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CLINICAL INVESTIGATION

Multicenter prospective observational study of fungal keratitis in Japan: analyses of culture-positive cases

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Abstract

Purpose To investigate the clinical characteristics and causative fungi in patients with fungal keratitis in Japan, and to determine factors related to the prognosis.

Study design Multicenter prospective observational study.

Methods Eligible patients were enrolled from November 2011 to October 2013 at the 1st stage and from April 2015 to March 2016 at the 2nd stage. The corneal foci were scraped, and the scrapings were cultured in potato dextrose agar. The isolated fungi were identified by gene analyses. Data were collected from the clinical records and statistically analyzed by Cox and logistic regression analyses.

Results Ninety-four fungal strains were isolated from 93 cases, including 42 yeast-like fungi and 52 filamentous fungi. The fungi affected the deep layers of the cornea in 23 cases (24.7%) and the peripheral cornea in 29 cases (31.2%). The incidences of lid swelling/redness, ciliary injection, anterior chamber cells/flare, anterior chamber fibrin, and hyphate ulcer in cases of filamentous fungi were significantly higher than in yeast-like fungi. No history of topical steroids, absence of a main lesion in the peripheral cornea, and best-corrected visual acuity (BCVA) of more than 0.04 at the first visit were related to a shorter healing time. No history of ocular surgery, absence of lesion at one-third deep stromal layer and BCVA of more than 0.04 at the first visit were correlated with BCVA at 3 months after the initial examination.

Conclusion Fungal keratitis is caused by various species of fungi and can become refractory due to poor prognosis factors.

Keywords Fungal keratitis · Multicenter study · Prognosis · Filamentous fungi · Yeast-like fungi

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Introduction

Fungal keratitis is caused by filamentous or yeast-like fungi which infect the cornea and can be induced by trauma, contact lens (CL) wear, post-keratoplasty, and locally immunocompromised situations. In India and China, fungi are the main cause of infectious keratitis, however, in Japan bacteria are the main cause of infectious keratitis and fungal keratitis is rare. Studies of a large number of cases of fungal keratitis have been made in other countries [1–7], but reports of fungal keratitis in Japan are on a limited number of cases [8–12]. Although there have been a few studies on a larger number of cases in Japan more than 30 years earlier, such as that on the efficacy of pimaricin exist [13] as well as summaries of clinical reports in Japan [14, 15], the current situation of fungal keratitis in Japan is likely to be different from the findings reported in these earlier publications.

The guidelines for the diagnosis and treatment of fungal keratitis are published in Japan [16], written based on the experience and opinions of infectious keratitis experts, rather than on evidential data from Japanese patients.

In 2003, a National Survey of Infectious Keratitis in Japan was conducted by the Japanese Association for Ocular Infection (JAOI) group at 24 ophthalmological institutions in Japan. However, there were only 12 cases of fungal keratitis out of 261 total cases of infectious keratitis, and no cases of *Fusarium* spp. [17]. Also, only a few cases of fungal keratitis are reported in the Survey of Severe Contact Lens-associated Microbial Keratitis in Japan conducted by the Japan Contact Lens Society and JAOI at 224 institutions [18].

Thus, much more information is needed to establish Japanese guidelines on the management of fungal keratitis. To accomplish this, the JAOI conducted a multicenter prospective observational study of fungal keratitis from November 2011 to October 2013 to investigate the clinical history, findings, treatment, and especially the in vitro drug susceptibility, including that of pimaricin. In that study, 133 cases of fungal keratitis were analyzed, and the results were published in the Journal of the Japanese Ophthalmological Society [19, 20]. However, in one-half of the cases (60 cases) the causative fungi were not isolated, and the culture-positive and culture-negative cases were analyzed together. The analyses of culture-positive cases should be more accurate than of all of the cases including the presumed ones. In addition, the drug susceptibility test was conducted for each drug, even though many cases had been treated by a combination of two or more antifungal drugs. Thus, in vitro drug susceptibility tests of a combination of two antifungal drugs are needed. Based on these findings, a second stage of the multicenter prospective observational study of fungal keratitis was conducted from April 2015 to March 2016.

The purpose of this study was to investigate the clinical characteristics and causative fungi of patients with fungal keratitis in Japan, and to determine the factors related to the prognosis. To accomplish this, we analyzed the demographics of the patients, the clinical findings, identification of the isolated species of fungi, and the results of treatment of culture-positive cases in the first and second stage of this study; the data were statistically analyzed to determine the factors of prognosis. The results of the in vitro combination effects in drug susceptibility tests are reported in another paper [21].

Materials and methods

Patients

Patients with culture-positive fungal keratitis diagnosed from November 1, 2011 to October 31, 2013 in 35 facilities at the first stage, and from April 1, 2015 to March 31, 2016 at the second stage in 15 participating facilities were studied. In bilateral cases, only the more severely affected eye was studied.

Study design

This was a multicenter prospective observational study. Participating ophthalmological institutions and clinics were listed at the end of this report.

Ethical approval

The procedures used in this study were approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Tottori University, Tottori, Japan as a representative facility, and were also approved by the IRBs of each participating institute except the ones that did not have an IRB. Informed consent was obtained from all participants after an explanation of the procedures to be used prior to the beginning of the procedures.

Clinical information of patients

The clinical information of the patients was collected from the medical records of each patient. The information collected was; date of the first visit defined as the date of initial examination for fungal keratitis, sex, age, residence location, occupation, history of trauma, contact lens use, history of systemic diseases, history of ocular diseases, history of ocular surgery, history of medications, laterality, interval between the onset and diagnosis of fungal keratitis, the presence or absence of the previous doctors, symptoms, best-corrected visual acuity (BCVA) at the first examination, clinical

findings at the initial examination (eyelids, conjunctiva, cornea, anterior chamber, iris, the depth, size, and location of corneal main lesion, the size of epithelial defect), presence of surgical therapy, prognosis, and BCVA at 3 months after the first visit.

