Fungal keratitis is a common cause of blindness worldwide, especially in some developing countries with a warm and wet climate. In the past 4 decades, the incidence of fungal keratitis has increased greatly in China; fungal keratitis has even become the leading cause of corneal blindness in some areas. However, fungal keratitis is difficult to manage merely with antifungal agents. Corneal transplantation is usually employed to eradicate the infectious tissues, including penetrating keratoplasty (PK) and lamellar keratoplasty (LK). Surgical injuries and serious complications, such as secondary glaucoma, corneal endothelial dysfunction, and immune rejections, occur more often in PK than in LK. Many cases undergoing PK cannot sustain useful visual acuities (VAs) for a long period. Therefore, LK is regarded as a better choice for patients with deep corneal stroma uninvolved at the early stage of fungal keratitis.

We previously reported different growth patterns of fungal hyphae in corneas and clinical therapeutic effects of LK. The horizontal growth pattern was seen as an important indication for LK in the treatment of fungal keratitis. However, differences in fungal recurrence after LK in cases of various hyphal growth patterns have not been investigated. In this study, we retrospectively reviewed the medical records of patients who underwent LK for fungal keratitis at Shandong Eye Institute from January 2000 through November 2006 and attempted to provide appropriate selection criteria of surgical procedures by evaluating hyphal growth patterns and fungal recurrence after LK.

Materials and Methods

We adhered to the principles outlined in the Declaration of Helsinki. All patients who met the following criteria were included:

- Hyphal growth patterns in corneas differ not only in the same fungal genus but also in the same species. The fungal recurrence rate after LK in patients with hyphae growing horizontally is much lower than that in those with hyphae growing vertically. Growth patterns of fungal pathogens may be an important factor for fungal recurrence after LK. Ophthalmology 2008;115:983–987 © 2008 by the American Academy of Ophthalmology.
Figure 1. Fungal keratitis infected by *Fusarium solani*. A, Microscopic image of preoperative corneal ulceration. B, Hyphae grow horizontally and locate in the superficial corneal stroma (stain, periodic acid–Schiff; original magnification, ×400). C, Slightly opaque graft on day 16 after lamellar keratoplasty, without fungal recurrence. D, Clear graft at 3 years after lamellar keratoplasty.

Figure 2. Fungal keratitis infected by *Aspergillus flavus*. A, Microscopic image of preoperative corneal ulceration. B, Hyphae grow vertically and invade deep corneal stroma (stain, periodic acid–Schiff; original magnification, ×400). C, Opaque graft on day 2 after lamellar keratoplasty, with fungal recurrence. D, Clear graft at 2 months after secondary penetrating keratoplasty.
1. Fungus was cultured positive in the microbial culture, and no other pathogens were mixed; clinically dominating pathogens, including Fusarium and Aspergillus, were identified to the species level.

2. Hyphae were detected by histopathological examination, and hyphal growth patterns could be identified.

3. Lamellar keratoplasty was performed, for which the indications were (a) only the upper or middle corneal stroma was infected, (b) administration of antifungal medications was not <1 week but ineffective, (c) uncorrected VA (Snellen) was 20/200, and (d) no hypopyon was present or hypopyon had reduced significantly with antifungal medications.

Specimens obtained from corneal scrapings or during LK were inoculated onto Sabouraud’s plain culture medium and cultured for 14 days at 27°C. The pathogens cultured positive were identified to the genus level; some were then inoculated onto culture-specific media, potato dextrose agar for Fusarium and Czapek–Dox agar for Aspergillus. Fungal species were identified according to the characteristics of the hyphae and spores. The identifications were performed at the Fungus Laboratory, Dermatology Department, Beijing University Affiliated Hospital and Medical Microbial Fungal Species Collection Center of China, Nanjing.

Partial corneal buttons obtained during LK were formalin fixed, paraffin processed, and serially sliced, with anatomical layers of the corneas shown in each sample (4 μm). The samples were stained with periodic acid–Schiff. Two fungal growth patterns were determined according to the growing directions of hyphae: horizontal when most hyphae grew at an angle <45° to the plane of stromal lamellae (Fig 1B) and vertical when the angle of most hyphae and the stromal lamellae was >45° (Fig 2B).

