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The Utility of Repeat Culture in Fungal Corneal Ulcer Management: A Secondary Analysis of the MUTT I Randomized Clinical Trial

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Abstract

PURPOSE—To determine whether patients who had a positive repeated culture was predictive of worse clinical outcome than those who achieved microbiological cure at 6 days in the Mycotic Ulcer Treatment Trial I (MUTT-I).

DESIGN—Secondary analysis from a multicenter, double-masked, randomized clinical trial.

METHODS

Setting: Multiple hospital sites of the Aravind Eye Care System, India.

Study Population: Patients with culture-positive filamentous fungal ulcers and visual acuity of 20/40 to 20/400 re-examined 6 days after initiation of treatment

Intervention: Corneal scraping and cultures were obtained from study participants at day-6 after enrollment.

Main outcome Measures: We assess 3-month best spectacle corrected visual acuity (BSCVA), 3-month infiltrate/scar size, corneal perforation and re-epithelialization rates stratified by culture positivity at day 6.

RESULTS—Of the 323 patients with smear positive ulcers enrolled in MUTT-I, 299 (92.6%) were scraped and cultured six days after enrollment. Repeat culture positivity was 31% (92/299). Among patients who tested positive at enrollment, those with positive 6-day cultures had

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significantly worse 3-month BSCVA (0.39 LogMAR; 95% CI: 0.24 to 0.44; $P<0.001$), larger 3-month scar-size (0.39 mm; 95% CI: 0.06 to 0. 73; $P=0.02$), were more likely to perforate or require therapeutic penetrating keratoplasty (OR: 6.27; 95% CI: 2.73 to 14.40; $P<0.001$), and were slower to re-epithelialize (HR: 0.33; 95% CI: 0.21 to 0.50; $P<0.001$) than those with a negative 6-day culture result.

CONCLUSIONS—Early microbiological cure on culture is a predictor of clinical response to treatment.

TRIAL REGISTRATION clinicaltrials.gov Identifier—NCT00996736

INTRODUCTION

Fungal keratitis represents up to 50% of corneal infections in the tropics and remains one of the most challenging categories of ocular infection to treat.[1,2] Although corneal cultures are still the gold standard for diagnosis of keratitis, their use for assessing treatment response and clinical prognosis may prove to be valuable.[3] Monitoring response to therapy is often complicated by toxicity of topical drops, and/or host immune/inflammatory response, which may appear to worsen the corneal opacity, although they are controlling the infection.[4–6] Typical characteristics of ulcer healing such as epithelialization do not always indicate that a fungal ulcer is responding; and in fact may even hinder the penetration of topical fungicide.[7]

Clinicians also look for ways to determine which patients are at highest risk of a poor outcome and need closer monitoring, particularly in resource poor settings.[4] One study of both suspected fungal and bacterial corneal ulcers found that baseline smear-negative and culture-negative microbial keratitis had a decreased risk of requiring surgical intervention compared with culture-positive keratitis. In the Steroids for Corneal Ulcer Trial (SCUT), baseline culture positivity in bacterial ulcers despite prior appropriate antibiotic treatment was associated with worse visual acuity outcomes.[8–12] Here, we investigate the utility of repeat culture for determining prognosis and management of fungal ulcers.

METHODS

The methods for the MUTT-I have been discussed in detail previously.[13] Briefly, patients presenting to the Aravind Eye Hospital or the University of California San Francisco Eye Clinics with a smear-positive fungal corneal ulcer and baseline vision of 20/40 to 20/400 were enrolled and randomized to receive either topical natamycin 5% (Natacyn; preserved with benzalkonium chloride, 0.01%) or topical voriconazole, 1% (Vfend IV; reconstituted in sterile water for injection with benzalkonium chloride, 0.01%, by Aurolab). Baseline and 6-day scraping and cultures were obtained and detailed methods for the handling of microbiological specimens have been outlined in a prior publication.[13] Ethical approval was obtained from the Aravind Eye Care System Institutional Review Board, the University of California, San Francisco Committee on Human Research, and the Dart-mouth-Hitchcock Medical Center Committee for the Protection of Human Subjects. Informed written consent was obtained from all participants, and the trial conformed to the Declaration of Helsinki. MUTT-I was registered at clinicaltrials.gov under NCT00996736.

OUTCOME MEASURES

The primary outcome for this non- pre-specified secondary analysis was best spectacle-corrected visual acuity (BSCVA) at 3 months. Secondary outcomes included infiltrate/scar size at 3 months, the occurrence of corneal perforation and/or the need for therapeutic penetrating keratoplasty (TPK) and rate of re-epithelialization.