Smear, culture, identification, and drug susceptibility tests

The foci of the corneal infection were scraped, and the scrapings were directly inoculated into potato dextrose agar, which was then sent to the Laboratory for Clinical Investigation, Osaka University Hospital (Osaka University Laboratory). It was then incubated for 3weeks. In addition, corneal scrapings collected similarly were used for cultures and smears for direct microscopic examinations at the laboratories of the respective treating institutions. Isolated *Candida* spp. were identified by API 20C AUX (bioMérieux) at Osaka University Laboratory, and isolated yeast-like fungi other than *Candida* spp. and all filamentous fungi were sent to Institute of Medical Mycology, Teikyo University or Medical Mycology Research Center, Chiba University for identifications based on morphological and phylogenic characteristics. The sequences of the β-tubulin gene for *Aspergillus* spp., the elongation factor-1α gene for *Fusarium* spp. and the internal transcribed spacer regions of rDNA for other fungi were determined. Searches of the sequences data were performed using the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Drug susceptibility levels were measured at Osaka University Laboratory by the broth microdilution method and performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards (M27-A3 and M38-A2), using specially manufactured minimum inhibitory concentration (MIC) plates for fungi (Eiken Chemical Co., Ltd.). Details of these procedures will be described in another report.

Statistical analyses

Cox regression analyses were used to determine the factors significantly correlated with the healing time, defined as the time when the antifungal eye drops were reduced to three times/day until 91 days. Cases that underwent therapeutic keratoplasty were treated as withdrawn at 92 days. Factors affecting the BCVA at 3 months after the first visit were also determined using logistic regression analyses. All cases requiring therapeutic keratoplasty were excluded from these analyses. The statistically analyzed factors are listed in Table 1.

Table 1 Statistically analyzed factors

Types of Causative Agent	Yeast-like fungi	Filamentous fungi
Trauma	(-)	(+)
CL use	(-)	(+)
Past history of corneal diseases	(-)	(+)
Systemic complications: DM, collagen diseases and other immunocompromised diseases	(-)	(+)
Past history of ocular surgery	(-)	(+)
Past history of topical steroid use	(-)	(+)
The interval from onset to diagnosis	Less than 2 weeks	More than 2 weeks
Symptoms: ocular pain	(-)	(+)
Symptoms: visual impairment	(-)	(+)
Best-corrected visual acuity at the first visit	Less than 0.03	More than 0.04
Hyphate ulcer	(-)	(+)
Hard infiltrates	(-)	(+)
Hypopyon	(-)	(+)
Endothelial plaque	(-)	(+)
Lesion involving one-third deep stromal layer	(-)	(+)
Size of main lesion	Smaller than 5mm^2	More than 5 mm^2
Main lesion on peripheral cornea	(-)	(+)
Size of epithelial defect	None or smaller than main lesion	Same as or larger than main lesion
Age (per 10-year-old)		
Use of sensitive topical antifungal drugs	(-)	(+)
Sex	Female	Male

Results

Isolation and identification of fungi

Ninety-four strains of fungi were isolated from 93 cases with two strains isolated from 1 case. Forty-two yeast-like fungi were isolated from 41 cases, and 52 filamentous fungi were isolated form 52 cases. The most abundant filamentous fungi were *Fusarium* spp. (23 strains), and the most abundant yeast-like fungi were *Candida albicans* (15 strains) and *Candida parapsylosis* (15 strains). Gene analyses revealed that a variety of fungal species can cause fungal keratitis (Table 2).

Demographics of patients

The mean age of the patients was 68.9 ± 15.4 years with a range of 13 to 94 years, and the peak age at the initial examination was in the 70 s (Fig. 1). Forty-nine cases (52.7%) were men and 44 cases (47.3%) were women. Eighty-nine cases were unilateral (52 right and 37 left), and 4 were bilateral (more severely affected eyes: OD 1, OS 3). The occupation with the highest number of cases was listed as 'no occupation' (23 cases, 24.7%) followed by agriculture (21 cases, 22.6%).

Seventy cases (75.3%) did not have causative trauma and 21 (22.6%) reported trauma as the cause of the keratitis. At the time of keratitis onset, seventy-five cases (80.6%) were not contact lens users whereas 18 (19.4%) were.

There were 51 cases (54.8%) with prior corneal diseases including 9 cases of herpetic keratitis, 7 of bullous keratopthy, and 4 cases of Mooren ulcer. There were 27 cases (29.0%) of prior systemic diseases including 12 cases of diabetes mellitus and 9 of collagen disease.

Prior ocular surgery was reported in 55 cases (59.1%) including 36 cases of cataract surgery, 31 of keratoplasty, 4 of vitreous surgery, and 3 of glaucoma surgery. The use of medications before the first visit was reported in 65 cases (69.9%) including 60 cases of steroid eye drops, 44 of antibiotics eye drops more than one month, 7 of other topical drugs related to infections such as tacrolimus, acyclovir, 8 of systemic steroid, and 4 of other systemic drugs causing immunosuppression. Steroid eye drops were used in 54 eyes before the onset of fungal keratitis and in 5 eyes after the onset.

The interval between the onset of symptoms and signs and the diagnosis of fungal keratitis is shown in Fig. 2. Cases with less than 1 week's duration were only 35.5%. Forty-three percentage of the cases were referred by previous doctors. The previous doctors had prescribed

Table 2 Causative fungi

Fungal name	No. of strains	
Fusarium solani (include one strain of species complex)	13	
Fusarium oxysporum	3	
Fusarium concentricum	1	
Fusarium equiseti	1	
Fusarium incarnatum	1	
Fusarium usarium nematophilum	1	
Fusarium proliferatum	1	
Fusarium spp.(Unidentifiable fungal name)	2	
Total numbers of *Fusarium* spp.	23	(24.5%)
Alternaria alternata	5	
Alternaria sp.(Unidentifiable fungal name)	1	
Total numbers of *Alternaria* spp.	6	(6.4%)
Aspergillus fumigatus	1	
Aspergillus flavus	1	
Aspergillus udagawae	1	
Ttotal numbers of *Aspergillus* spp.	3	(3.2%)
Paecilomyces lilacinus *[1]	4	
Beauveria bassiana	3	
Scedosporium apiospermum	2	
Acremonium sp. (Unidentifiable fungal name)	1	
Cladosporium cladosporioides	1	
Colletotrichum gloeosprioides	1	
Curvularia lunata	1	
Exophiala dermatitidis	1	
Nigrospora sphaerica	1	
Pestalotiopsis clavispora	1	
Phialemonium curvatum	1	
Stemphylium sp.	1	
Roussoella sp. (Unidentifiable fungal name)	1	
Filamentous fungi (Unidentifiable fungal genus)	1	
Total numbers of filamentous fungi	52	(55.3%)
Candida albicans	15	
Candida parapsilosis	15	
Candida guilliermondii *[2]	3	
Candida tropicalis	2	
Candida duobushaemulonii	1	
Candida nivariensis	1	
Candida andida pararugosa	1	
Candida pseudohaemulonii	1	
Kodamaea ohmeri *[3]	1	
Wickerhamomyces anamalus *[4]	1	
Total numbers of *Candida* spp.	41	(43.6%)
Rhodotorula slooffiae	1	
Total numbers of yeast-like fungi	42	(44.7%)
Total	94	

