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The Significance of Repeat Cultures in the Treatment of Severe Fungal Keratitis

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Abstract

PURPOSE—To identify fungal keratitis patients who are at risk of a poor outcome and may benefit from closer follow-up or more aggressive treatment.

DESIGN—Secondary analysis of randomized clinical trial data.

SUBJECTS—Patients presenting with a smear-positive filamentous fungal ulcer, visual acuity of 20/400 or worse, and who subsequently had a 6-day fungal culture performed at the Aravind Eye Care system (India), Lumbini Eye Hospital (Nepal), or Bharatpur Eye Hospital (Nepal).

METHODS—We compare the clinical outcomes of patients who had positive 6-day fungal cultures compared to those who did not, using backwards-stepwise regression with co-variates for all baseline clinical characteristics.

MAIN OUTCOME MEASURES—The primary outcome is rate of corneal perforation and/or the need for therapeutic penetrating keratoplasty. Secondary outcomes include 3-month best spectacle corrected visual acuity (BSCVA), 3-month infiltrate and/or scar-size, and rate of re-epithelialization.

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RESULTS—Patients who tested positive at their 6-day culture had twice the hazard of experiencing a corneal perforation or the need for therapeutic penetrating keratoplasty *(P*=0.002) than those who tested negative even after controlling for baseline ulcer characteristics. These patients also had on average 0.26 LogMAR lines worse BSCVA at 3-months (*P*=0.001). Culture positivity at day-6 was not a statistically significant predictor of 3-month infiltrate/scar-size (−0.24 mm^1; P=0.45) or time to re-epithelialization (HR=.81; *P*=0.31).

CONCLUSIONS—Here we identify a uniquely valuable clinical tool, day 6 culture results, for the treatment of severe fungal keratitis. Risk stratification based on repeat culture positivity is an objective way to assess response to medical therapy and identify patients who are at high risk of a poor clinical outcome. This establishes a new standard of care for severe fungal keratitis management.

INTRODUCTION

Studies have suggested that in addition to providing an initial diagnosis, repeated culture can be used to assess response to treatment and potentially even have prognostic value.[1–4] A secondary analysis of the Mycotic Ulcer Treatment Trial–I (MUTT-I) demonstrated that fungal ulcers that were culture positive after starting antifungal therapy had worse 3-month visual acuity, scar size and, rate of perforation and/or need for therapeutic penetrating keratoplasty (TPK).[1] This association remained significant even after accounting for baseline characteristics known to correlate with outcomes.[1,5] Here, we evaluate the utility of baseline and repeat cultures to predict clinical outcomes in Mycotic Ulcer Treatment Trial – II (MUTT II), a new and independent dataset comprised of patients with more severe fungal corneal ulcers.

METHODS

The methods of MUTT-II have been described in detail previously.[6] Briefly, patients presenting with smear-positive filamentous fungal ulcers and visual acuity of 20/400 or worse were randomized to oral voriconazole versus placebo; all patients were treated with topical antifungals.[6] Scrapings and cultures were obtained from the corneal ulcers at baseline and 6 days (+/−1 day) after enrollment. Fungal cultures were defined as positive if any growth occurred on any 2 media or moderate to heavy growth on 1 medium.[7] The primary outcome for the trial was rate of corneal perforation and/or the need for TPK. Secondary outcomes included 3-month best spectacle corrected visual acuity (BSCVA), 3-month infiltrate/scar-size, and rate of re-epithelialization.

The primary analysis for this study used a backward stepwise elimination procedure with Cox regression to estimate the hazard of perforation or need for therapeutic penetrating keratoplasty (TPK) assessing 6-day culture positivity as the predictor of interest. In our initial model we included co-variates for multiple clinical baseline factors, including culture positivity, visual acuity, infiltrate/scar-size, ulcer depth, epithelial defect size, presence of hypopyon, treatment arm and organism categorized as *Fusarium, Aspergillus*, or other filamentous fungus. The stepwise elimination rule was pre-specified and had a significance level for removal of terms from the model of *P*>0.20. A corrected *p*-value for the repeat-positivity was determined using 10,000 Monte-Carlo simulations of the backward step-wise

subroutine, permuting repeat-positivity, and including all covariates in each initial model. For each permutation of repeat-culture, the backward stepwise regression subroutine sequentially removed variables from the model until the significance of each term satisfied $P<0.20$.