The patients were followed up at our institution within the first month after LK. Postoperative fungal recurrence was regarded as the most important index in evaluating the success of surgery. If there was no fungal recurrence within 1 month, the LK was considered successful (Fig 1). In patients with corneal ulceration after LK, fungal recurrence was diagnosed if the same pathogen as that found preoperatively or intraoperatively was identified. In patients with corneal ulceration after LK, if the same pathogen as that found preoperatively or intraoperatively was identified, fungal recurrence was diagnosed and the LK was regarded as a failure (Fig 2); if a different pathogen was found, postoperative infection was diagnosed.

All data were analyzed with SPSS software (version 10.0, SPSS Inc., Chicago, IL). Chi-square tests were used to compare the fungal recurrence rates in patients with different hyphal growth patterns and pathogens. A P value < 0.05 was considered statistically significant. Reproducibility of fungal recurrence rate was assessed by Cohen κ statistics. The κ values were interpreted with the guideline proposed by Altman: <0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good; and 0.81 to 1.00, very good.

Results

There were 174 fungal keratitis patients, from 13 to 72 years old (mean, 48.2 ± 10.6), included in this study. Of the 85 women and 89 men, 156 were farm workers, and 10 were factory laborers; 128 had ocular trauma histories, and 25 had been treated with corticosteroids. The disease had been present 5 to 72 days (mean, 22.4 ± 8.9). The ulceration area was from 2 × 3 mm to 9 × 9 mm. Fourteen cases were combined with hypopyon.

The pathogens were Fusarium (148 cases [85.1%]), Aspergillus (11 [6.3%]), Alternaria (8 [4.6%]), Penicillium (4 [2.3%]), and Candida (3 [1.7%]). Fusarium and Aspergillus were the dominating pathogens, with the major species being F. solani (38.5%), F. moniliforme (33.1%), and F. oxysporum (26.4%) in the Fusarium genus and A. flavus (54.5%) and A. fumigatus (27.3%) in the Aspergillus genus (Table 1).

Hyphae grew horizontally in the corneas of most patients (85.1%). Fusarium, Alternaria, and Penicillium hyphae mainly lay parallel to the stromal lamellae, whereas Aspergillus and Candida grew vertically. In the cases infected by Fusarium, 91.2% had a horizontal growth pattern. The percentages of horizontal pattern were 94.7%, 95.9%, and 79.5% in F. solani, F. moniliforme, and F. oxysporum, respectively. A vertical pattern was observed in 90.9% of the Fusarium cases and in 83.3% and 100% of the Aspergillus flavus and Aspergillus fumigatus cases, respectively.

Fungal keratitis recurred in 15 cases (8.6%) on day 1 to day 20 (mean, 8.4 ± 5.3) after LK. The hyphae grew vertically in 12 cases (80%) and horizontally in 2 cases infected by F. solani and in 1 case by F. moniliforme. Eleven eyes were infected by Fusarium, in which the species were F. oxysporum (5 cases), F. solani (4), and F. moniliforme (2). The other 4 were infected by Aspergillus, in which the species were A. flavus (2), A. fumigatus (1), and A. terreus (1).

In the 174 cases, the recurrence rate of fungal infection in the patients with horizontally growing hyphae (3/148 [2%]) was much lower than in those with vertically growing hyphae (12/26 [46.2%]), and the difference was statistically significant (χ² = 54.664, P < 0.001). The rate was similar in patients infected by species of F. solani (χ² = 17.267, P < 0.001), F. moniliforme (χ² = 11.230, P = 0.001), and F. oxysporum (χ² = 22.224, P < 0.001). The recurrence rate in the patients infected by Fusarium (11/148 [7.4%]) was lower than that in those infected by Aspergillus (4/11 [36.4%]), and the difference was statistically significant (χ² = 10.031, P = 0.002). The reproducibility of fungal recurrence rate was moderate in the patients with different hyphal growth patterns (κ = 0.534) but poor in those with different fungal pathogens (κ = −0.044).

Discussion

Recurrence of fungal keratitis often occurs within 2 weeks after surgical treatment. If there is no sign of fungal recurrence during this period, topical corticosteroids are generally administered to prevent immune rejection of corneal grafts. It has been proven that corticosteroids can weaken the ability of the hosts to eradicate fungal pathogens, and if the pathogens remain during LK, they will grow rapidly and cause fungal recurrence within 2 weeks after the administration of corticosteroids. Therefore, patients are usually not significantly at risk for recurrence after the first postsurgical month.