*1: *Purpureocillium lilacinum* is an alias

*2: Asexual generation name of *Meyerozyma guilliermondii*

*3: Sexual generation name of *Candida guilliermondii* var. *manbranifaciens*

*4: Sexual generation name of *Candida pelliculosa*

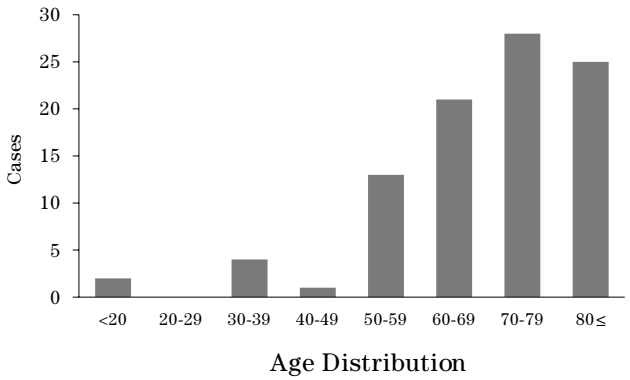

Fig. 1 Age distribution. The peak of age distribution is in the 70 s

various drugs including topical antibiotics in 51 cases, systemic antibiotics in 15, topical fluconazole in 5, topical voriconazole in 5, topical pimaricin in 3, other topical antifungal drugs in 4, other systemic antifungal drugs in 3, acyclovir ointment in 5 cases, and steroid eye drops in 22, including some combinations of drugs.

Symptoms and best-corrected visual acuity (BCVA) at initial examination

The main signs and symptoms at the first visit were conjunctival injection, visual impairment, ocular pain, and foreign body sensations (Fig. 3a). The decimal BCVA was less than 0.09 in 63 cases (67.7%), and less than 0.03 in 48 cases (51.6%; Fig. 3b).

Clinical findings at the first visit

The clinical findings at the first visit are presented in Tables 3, 4, 5. Signs of the severity of the keratitis observed in the anterior chamber were hypopyon in 22 cases and endothelial plaque in 14. The typical corneal lesions of fungal keratitis were hard infiltrates in 30 cases, hyphate ulcer in 23, elevated lesions in 17, and satellite lesions in 9. The incidence of lid swelling/redness, ciliary injection, anterior chamber cells/flare, anterior chamber fibrin, and hyphate ulcer in cases due to filamentous fungi were significantly higher than the ones due to yeast-like fungi.

The depth, size, location of main lesion, and comparisons of the sizes of corneal lesions and epithelial defects are presented in Fig. 4. In more than one-half of the cases (52 cases; 55.9%), the size of the lesion was less than 10 mm^2 (Fig. 4b). On the other hand, the lesions in 23 of the cases (24.7%) affected the deep layer of the corneal stroma (Fig. 4a), and those in 29 cases (31.2%) affected the peripheral cornea (Fig. 4c). Epithelial defects were not observed in 8 cases (8.6%), and epithelial defects less than the main corneal lesion was found in 28 cases (30.1%; Fig. 4d).

Treatment and clinical course

Various antifungals had been used; mainly voriconazole and pimaricin (Table 6). Voriconazole had been administered by various routes. Pimaricin is available as topical and ointment preparations, and both were used in 14 cases with filamentous fungi. In total, 62 cases used pimaricin, including 18 with yeast-like fungi and 44 with filamentous fungi. It should be noted that the combined use of drugs was common, e.g., 45 cases (48.4%) used 2 drugs, 31 cases

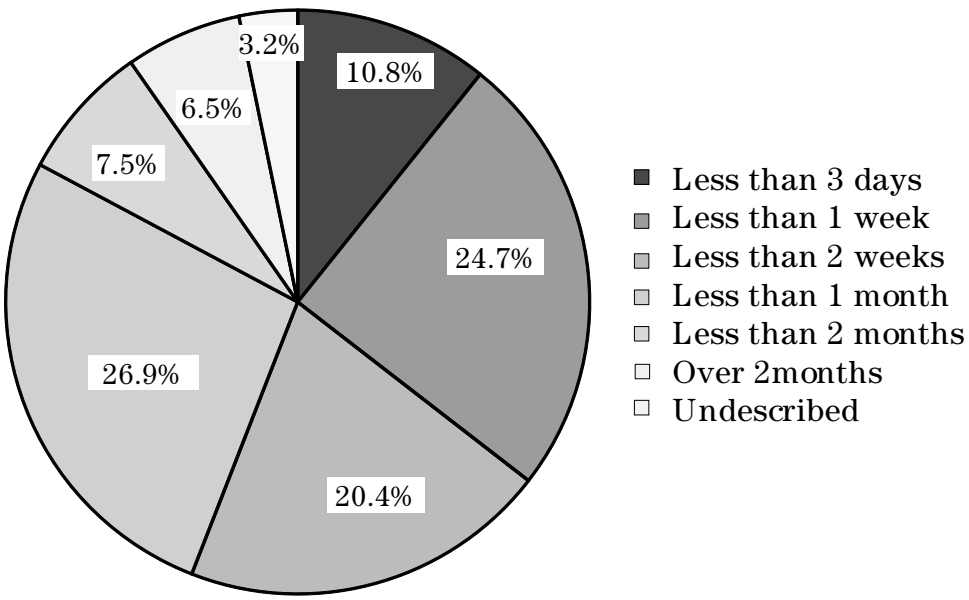

Fig. 2 Interval between the onset and the diagnosis of fungal keratitis. Cases with less than 1 week's interval were 35.5%