Secondary analyses were performed using backward stepwise procedures with linear regression to estimate BSCVA and infiltrate/scar-size assessing repeat culture-positivity, while controlling for enrollment clinical factors (as above), organism, and study treatment arm. Likewise, the stepwise procedure with Cox regression to estimate time to re-epithelialize with the same covariates listed above was performed. Each of the secondary models used a pre-specified significance level for removal of terms from the model set to $P>0.20$. We obtained a corrected *p*-value for the repeat-positivity using Monte-Carlo simulations permuting 6-day culture positivity 10,000 times. We also performed a sensitivity analysis of our results by adjusting for day-6 clinical characteristics (BSCVA, infiltrate/scar size, epithelial defect size, presence of hypopyon, and depth of ulcer) instead of enrollment clinical characteristics.

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 Dartmouth-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-II was a registered at clinitcaltrials.gov under NCT00997035.

RESULTS

A total of 208/237 (87.8%) corneas were scraped and samples cultured 6 days after enrollment and initiation of medical therapy. Repeat cultures were positive in 48.0% (100/208) of cases. Table 1 compares the baseline characteristics of study participants who underwent repeat cultures at day 6. There were 106 males (51.0%), with a mean age of 52.2 (SD 13.0), and 110 (52.8%) reported topical antifungal use prior to enrollment. Mean baseline visual acuity was logMAR 1.54 (SD 0.38) and mean baseline infiltrate/scar size was 5.55 (SD 1.59). Those who had a positive culture at day 6 had larger epithelial defect size at baseline as well as slightly decreased visual acuity, increased scar size, and were more likely to have a hypopyon than those who were culture negative at day 6. Table 2 outlines the infectious organisms isolated in the 208 patients undergoing both enrollment and repeat cultures, which included 67 (32.2%) Fusarium, 59 (28.4%) Aspergillus, and 52 (25.0%) other filamentous fungi. Thirty (14.4%) patients tested fungal culture negative both at baseline and repeat culture.

In multiple regression analysis, study participants who tested positive at their 6-day culture had 1.99 times the hazard of experiencing a corneal perforation or the need for therapeutic penetrating keratoplasty (95% CI: 1.29 to 3.08; $P<0.002$; Table 3). Variables remaining in the final stepwise regression model included enrollment infiltrate/scar-size (HR: 1.31; 95%CI 1.14 to1.51: P<0.001), ulcer depth (HR 1.50; 95% CI: 1.09 to 2.05; P=0.11), species (Aspergillus vs. Fusarium HR 0.63; 95% CI: 0.39 to 1.04; P=0.07), presence of hypopyon (HR: 1.56; 95% CI: 0.92 to 2.64; P=0.10), and treatment (oral Voriconazole vs oral placebo

HR 0.75; 95% CI: 0.50 to 1.24; P=0.16). Variables that dropped out of the stepwise procedure for this primary analysis included baseline culture positivity, visual acuity, and epithelial defect size.

Those with positive 6-day cultures had on average 0.26 LogMAR lines worse BSCVA at 3-months (95% CI: 0.10 to 0.42; *P*=0.001; Table 3) after controlling for baseline BSCVA, infiltrate/scar size, depth of ulcer and presence of hypopyon. Culture positivity at day 6 was not a statistically significant predictor of 3-month infiltrate/scar-size (−0.24 mm^1; 95% CI −0.86 to 0.38; P=0.45) or time to re-epithelialization (HR=.81; 95% CI: 0.46 to 1.28; *P*=0.31).

Variables selected from the stepwise procedure are shown in Table 3 for each of the secondary outcomes. For BSCVA at 3-months, selected variables included enrollment BSCVA (0.56 LogMAR; 95% CI: 0.30 to 0.81; *P*<0.001), infiltrate/scar-size (0.11 LogMAR; 95% CI: 0.06 to 0.17; P<0.001), depth of ulcer (−0.23 LogMAR; 95% CI: −0.39 to −0.07; *P*=0.01), and presence of hypopyon (0.15 LogMAR; 95% CI: −0.04 to 0.34; *P*=0.12). Predictors remaining for 3-month infiltrate/scar-size included baseline infiltrate/scar-size (0.11 mm^1; 95% CI: 0.06 to 0.17; *P*<0.001), presence of hypopyon (0.15 mm^1; 95% CI: −0.04 to 0.34; P=0.12) and treatment arm (0.75 mm^1; 0.50 to 1.24; *P*=0.16). Finally, variables selected from the stepwise procedure predicting hazard to re-epithelialization included epithelial defect size (HR 0.59; 0.50 to 0.71; *P*<0.001), ulcer depth (HR 0.73; 95%CI 0.50 to 1.06; *P*=0.10), and species (Other vs. Fusarium HR 0.67; 0.40 to 1.14; *P*=0.14).

