae approaching Descemet’s membrane, we concluded that the presence of hypopyon or endothelial plaque is a risk factor for LK treatment failure.

Moreover, the viability of donor corneal tissue is not required at the time of LK surgery. It is more convenient to obtain necessary donor corneal tissues for LK than full-thickness transplantation. Because of the low rate of treatment failure and fewer associated surgical complications,12 we recommend LK as the first choice for the treatment of fungal keratitis that could not be cured by antifungal therapeutics, even if sufficient provision of viable donor tissues can be acquired.

In summary, this study focused on the clinical features of treatment failure after LK for fungal keratitis. The treatment failure may be significantly reduced with adept surgical skill. Prompt recognition and management of failed LK with PK can achieve successful outcomes. Analysis of various risk factors also may help surgeons to evaluate LK for fungal keratitis.

References