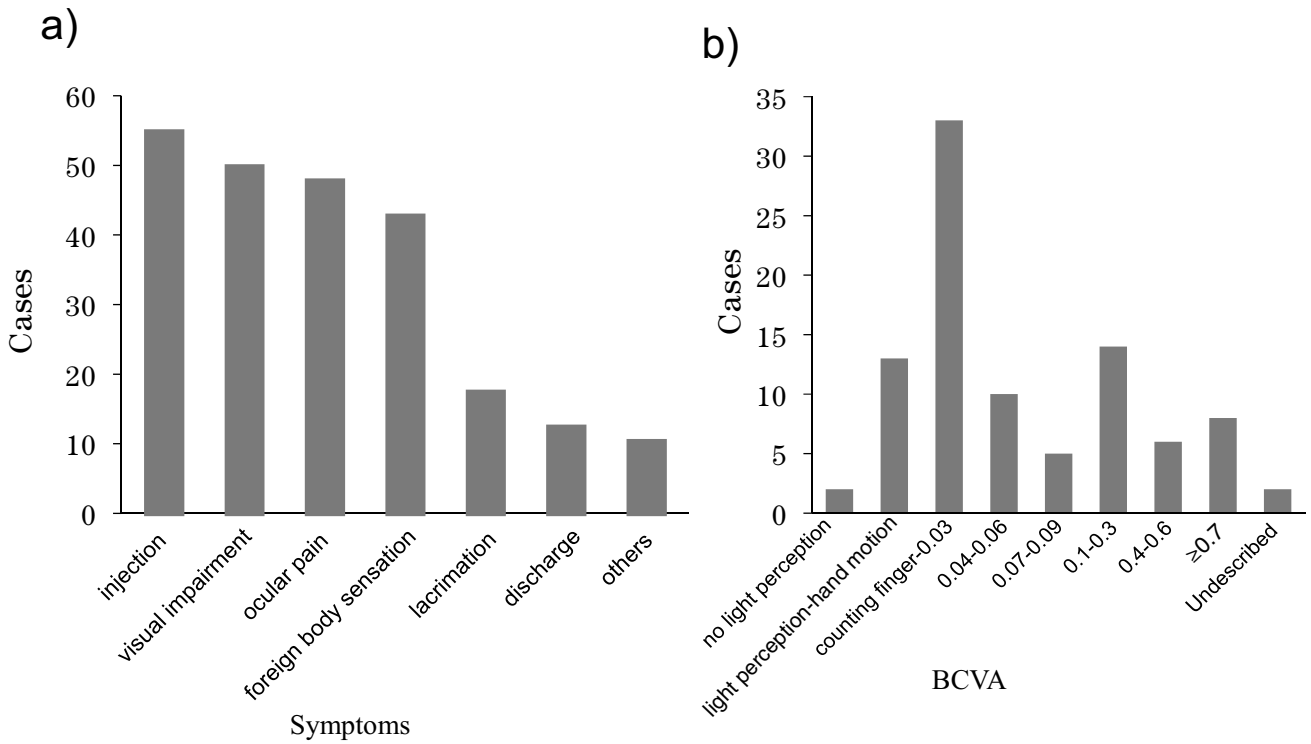

Fig. 3 Symptoms and best-corrected visual acuity (BCVA) at the first visit. **a** Symptoms. There are overlaps. **b** BCVA. The BCVA was less than 0.09 in 63 cases (67.8%), and less than 0.03 in 48 cases (51.6%)

Table 3 Clinical findings at the first visit (eyelid•conjunctiva)

	All cases 93 cases		Yeast-like fungi 41 cases		Filamentous fungi 52 cases	
	Eyes	%	Eyes	%	Eyes	%
Lid swelling •redness * [1]	22	23.7	5	12.2	17	32.7
Discharge	20	21.5	5	12.2	15	28.8
Ciliary injection * [2]	59	63.4	17	41.5	42	80.8
Others	2	2.2	1	2.4	1	1.9

Overlap (+)

*1: yeast-like fungi vs filamentous fungi, $P=0.0209$, $\chi 2$ test

*2: yeast-like fungi vs filamentous fungi, $P=0.0001$, $\chi 2$ test

(33.3%) used 3 drugs, and 4 cases (4.3%) used 4 drugs. A combination of pimaricin and voriconazole was used in 23 cases (24.7%).

Surgery, other than keratoplasty, was performed in 37 cases (39.8%) including 34 of debridement and 1 of anterior chamber irrigation. In 10 cases, therapeutic keratoplasty was performed, including 9 of penetrating keratoplasty and 1 of deep anterior lamellar keratoplasty. Fungi were detected in the excised cornea in 2 cases. The causative organisms of cases requiring keratoplasty were; 5 yeast-like fungi (1 *Candida albicans*, 2 *Candida parapsilosis*, 1 *Candida duobushaemulonii,* and 1 *Wickerhamomyces anamalus*), and 5 filamentous fungi (1 *Aspergillus udagawae*, 2 *Fusarium solani*, 1 *Fusarium oxysporum*, and 1 *Paecilomyces lilacinus*).

The distribution of the BCVAs at 3 months after the first visit had a bimodal shape (Fig. 5). The BCVA in 39 eyes (41.9%) was less than 0.03.

Statistical analyses

Statistical analyses were performed on the data collected from 90 cases (40 cases of yeast-like fungi and 50 cases of filamentous fungi) and not on 3 cases with unknown outcomes. Univariate Cox regression analyses found that filamentous fungi, no prior topical steroid use, visual

Table 4 Clinical findings at the first visit (cornea)

	All cases 93 cases		Yeast-like fungi 41 cases		Filamentous fungi 52 cases	
	Eyes	%	Eyes	%	Eyes	%
Immune ring	3	3.2	0	0.0	3	5.8
Satellite lesions	9	9.7	5	12.2	4	7.7
Hyphate ulcer*	23	24.7	4	9.8	19	36.5
Pseudodendrites	2	2.2	1	2.4	1	1.9
Elevated lesions	17	18.3	8	19.5	9	17.3
Hard infiltrates	30	32.3	13	31.7	17	32.7
Corneal perforation	3	3.2	2	4.9	1	1.9
Others	9	9.7	5	12.2	4	7.7

Overlap (+)

*: yeast-like fungi vs filamentous fungi, $P=0.0030$, $\chi 2$ test

Table 5 Clinical findings at the first visit (Anterior chamber•Iris)

	All cases 93 cases		Yeast-like fungi 41 cases		Filamentous fungi 52 cases	
	Eyes	%	Eyes	%	Eyes	%
AC cells•flare*1	55	59.1	17	41.5	38	73.1
AC fibrin*2	7	7.5	0	0.0	7	13.5
Hypopyon	22	23.7	6	14.6	16	30.8
Iris NV	2	2.2	0	0.0	2	3.8
Keratic precipitates	21	22.6	9	22.0	12	23.1
Endothelial plaque	14	15.1	4	9.8	10	19.2
Posterior synechia	5	5.4	2	4.9	3	5.8
Anterior synechia	4	4.3	2	4.9	2	3.8
Others	2	2.2	2	4.9	0	0.0

Overlap (+)

AC: anterior chamber

NV: neovascularization

*1: yeast-like fungi vs filamentous fungi, $P=0.0020$, $\chi 2$ test

*2: yeast-like fungi vs filamentous fungi, $P=0.0146$, $\chi 2$ test

impairment as a symptom at the first visit, and BCVA of more than 0.04 at the first visit were factors related with a shorter healing time (Fig. 6). Multivariate Cox regression analysis found that no prior topical steroid use, BCVA of more than 0.04 at the first visit, and no main lesion at the peripheral cornea were factors related with a shorter healing time (Table 7). Univariate logistic regression analyses found that no past history of corneal diseases, no past history of ocular surgery, no prior topical steroid use, ocular pain at the first visit, and BCVA of more than 0.04 at the first visit, and no lesion involving one-third depth of the stromal layer were factors correlated with BCVA of more than 0.1 at 3 months after the first visit (Fig. 7). Multivariate logistic regression analysis showed that no history of ocular surgery, BCVA of more than 0.04 at the first visit, and no lesion involving one-third deep stromal layer were factors correlated with BCVA of more than 0.1 at 3 months after the first visit (Table 8).

Discussion

Our results show that 54.8% of the cases of fungal keratitis had prior corneal diseases, and 59.1% had prior ocular surgery. These percentages are much higher than in other countries such as the 6.4% in India [1]. We suggest that this difference is related to the higher incidence of yeast-like fungi which was 0.7% in India [1], 2.0% in China [2], 22% in United States [3], and 44.1% in Japan. In Japan, many cases of fungal keratitis are caused by topical steroid use after ocular surgery, especially corneal surgery.

A history of systemic diseases was observed in 29.0%, not too high considering that 79.6% of the patients were more than 60-years-of-age.

One reason for the difficulty of diagnosing fungal keratitis in Japan is the rarity of cases, and another is the

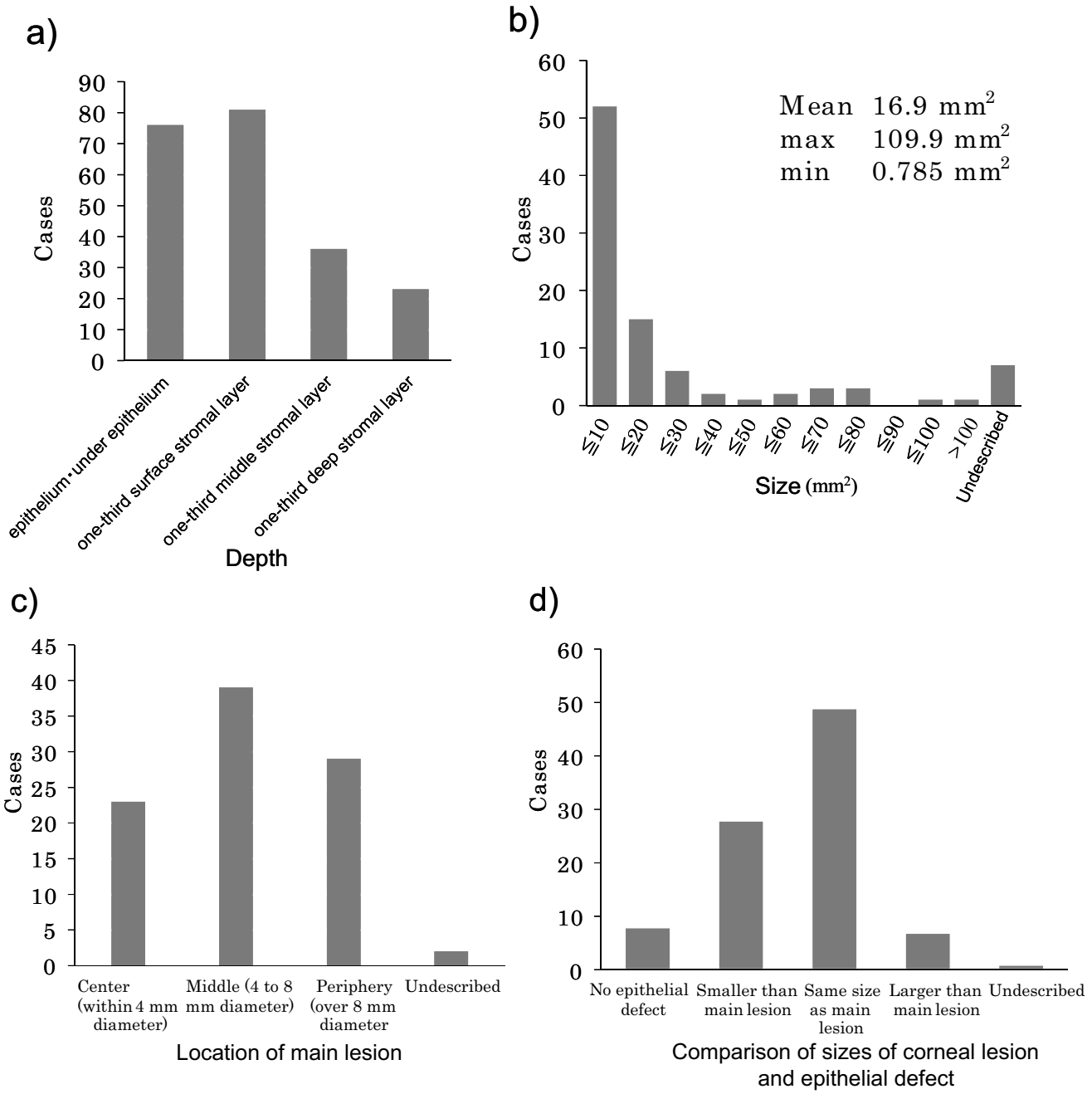

Fig. 4 Corneal lesions. **a** Depth. There are overlaps. Twenty-three cases (24.7%) were affected in the deep layer of the corneal stroma. **b** Size. In 52 cases (55.9%), the size of lesion was less than 10 mm^2. **c** Location of main lesion. The lesion in 29 cases (31.2%) affected the peripheral cornea. **d** Comparison of sizes of corneal lesions and epithelial defect. No epithelial defect was observed in 8 cases (8.6%), and epithelial defect was smaller than corneal main lesion in 28 cases (30.1%)

difficulty in culturing fungi compared to that of bacteria. In this sense, the collection of culture-proven 93 cases in this study was important. Direct inoculation of scraped specimens into the media and longer incubation period of up to 3 weeks were helpful in increasing the positive rate of the cultures.