Permutation p-values for day 6 culture positivity in each of the stepwise regression techniques yielded adjusted p-values (P_{adj}) of 0.013, 0.004, 0.46 and 0.38 for hazard of corneal perforation or need for TPK, 3-month BSCVA, 3-month Infiltrate/Scar, and time to re-epithelialization, respectively (Table 3, column 5). Sensitivity analysis adjusting for day 6 clinical characteristics (infiltrate/scar size, epithelial defect size, presence of hypopyon, and depth of ulcer) found a 1.97 times the hazard of experiencing a corneal perforation/TPK (Coef 1.97, 95% CI: 1.21 to 3.23, *P*=0.007) and 3-lines worse 3-month visual acuity (Coef 0.30, 95% CI 0.03 to 0.58, *P*=0.03)

DISCUSSION

In this study, we found that repeat culture positivity is an important predictor of clinical outcome in severe fungal ulcers. Those who were culture positive at 6 days despite appropriate medical treatment had a 2-fold risk of corneal perforation and/or the need for TPK and more than 2.5 lines worse BSCVA at 3 months, even after adjusting for other baseline clinical features such as treatment arm and infectious organism. Although at baseline these patients were clinically indistinguishable by day 6 those who were repeat culture positive had larger epithelial defects and infiltrate/scar size, deeper ulcers, and more often had a hypopyon. However, 6-day culture positivity continued to predict a 2-fold increase in corneal perforation and/or TPK and 3 lines worse 3-month visual acuity even after controlling for other 6-day characteristics suggesting that it is a uniquely valuable clinical tool and an objective measure of treatment response. Given the high correlation with

clinical outcomes, culture status may also have potential as an early surrogate outcome for clinical trials.

Culture positivity despite treatment has previously been found to be an important predictor of clinical outcome in bacterial keratitis and in less severe fungal ulcers in MUTT I.[1–3] This study validates the importance of repeat culture positivity in fungal ulcers in a second independent dataset of more severe ulcers with few overlapping baseline characteristics compared with MUTT I. Fungal organisms can represent 60–70% of infectious keratitis cases in tropical regions and fungal keratitis often carries a worse prognosis than bacterial keratitis.[8–11] Corneal opacity from prior infectious keratitis is an important cause of blindness worldwide.[12,13] In MUTT II, which found no benefit to adding oral voriconazole for severe fungal ulcers, had a rate of perforation and/or the need for TPK that was over 50%.[14] Baseline ulcer characteristics such as hypopyon, large infiltrate size and infiltrates involving the posterior 1/3rd of the stroma have previously been identified as risk factors for full thickness corneal perforation or the need for TPK.[15] Although these clinical characteristics are helpful in predicting outcomes, they do not provide as much insight into the best clinical management. For example, the association between hypopyon and culture positivity is statistically significant (P=0.004) however the presence of a hypopyon could mean that there is ongoing infection or it could be due to an inflammatory response to the infection. Repeat cultures provide important additional information to assess response to treatment and guide therapy.

The utility of repeat culture positivity to identify patients at greatest risk has significant implications for clinical practice. It is an indication to follow patients more closely and to consider an increase in therapy. Although current treatments in fungal keratitis are limited, intrastromal injection of voriconazole and corneal cross-linking are potential treatments that might be considered in these cases.[16–19] Repeat culture positivity along with the presence of hypopyon, or large, deep ulcers at baseline may also select patients who might benefit from early surgical intervention to eliminate infection such as TPK or therapeutic deep anterior lamellar keratoplasty.[20–22]

Strengths of our study include the prospective nature of our data collection and our rigorous statistical methods. