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Effect of pretreatment with antifungal agents on clinical outcomes in fungal keratitis

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Abstract

Background—To determine if pretreatment with antifungal agents is predictive of worse clinical outcome in a fungal keratitis clinical trial.

Design—Non-pre-specified subgroup analysis of a randomized controlled trial in a tertiary hospital.

Participants—323 fungal ulcer cases with enrollment visual acuity of 20/40 to 20/400.

Methods—The Mycotic Ulcer Treatment Trial I was a randomized, double-masked trial to determine the optimal treatment for filamentous fungal keratitis at the Aravind Eye Care System, India. Enrolled cases were randomized to receive topical natamycin or voriconazole. Prior antifungal medication use, dose and duration were collected at enrollment. A subgroup analysis was performed to determine if patients using natamycin or azoles at presentation have worse clinical outcomes compared to those who were not pretreated.

Main Outcome Measures—3-month visual acuity (primary), 3-month infiltrate or scar size, corneal perforation and/or transplant, and re-epithelialization time.

Results—Of the 323 patients enrolled, 44% presented on an antifungal agent. Pretreated patients had larger mean baseline infiltrate size ($P<0.001$) and epithelial defect size ($P=0.02$). Multivariate regression analysis demonstrated that pretreatment was associated with significantly worse 3-month visual acuity ($P=0.006$), larger 3-month scar size ($P<0.001$) and increased odds of corneal perforation and/or transplant ($P=0.001$).

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Conclusions—Fungal keratitis that is smear-positive despite being pretreated with appropriate antifungal agents appears to be a risk factor for worse outcomes, likely due to initial ulcer severity and treatment failure. These patients may benefit from more aggressive multimodal therapy at a tertiary center.

Keywords

Fungal Keratitis; Fungal disease; Anti-infective agents

INTRODUCTION

Although treatment of bacterial corneal ulcers with antibiotics prior to presentation has been associated with increased time to healing[1] and increased antibiotic resistance, it has not been shown to worsen clinical outcomes.[1–3] In contrast to bacterial ulcers, pretreatment with antifungal agents has not been shown to promote resistance.[4] However, it is unknown if patients with fungal corneal ulcers who present to tertiary ophthalmic centers already on treatment with antifungal agents have worse clinical outcomes.

The Mycotic Ulcer Treatment Trial I (MUTT I) was a multicenter, randomized, double-masked clinical trial, which found that natamycin is superior to voriconazole for the treatment of filamentous fungal ulcers.[5] In this study, we performed a non-pre-specified subgroup analysis to determine if patients using natamycin or azoles at presentation have worse clinical outcomes compared to those who were not pretreated.

METHODS

Detailed methods for MUTT I have been reported previously.[5] Briefly, 323 smear-positive fungal ulcer cases with enrollment visual acuity of 20/40 (0.3 logMAR) to 20/400 (1.3 logMAR) seeking treatment at the Aravind Eye Care System in India were randomized to receive 5% topical natamycin (Natacyn, Alcon, Fort Worth, TX) or 1% topical voriconazole (VFEND I.V., Pfizer, New York, NY). One drop of medication was applied every hour while awake for one week, then every two hours while awake until three weeks post-enrollment. Pre-specified outcomes included visual acuity at 3 months (primary), infiltrate or scar size at 3 months, corneal perforation and/or transplant, and re-epithelialization time.

Enrolled patients were 10% potassium hydroxide (KOH) smear-positive for filamentous fungus, Gram stain-negative for bacteria and Giemsa stain-negative for Acanthamoeba. Three additional corneal scrapings were obtained for bacterial and fungal culture on day 1 and day 7 post-enrollment. Baseline characteristics between cases pretreated with antifungal agents versus those not pretreated were compared using chi-square or Fisher’s exact test for categorical variables, and t-test for continuous variables. Multivariate regression was performed, predicting clinical outcome with prior natamycin, topical or systemic azole, and natamycin plus azole compared to no pretreatment. Sensitivity analyses controlled for fixed effects of treatment arm, associated baseline variable, and organism. All statistical analyses were conducted using Stata 10.0 (College Station, TX: StataCorp LP). The MUTT I trial adhered to the Declaration of Helsinki and received approval from the Institutional Review Boards (IRB) at Aravind, Dartmouth, and University of California San Francisco (UCSF).

RESULTS

Of the 323 patients enrolled, 143 (44%) presented on an antifungal agent. Of those 143 patients who were pretreated, 79 (55%) used only topical natamycin, 17 (12%) used only topical or systemic azole, and 47 (33%) used both topical natamycin and an azole. The length of pretreatment varied between 6 days for those presenting only on natamycin (standard deviation [SD] 6 days), 4 days for those only on azole (SD 3 days) and 6 days for those on both natamycin and an azole (SD 5 days). The most common topical azoles at presentation were itraconazole (N=38), econazole (N=10) and voriconazole (N=6).