STATISTICAL ANALYSIS

Patients enrolled in MUTT-I who did not have a repeat corneal scraping and culture were excluded from the analysis. Baseline characteristics were compared using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. Multiple linear regression predicting patient's 3-month BSCVA with covariates including 6-day culture-positivity (yes/no), treatment arm, and baseline BSCVA was performed. The geometric mean of the longest diameter and the longest perpendicular was used to assess infiltrate/scar size and epithelial defect size. Multiple linear regression was fit predicting patient's 3-month infiltrate/scar size using 6-day culture positivity, treatment arm, and enrollment infiltrate/scar size as covariates. Time to re-epithelialization was analyzed using a Cox proportional hazards model with 6-day culture positivity, treatment arm, and enrollment epithelial defect size as covariates. A logistic regression model with covariates for 6-day culture positivity, treatment arm, and baseline infiltrate depth was used to assess the odds of corneal perforation and/or TPK. Separate sensitivity analyses of the models were performed by controlling for baseline culture positivity (yes/no), bacterial organism and baseline clinical characteristics including BSCVA, infiltrate/scar size, epithelial defect size, presence of hypopyon, and depth of ulcer.

RESULTS

Six days after trial enrollment, 299 of the 323 patients were scraped and cultured again, resulting in a repeated culture positivity of 31% (92/299). Table 1 compares the baseline characteristics of study participants who were repeat culture positive versus repeat culture negative. There were 130 males (43.5%) with a median age was 47 (IQR 38, 56). Median baseline visual acuity was logMAR 0.66 (IQR 0.38, 0.90), and median baseline infiltrate/scar size was 3.19 (IQR 2.50, 4.00). Those who did not have a negative culture by day 6 were slightly younger and somewhat less likely to have been on topical antifungals at presentation. They also had overall slightly worse baseline clinical features such as decreased visual acuity, increased scar size, increased epithelial defect and more likely to have a hypopyon than those who were culture negative at 6 days.

Table 2 outlines the infectious organisms isolated in the 299 patients undergoing repeat culture, which included 122 (40.8%) Fusarium, 49 (16.4%) Aspergillus and 72 (24.1%) other filamentous fungi. Fifty-six (18.7%) patients tested fungal culture negative both at baseline and repeat culture. Baseline cultures did not predict 3-month visual acuity (P = 0.11), 3-month infiltrate/scar size (P = 0.30), rate of re-epithelialization (P=0.08) or rate of corneal perforation or the need for TPK (P = 0.07) after correcting for baseline values and treatment arm (Table 3a &3b).

Study participants with positive 6-day cultures had on average 0.42 LogMAR lines worse BSCVA at 3-month after correcting for baseline visual acuity and treatment arm (95% CI: 0.28 to 0.56; P<0.001; Table 3a). They also had 0.39 mm larger 3-month scar-size (95% CI: 0.09 to 0. 70; P=0.01), had 7.15 times the odds of full thickness corneal perforation or the need for therapeutic penetrating keratoplasty (95% CI: 3.38 to 15.13; P<0.001; Table 3b). Finally, they were also slower to re-epithelialize after correcting for baseline values and treatment arm than those with a negative 6-day culture result (HR: 0.32; 95% CI: 0.22 to 0.47; P<0.001; Figure 1).

Enrollment culture results showed a higher percentage of positivity (79%; 252/323) as compared to day-6 cultures 31% (92/299) and culture-positivity between baseline and 6-days was significantly correlated (R=0.22; P<0.001). Sensitivity of our models were tested by adjusting for enrollment culture-positivity, microorganism, and baseline clinical characteristics (BSCVA, infiltrate/scar size, epithelial defect size, presence of hypopyon, and depth of ulcer). Sensitivity models produced very similar results and culture-positivity remained statistically significant.

Day 6 smear-positive results were highly correlated with day-6 culture-positive results (R=0.48; P<0.001). Smear positive patients had on average 0.35 LogMAR lines worse BSCVA at 3-month (95% CI: 0.22 to 0.48; P<0.001, Table 3a), 0.49 mm larger 3-month scar-size (95% CI: (0.20 to 0.77); P=0.001), were slower to re-epithelialize (HR: 0.40; 95% CI: 0.28 to 0.57; P<0.001), and had 4.36 times the odds of full thickness corneal perforation or the need for therapeutic penetrating keratoplasty (95% CI: 2.21 to 8.60; P<0.001) after correcting for baseline values and treatment arm than those with a negative 6-day smear result. (Table 3b).

DISCUSSION

The importance of corneal cultures in the diagnosis of infectious keratitis has been well established. Here, we demonstrate that there is a potential role for corneal cultures in assessing treatment response and determining clinical prognosis as well. In our study, corneal ulcers that did not have negative cultures results by day 6 had worse 3-month visual acuity, larger scar size, increased risk of corneal perforation and slower rates of re-epithelialization. Although our ultimate goal in treatment of corneal ulcers is ulcer healing, our immediate objective of therapy is to eliminate the infection. Baseline culture positivity despite antibiotic therapy has been associated with poor outcomes in bacterial and fungal keratitis[8,9,12,14] Similarly, positive fungal donor rim cultures have also been shown to predict increased risk of fungal endophthalmitis after corneal transplant.[15] By contrast in our fungal smear positive population, baseline culture status did not predict clinical outcomes.