Many cases of severe keratitis were included in this study. In more than one-half of the cases, the BCVA at the first visit was less than 0.03, and more than 40% had a BCVA of less than 0.03 at 3 months after the first visit. Twenty-two cases of hypopyon, 14 cases of endothelial plaque, and 10 cases requiring therapeutic keratoplasty also indicated the

Table 6 Number of cases which used each type of antifungal drug

Antifungals	Administration route				
	Eye drops	Ointment	Subconjunctival injection	Intrastromal injection	Intravenous or Oral
Fluconazole	10 (10)	0	0	0	0
Miconazole	6 (2)	0	0	0	0
Voriconazole	80 (35)	6 (3)	16 (7)	12 (3)	53 (20)
Micafungin	25 (17)	0	3 (2)	0	7 (3)
Pimaricin	21 (3)	55 (15)	0	0	0
Amphotericin B (including liposomal)	18 (2)	1 (1)	0	0	5 (1)
Itraconazole	0	0	0	0	21 (11)
Flucytosine	0	0	0	0	0

Numbers in parentheses indicate the number of cases of yeast-like fungi

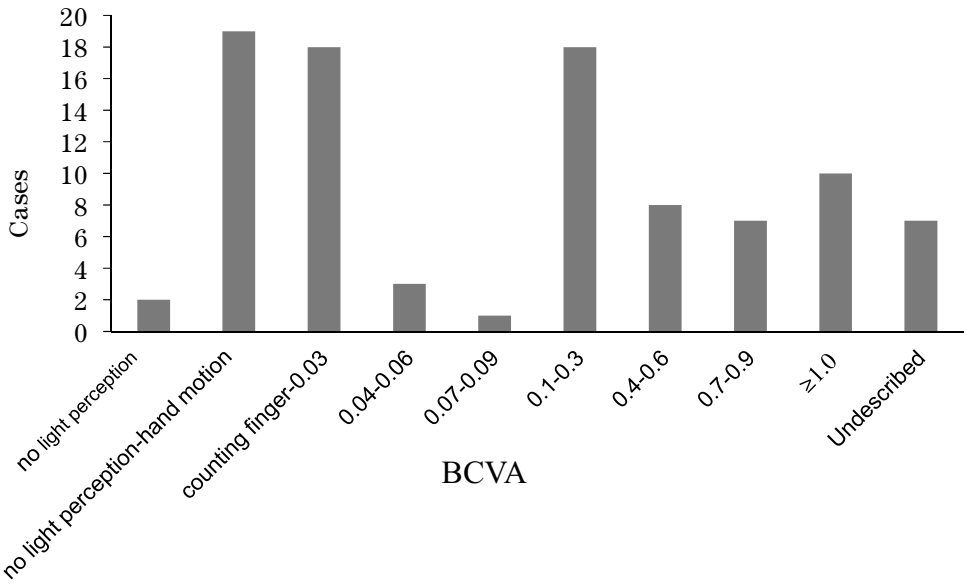

Fig. 5 Best-corrected visual acuity (BCVA) at 3 months after the first visit. The BCVA was less than 0.03 in 39 cases (41.9%)

severity of our cases. The ratio of therapeutic keratoplasty was higher than cases of bacterial keratitis, however. it was lower than that of cases of fungal keratitis in other countries (15–88%) [2, 4, 5, 22]. Also, no enucleation was performed in this cohort. This is remarkable compared with the data in other countries, such as the 4.3% [2] in China and 6.8% [22] in India. These data indicate that the management of fungal keratitis is not so inferior in Japan. The appropriateness of the management of fungal keratitis was also indicated by no use of fluconazole in cases of filamentous fungi (Table 6), because the low efficacy of fluconazole on filamentous fungi is generally recognized by cornea specialists in Japan.

The differences in the clinical findings of yeast-like fungi and filamentous fungi are important, especially in Japan because of high rates of cases due to yeast-like fungi. The differences between the two were statistically confirmed. Statistically higher incidences of lid swelling/redness, ciliary injection, anterior chamber cells/flare, anterior chamber fibrin in cases of filamentous fungi, suggested that filamentous fungi cause more inflammation than yeast-like fungi, or the inflammation was masked by the steroid use in cases of yeast-like fungi.

Although *Candida* and *Fusarium* spp. are the main causative agents, gene analyses revealed that various fungal species can cause fungal keratitis, which is one of the factors of the diversity of fungal keratitis, leading to the difficulty in establishing uniform treatment. This diversity is not surprising, because more than one million fungal species exist, and modern medical advancement provides numerous immunosuppressed situations to allow the fungi to grow in the cornea.