Table 1 shows baseline characteristics by pretreatment status. At enrollment, patients pretreated had on average 9 days of symptoms compared to 6 days for those not pretreated (P<0.001). Pretreated patients had larger ulcers compared to not pretreated, as measured by mean baseline infiltrate size (3.7mm vs. 3.0mm, P<0.001) and mean baseline epithelial defect size (2.7mm vs. 2.4mm, P=0.02). Pretreated ulcers were significantly less likely to be fungal-culture positive based on post-enrollment cultures obtained on day 1 (P=0.04) and day 7 (P=0.046). There was no significant difference in the number of *Fusarium* (P=0.45) or *Aspergillus* ulcers (P=0.98) between the two groups. Pretreatment status was not significantly associated with treatment arm (P=0.10), positive bacterial culture (P=0.12), steroid (P=0.12) or acyclovir use prior to enrollment (P=0.59).

Multivariate regression analysis (Table 2) demonstrated that pretreatment with an antifungal agent was associated with significantly worse 3-month visual acuity (0.23 logMAR, 95% confidence interval [CI] 0.10 to 0.35, P<0.001), larger 3-month scar size (0.62mm, 95% CI 0.28 to 0.95, P<0.001) and an increased odds of perforation and/or corneal transplant (odds ratio [OR] 4.26, 95% CI 1.89 to 9.61, P<0.001), after controlling for baseline variables, including baseline ulcer severity. Specifically, patients pretreated with both natamycin and an azole had significantly worse 3-month visual acuity, 3-month infiltrate/scar size and increased odds of perforation and/or therapeutic penetrating keratoplasty (TPK). Patients pretreated with an antifungal agent did not have significantly longer re-epithelialization time (hazard ratio [HR] 0.79, 95% CI 0.57 to 1.10, P=0.16) after controlling for epithelial defect size. As a sensitivity analysis, we included a term for frequency of dosing and duration of pretreatment to determine if inadequate pretreatment may have contributed to worse outcomes, but we did not find an association.

DISCUSSION

In this study we investigate whether pretreatment with antifungal medications prior to presentation at a tertiary ophthalmic center is a risk factor for worse clinical outcomes. We found that study participants treated with antifungal agents prior to trial enrollment had significantly worse 3-month visual acuity, increased scar size and increased risk of corneal perforation or need for penetrating keratoplasty after controlling for baseline ulcer severity. All patients included in the trial were KOH smear-positive for filamentous fungus at the time of enrollment.

One could consider smear-positivity in the setting of prior antifungal treatment to be a sign of treatment failure. As such, there may be a selection effect, with referral of more advanced corneal ulcers, which are doing poorly despite appropriate therapy, to tertiary centers. In support of this finding is the fact that study participants on pretreatment had worse clinical characteristics at presentation, including epithelial defect size and infiltrate or scar size. While other factors may contribute to delayed presentation at a tertiary care center, the main ones found to be significantly associated were increasing age, non-*Fusarium* cases, worse visual acuity at baseline and pretreatment with antifungal agents. It does not appear that a delay in treatment contributed to poor outcome since the mean time to initiation of antifungal therapy was 4 days in pretreated patients compared to 6.5 days in non-pretreated patients ($P<0.001$). Therefore, pre-treated patients actually had a shorter time between symptom onset and initiation of appropriate antifungal therapy.

It is also unlikely that inadequate initial treatment contributed to worse outcome. The majority of pretreated patients were on topical natamycin or a combination of natamycin and an azole. As demonstrated in MUTT I, natamycin treatment was superior to voriconazole treatment for filamentous fungal keratitis, especially for *Fusarium* cases.[5] The causative organism in pretreated compared to non-pretreated cases were not significantly different. The fact that these patients were pretreated with appropriate therapy and still smear-positive at the time of enrollment could be considered a sign of treatment failure. In support, our multivariate regression found an association between use of both natamycin and azole with worse outcomes. These patients who failed initial therapy and then were referred to a tertiary care center, likely had the most severe ulcers.

Another possible explanation could be the development of antifungal resistance during treatment. However, a previous analysis from MUTT I demonstrated that there was no association between pretreatment and antifungal resistance as measured by minimum inhibitory concentration.[4]

Therefore, clinicians should recognize that fungal keratitis patients referred despite receiving appropriate treatment and who continue to be smear-positive have a worse prognosis and therefore may warrant more aggressive treatment with multimodal therapy, which may include a combination of topical antifungals, oral antifungals, intrastromal antifungal injections as well as other surgical interventions.

Limitations to this study include that it is a non-pre-specified subgroup analysis with a relatively small sample size of patients who were pretreated. Data on patient compliance to pretreatment medications was not collected at the initial visit. As such, initial non-compliance to pretreatment medications may have been a risk factor contributing to worse clinical outcomes. However, upon enrollment in the study, patients were hospitalized, medications were administered by nurses and compliance was recorded. The trial enrolled patients in South India with filamentous fungal ulcers, which may limit the generalizability of the study to other geographic locations.

In summary, continued baseline KOH smear-positivity despite treatment with antifungal medications was associated with worse clinical characteristics at baseline and worse clinical

outcomes, which we attribute to a selection effect. These findings are significant because patients and clinicians seek presenting factors that will predict final outcome. Results of this study suggest that clinicians should inquire about pretreatment antifungal agents, obtain smear and culture at presentation in these ulcers despite pretreatment, and consider initiating more aggressive multimodal therapy.

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Table 1

Baseline Characteristics of Pretreated and Non-pretreated Isolates (*N*=323)

Characteristicss	*Pretreated (N=143)*	*Not Pretreated (N=180)*	*P-value*
Male, N (%)	83 (58)	100 (56)	0.65
Age (years), mean (SD)	46 (13)	48 (13)	0.12
Agriculture occupation, *N* (%)	63 (44)	93 (52)	0.17
Trauma or Injury, *N* (%)	95 (66)	111 (62)	0.38
Vegetative matter or wood	36 (25)	46 (26)	0.94
Metal or other[†]	55 (39)	56 (31)	0.17
Unknown object	5 (4)	9 (5)	0.59
Duration of symptoms (days), mean (SD)[‡]	9 (8)	6 (6)	**<0.001**
Systemic disease, *N* (%)	14 (10)	8 (4)	0.06
***Fusarium*, *N* (%)**	60 (42)	68 (38)	0.45
***Aspergillus*, *N* (%)**	24 (17)	30 (17)	0.98
Visual acuity (logMAR), mean (SD)	0.74 (0.4)	0.66 (0.4)	0.06
Infiltrate or scar size (mm), mean (SD)	3.7 (1.2)	3.0 (1.1)	**<0.001**
Hypopyon, *N* (%)			0.051
None	84 (59)	129 (72)	
<0.5mm	31 (22)	26 (14)	
>0.5mm	28 (20)	25 (14)	
% of Ulcer Depth, *N* (%)			0.28
>0–33	72 (50)	102 (57)	
>33–67	54 (38)	65 (36)	
>67–100	17 (12)	13 (7)	
Epithelial defect size (mm), mean (SD)	2.7 (1.4)	2.4 (1.2)	**0.02**
Positive fungal culture, *N* (%)[§]			
Day 1 enrollment culture	106 (74)	150 (83)	**0.04**
Day 7 repeat culture (*N*=299)	33 (25)	59 (36)	**0.046**
Natamycin treatment arm, *N* (%)	79 (55)	83 (46)	0.10
Positive bacterial culture, *N* (%)	0 (0)	3 (2)	0.12

Abbreviations: Number (N), Standard Deviation (SD), millimeter (mm), Logarithm of the Minimal Angle of Resolution (logMAR)

[†]Includes dust, finger, kerosene, cement, fingernail, chili powder, sand, cow's tail, and insect.

[‡]The *P*-value for duration of symptoms was calculated by Wilcoxon rank sum test; all other *P*-values were calculated by Chi square or Fisher's exact test for categorical variables and t-test for continuous variables.

[§]Results read after incubation for 1 week

Table 2

Relationship between Pretreatment and Clinical Outcomes[†]

Covariate	Clinical Outcome (95% CI)	*P*-value
Linear Regression Model Predicting 3-Month Visual Acuity (logMAR)		
Any antifungal agent	0.23 (0.10 to 0.35)	<0.001
Natamycin and topical azole use at presentation	0.36 (0.11 to 0.62)	0.006
Only natamycin use at presentation	0.22 (0.02 to 0.42)	0.03
Only topical azole use at presentation	0.06 (−0.36 to 0.48)	0.77
Linear Regression Model Predicting 3-Month Scar Size (mm)		
Any antifungal agent	0.62 (0.28 to 0.95)	<0.001
Natamycin and topical azole use at presentation	1.47 (0.82 to 2.13)	<0.001
Only natamycin use at presentation	0.47 (−0.03 to 1.00)	0.07
Only topical azole use at presentation	−0.10 (−1.15 to 0.96)	0.86
Logistic Regression Model Predicting Perforation and/or TPK (Odds Ratio)		
Any antifungal agent	4.26 (1.89 to 9.61)	<0.001
Natamycin and topical azole use at presentation	12.87 (2.68 to 61.70)	0.001
Only natamycin use at presentation	3.30 (0.91 to 12.02)	0.07
Only topical azole use at presentation	3.07 (0.31 to 29.88)	0.34
Cox Proportional Hazards Regression Model Predicting Time to Re-epithelialization (Hazards Ratio)		
Any antifungal agent	0.79 (0.57 to 1.10)	0.16
Natamycin and topical azole use at presentation	0.64 (0.32 to 1.27)	0.20
Only natamycin use at presentation	0.91 (0.47 to 1.38)	0.43
Only topical azole use at presentation	1.69 (0.62 to 4.55)	0.30

Abbreviations: Logarithm of the Minimal Angle of Resolution (logMAR), Confidence Interval (CI), millimeter (mm), Therapeutic Penetrating Keratoplasty (TPK)

[†]Multivariate regression adjusting for the following fixed effects: associated baseline variable (enrollment visual acuity, infiltrate size, ulcer depth, epithelial defect size), treatment arm, organism (*Aspergillus, Fusarium*, or all other), fungal culture-positive at day 7 of treatment, duration of symptoms minus pretreatment time (including interaction between this variable and pretreatment).