Obtaining repeat cultures allows clinicians to directly assess treatment response. If the patient has a positive repeated culture, clinicians could then consider augmenting current therapy by adding another medication topically, starting oral antifungal, or using innovative treatment techniques such as collagen cross-linking or intrastromal antifungal injection. In addition to altering therapy, positive repeat cultures identify patients who are at greatest risk of a negative outcome such as corneal perforation or the need for TPK and would benefit

from closer monitoring. This is particularly important in resource poor settings where these types of infections are most prevalent. Remarkably, a simple smear was also highly correlated with clinical outcomes in our study making it a quick and inexpensive alternative to full corneal cultures.

The potential for repeat culture status to be used as surrogate markers in clinical trials is also noteworthy. The use of surrogate endpoints has become increasingly common in clinical trials, particularly in the fields of oncology and infectious disease.[16] Advantages of surrogate trial endpoints include smaller sample sizes and faster trial completion as they allow detection of response to treatment at an early stage. The fact that repeat culture is easily obtained at an early stage of treatment and so highly correlated with all clinical outcomes of interest make it an excellent choice.

Limitations to this study include the fact that all patients in the study were enrolled in south India therefore organisms in this region may not be representative of infectious organisms in other regions or countries. Although all organisms in this study were filamentous fungi, a variety of species were represented, making it difficult to draw conclusions about the significance of culture positivity for each organism subtype.

Microbiological positivity on repeat culture appears to be a clinically useful tool for assessing treatment response and risk of poor clinical outcome. This simple measure also may serve as a valuable surrogate endpoint for corneal ulcer clinical trials given how highly it is correlated with clinical outcomes of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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b) FINANCIAL DISCLOSURES. There are no financial conflicts of interest to report. Kathryn Ray, Tom Lietman and Jennifer Rose-Nussbaumer had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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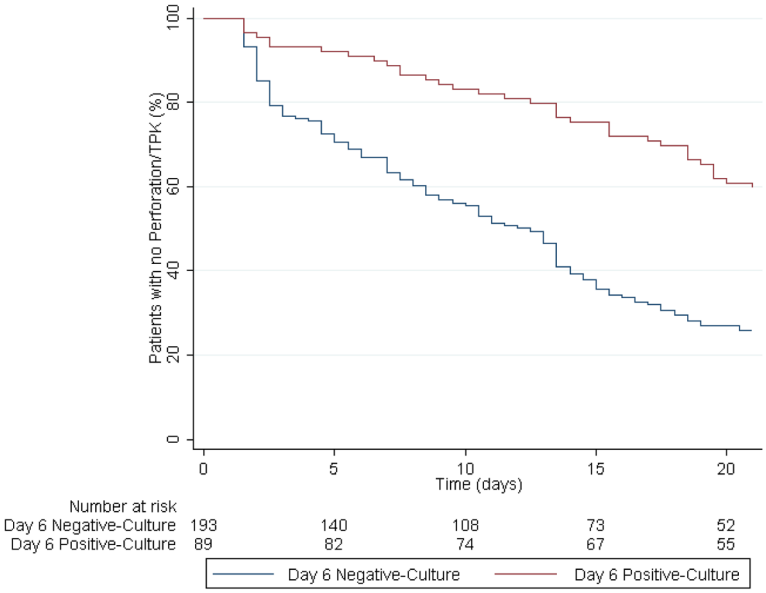

Figure 1. Kaplan-Meier Survival Curve for Corneal Re-Epithelialization

Kaplan-Meier survival curve comparing the probability of corneal re-epithelialization between patients enrolled in the Mycotic Ulcer Treatment Trial–I who had culture-positive results after 6 days of treatment (red) compared to those who had culture-negative results (blue).

Table 1
Baseline Characteristics of Patients by Repeated Culture Results

Baseline characteristic data for 299 patients with a repeated fungal culture performed 6 days after enrollment in the Mycotic Ulcer Treatment Trial-I. Patient characteristics are stratified by their day-6 culture results.