Factors correlated with shorter healing time were filamentous fungi, no prior topical steroid use, visual impairments as symptoms at the first visit, and BCVA of more

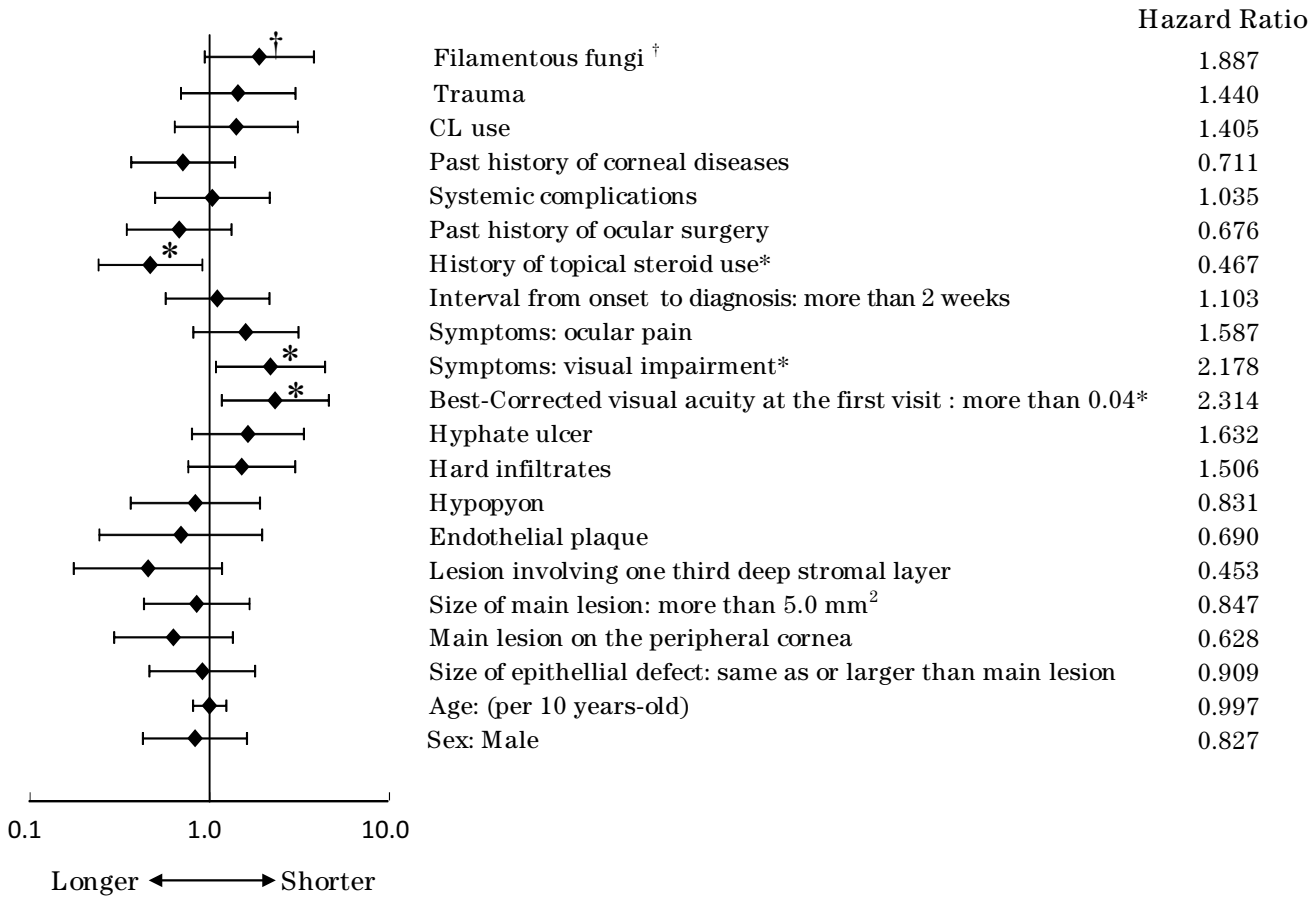

Fig. 6 Factors related with shorter healing time. * $P<0.05$; † $P=0.0746$; Univariate Cox regression analyses. All cases that necessitated keratoplasty were treated as withdrawal at 92 days

than 0.04 at the first visit as determined by univariate Cox regression analyses (Fig. 6). Of these, no prior topical steroid use and BCVA of more than 0.04 at the first visit were also found to be significant by the multivariate Cox regression analysis. The main lesion on the peripheral cornea was selected as a factor in multivariate Cox regression analysis (Table 7). From these data, it was considered that steroid use caused fungal growth, prolonging the duration of fungal keratitis, and the BCVA at the first visit was important for the prognosis. The main lesion on the peripheral cornea is obviously a negative factor for the management of fungal keratitis because therapeutic keratoplasty is difficult in such cases, necessitating continuation of medical treatments for long periods. Filamentous fungi and visual impairments at the first visit were unexpected factors related with shorter healing time, however conversely, it was believed that cases with yeast-like fungi had less inflammation due to topical steroid use, and occasionally no visual impairment as symptoms at the first visit. However, it required more time to heal because it is difficult to stop the use of steroids prescribed for the protection of corneal condition, such as a corneal graft. It was assumed that these two factors were not detected in the multivariate analysis because of their relationship with steroid use.

Table 7 Factors related with shorter healing date (Multivariate Cox regression analysis)

Parameter	P value	Hazard ratio	95% confidence Interval	
			Lower	Upper
History of topical steroid use	0.0179	0.446	0.229	0.870
Best-corrected visual acuity at the first visit: more than 0.04	0.0038	2.833	1.399	5.735
Main lesion on peripheral cornea	0.0628	0.477	0.219	1.040

To analyze factors related to healing, the date of the disappearance of epithelial defects was also determined. However, accurate results were not obtained (data not shown), because of various factors including the absence of epithelial