Fusarium and Aspergillus are the most common pathogens of fungal keratitis in this study, a result that is consistent with the reports from many countries and regions in the world. We previously found that most Fusarium grew horizontally and most Aspergillus grew vertically in corneas, whereas the hyphal growth patterns in the same fungal genus were not completely identical. However, the fungal pathogens had been identified only to the genus level, and we presumed that different species in the same genus might grow with various patterns. With the improvement in fungal identification techniques, the pathogens can be identified to...
In conclusion, hyphal growth patterns in corneas appear to differ not only in the same fungal genus but also in the same species. For example, in the most frequently encountered *Fusarium* most hyphae of *F. solani* (94.7%), *F. moniliforme* (95.9%), and *F. oxysporum* (79.5%) grew horizontally in corneas, although a minority demonstrated a vertical growth pattern.

To find the possible factors contributing diversity, we evaluated the 13 *Fusarium* keratitis patients with vertically growing hyphae and found that all had been administered topical corticosteroids at the early stage of the disease. However, not all species of *Fusarium* grew vertically in the patients administered corticosteroids. In this study, the hyphae continued to grow horizontally in 12 *Fusarium* keratitis patients despite corticosteroid administration. Therefore, corticosteroids may not be the only factor contributing to the discrepancy in growth patterns. Timing, dose, frequency, and duration of corticosteroid administration can also affect the growth patterns of the same fungal species. Nevertheless, these patients had been treated with corticosteroids in other hospitals, although details regarding their treatment were not available. The relation between corticosteroid treatment and hyphal growth patterns cannot be determined from these results.

Some investigators believe that fungal hyphae grow vertically in corneal stroma and soon penetrate Descemet’s membrane into the anterior chamber and, therefore, regard PK as the only choice in the treatment of fungal keratitis refractory to antifungal medications. However, our histopathologic study found that most hyphae grow horizontally in corneas and therefore provided a theoretical basis for using LK in the treatment of fungal keratitis.

In cases of horizontal hyphal growth, the lesions are generally superficial at the early stage of the disease and can be excised thoroughly by LK. However, the deep stroma is liable to be invaded in cases of vertical hyphal growth. The lesions are not easy to be cleared by LK, and the rudimentary fungi in the deep stroma may cause fungal recurrence postoperatively. In such cases, PK is an alternative treatment option. To evaluate this treatment in clinical practice, we retrospectively reviewed the hyphal growth patterns of the predominant pathogens in corneas and fungal recurrence after LK.

Of the 15 patients with fungal recurrence, the hyphae grew vertically in most (80%). The fungal recurrence rate in the patients with vertical hyphal growth was much higher than that in those with horizontal growth. Results were similar even when analysis was limited to *F. solani*, *F. moniliforme*, and *F. oxysporum*. Hyphal growth pattern appears to be an important factor contributing to fungal recurrence after LK. The fungal recurrence rate after LK in *Fusarium* keratitis was lower than that in *Aspergillus* keratitis, which may relate to their different growth patterns. However, the number of patients infected by *Aspergillus* is too small in this study to base conclusions. More cases need to be investigated.

Satisfactory therapeutic effects can be achieved by LK in a majority of fungal keratitis cases infected by *Fusarium*. In this study, fungal recurrence was observed in 3 *Fusarium* keratitis patients with horizontal hyphal growth. We conclude that horizontally growing hyphae may also invade the deep corneal lamellae in the progression of fungal infection. This finding points to the importance of surgical timing in the treatment of fungal keratitis with LK. For patients unresponsive to antifungal agents, LK should be employed as early as possible, because delayed treatment may increase the risk of postoperative fungal recurrence.

In conclusion, hyphal growth patterns in corneas appear to differ not only in the same fungal genus but also in the same species and may contribute to fungal recurrence after LK. Lamellar keratoplasty should be performed in time to reduce the risk of fungal recurrence.

### Table 1. Growth Patterns of Fungal Pathogens and Recurrence of Fungal Keratitis after Lamellar Keratoplasty

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Case No.</th>
<th>Recurrence No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horizontal</strong></td>
<td><strong>Vertical</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><em>Fusarium</em> (%)</td>
<td>135 (91.2)</td>
<td>13 (8.8)</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>54</td>
<td>3</td>
</tr>
<tr>
<td><em>F. moniliforme</em></td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td><em>F. nivale</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>F. poae</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>F. subglutinans</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Aspergillus</em> (%)</td>
<td>10 (90.9)</td>
<td>11 (100)</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Alternaria alternata</em></td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida</em></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total (%)</td>
<td>148 (85.1)</td>
<td>26 (14.9)</td>
</tr>
</tbody>
</table>

### References