Baseline Characteristic	**Fungal Culture Positive on Day 6 (*N*=92)**	**Fungal Culture Negative on Day 6 (*N*=207)**
Gender, N		
Male	43 (14%)	87 (29%)
Female	49 (16%)	120 (40%)
Age (years), median (25th, 75th Percentile)	45 (38, 55)	48 (38,58)
Occupation, N		
Agriculture	34 (28%)	100 (33%)
Non-Agriculture[a]	58 (19%)	107 (36%)
Medication use at enrollment[b], N		
Topical ocular antifungals	37 (12%)	101 (34%)
Other topical ocular drops[c]	50 (17%)	128 (43%)
Systemic antifungals	1 (1%)	10 (3%)
Other systemic	26 (9%)	54 (18%)
Trauma/Injury, N		
Vegetative Matter/Wood	25 (8%)	47 (16%)
Metal/Other[d]	33 (11%)	74 (25%)
Unknown Object	3 (1%)	11 (4%)
Contact Lens	0 (0%)	0 (0%)
Affected Eye, N		
Right	115 (38%)	40 (13%)
Left	92 (31%)	52 (17%)
Visual Acuity (logMAR), median (25th, 75th percentiles)	0.72 (0.46, 1.02)	0.64 (0.38, 0.88)
Infiltrate/Scar Size (mm^1), median (25th, 75th percentiles)	3.39(2.95, 4.08)	3.14(2.40, 3.99)
Hypopyon		
no	50 (17%)	147 (49%)
<0.5mm	20 (7%)	32 (11%)
>0.5mm	22 (7%)	28 (9%)
Depth		
>0–33%	47 (16%)	111 (37%)
>33–67%	36 (12%)	76 (25%)
>67–100%	9 (3%)	19 (6%)
Epithelial Defect (mm^1), median (25th, 75th percentiles)	2.97 (2.00,3.66)	2.30 (1.41,3.39)
Duration of Symptoms, days, median (25th, 75th percentiles)	5 (4,10)	6 (3,10)
Systemic disease, N	9 (3%)	31 (10.4%)

[a] Includes unemployed, retired, etc.

[b] Some patients were on more than one medication at enrollment

[c] Includes topical antibiotics, dilating drops, glaucoma medication, lubricating drops

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

Table 2

Microbiological Fungal Culture Results

Fungal microorganisms identified in isolates obtained from patients for cultures performed at enrollment and 6 days after enrollment in the Mycotic Ulcer Treatment Trial-I.

Fungal Culture Results		***Fusarium* (*N*=122)**	***Aspergillus* (*N*=49)**	**Other (*N*=49)**
Enrollment	**6 Days After Enrollment**	***N* (%)**	***N* (%)**	***N* (%)**
+	+	41 (34%)	28 (23%)	16 (13%)
+	−	79 (20%)	20 (16%)	52 (43%)
−	+	2 (1%)	1 (1%)	4 (3%)

[a] Not included are 56 patients who tested positive for fungal smear (inclusion criterion for trial enrollment) but tested negative for fungal culture at enrollment and 6 days after enrollment.

Table 3a

Multiple Linear Regression assessing Best Spectacle-Corrected Visual Acuity.Results from three separate multiple linear regression models predicting 3-month Best Spectacle-Corrected Visual Acuity (logMAR) for patients testing positive (vs. negative) for presence of fungus at day-6, while correcting for treatment arm and baseline Best Spectacle-Corrected Visual Acuity.

Predictor	*N*	3-month BSCVA[a] (logMAR)	95% CI	*P*
Enrollment Culture Positivity	292	0.12	(−0.03 to 0.27)	0.11
Day-6 Culture Positivity	274	0.42	(.28 to 0.56)	<0.001
Day-6 Smear Positivity	275	0.35	(0.22 to 0.48)	<0.001

[a]Best Spectacle-Corrected Visual Acuity;

Table 3b

Regression Models assessing secondary outcomes: 3-month infiltrate/scar and corneal perforation (or need for Therapeutic Penetrating Keratoplasty).Results from models predicting 3-month infiltrate/scar size and corneal perforation (or need for Therapeutic Penetrating Keratoplasty) among patients testing positive for presence of fungus from baseline cultures, day-6 cultures, and day-6 smear, while correcting for treatment arm and baseline measurements.

	Multiple linear regression [a] predicting 3-month infiltrate/scar (mm)				**Logistic regression[b] predicting perforation or need for TPK,[c]**				
Predictor	***N***	**Coef.**	**95% CI**	***P***	***N***	**HR**	**95% CI**		***P***
Enrollment Culture Positivity	290	0.17	(−0.15 to 0.48)	0.30	299	2.35	(0.94 to 5.89)		0.07
Day-6 Culture Positivity	282	0.39	(0.09 to 0.70)	0.01	299	7.15	(3.38	to 15.13)	<0.001
Day-6 Smear Positivity	273	0.49	(0.20 to 0.77)	0.001	300	4.36	(2.21 to 8.60)		<0.001

[a] Model included baseline infiltrate/scar size and treatment arm as covariates

[b] Model included baseline depth and treatment arm as covariates

[c] Therapeutic Penetrating Keratoplasty