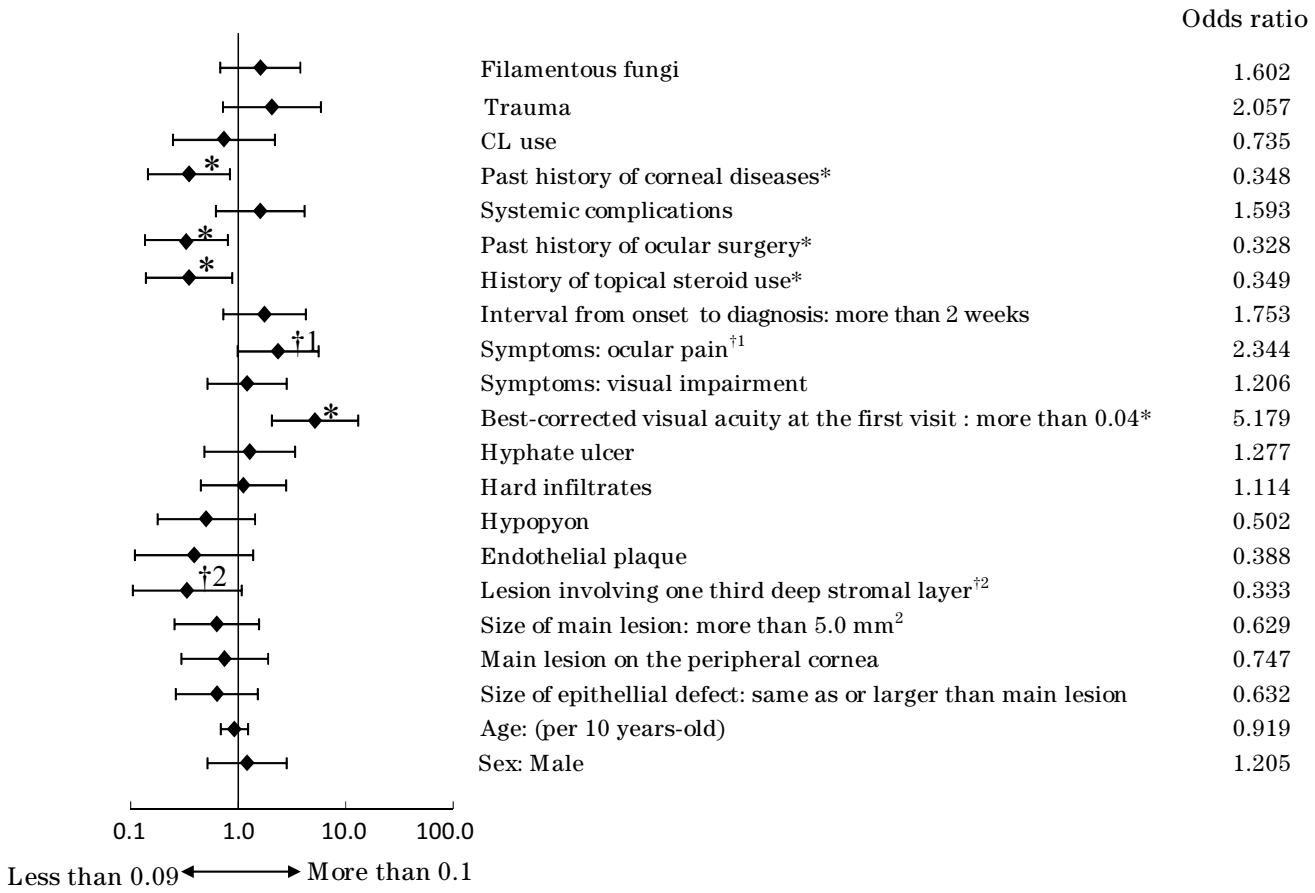

Fig. 7 Factors related with best-corrected visual acuity (BCVA) more than 0.1 at 3 months after the first visit. * $P<0.05$; †1 $P=0.0538$; †2 $P=0.0654$ Univariate logistic regression analyses. Cases that necessitated keratoplasty are excluded

Table 8 Factors related with best-corrected visual acuity more than 0.1 at 3 months after the first visit (Multivariate logistic regression analysis)

Parameter	P value	Odds ratio	95% confidence interval	
			Lower	Upper
Past history of ocular surgery	0.0168	0.177	0.043	0.733
Best-corrected visual acuity at the first visit: more than 0.04	0.0103	5.733	1.509	21.780
Lesion involving one third deep stromal layer	0.0731	0.264	0.061	1.133

defect at the first visit in some of cases, and no healing of epithelium because of presumed drug toxicity in other cases.

Factors related with BCVA of more than 0.1 at 3 months after the first visit, included no prior topical steroid use, no history of corneal diseases, no history of ocular surgery, ocular pain at the first visit, and BCVA of more than 0.04 at the first visit, and absence of a lesion involving one-third deep stromal layer were related factors as determined the univariate logistic regression analyses (Fig. 7). Of these, no prior ocular surgery, a BCVA of more than 0.04 at the first visit, and absence of the lesion involving one-third deep stromal layer were also found by the multivariate logistic regression analysis (Table 8). BCVA of more than 0.04 at the first visit and no prior topical steroid use were significantly correlated with both the healing time and visual prognosis. Thus, it can be said that the prognosis of fungal keratitis can be determined at the first visit. The lesion involving one-third deep stromal layer is an obvious poor factor for the treatment of fungal keratitis. A history of ocular surgery and corneal diseases were related with visual prognosis presumably due to basic visual impairment before the infection and vulnerability of the cornea to infection and inflammation.

As we could obtain the results of in vitro susceptibility tests provided by another report [21], the use of topical

antifungal drugs with the susceptibility by in vitro tests was also analyzed statistically. However, only two cases with no use of topical antifungal drugs with susceptibility in the analysis of healing time led to insignificant results. Only one case with no use of topical antifungal drugs with susceptibility in the analysis of visual prognosis led to an inability to perform statistical analyses. Even if drugs with susceptibility in vitro were used, the prognosis of fungal keratitis varies because different factors including basic corneal condition, basic systemic condition and differences of in vivo efficacy related with pharmacokinetics and drug toxicity. In a study comparing monotherapy of natamycin (pimaricin) and voriconazole in India [7], the relationship between the proportion of patients successfully treated with natamycin or voriconazol, and the MIC of each drug in vitro proved complicated [23]. Since our study included many cases with multidrug treatments, the relationships between the clinical effects and the results of in vitro susceptibility tests are not straightforward and even more complicated.

Although newly developed antifungal drugs, including voriconazole and micafungin are available, pimaricin is still the most powerful treatment for fungal keratitis. However, pimaricin is toxic to the ocular surface and has poor penetration to the deep layers of the cornea [24]. Accordingly, in the results of in vitro drug susceptibility tests in the first stage of the multicenter prospective observational study of fungal keratitis [20], the susceptibility to most antifungal drugs was good in cases of yeast-like fungi, however, in cases of filamentous fungi, drug susceptibility was mostly poor, and more than 50% of the strains were only susceptible to pimaricin. This is another reason why the treatment of fungal keratitis is difficult.

There are some limitations to our study. Despite this being a multicenter study, there were only 93 cases in the database. This is less than the study conducted by one institute in India and one in China. Also, this study was an observational study, therefore the analyses of antifungal drug efficacy were difficult.

In conclusion, fungal keratitis is caused by various species of fungi, leading to a diversity of findings and treatment results. Therefore, the ability to become refractory with various poor prognosis factors such as steroid use, and peripheral and deep stromal lesion make the treatment protocol difficult to codify.

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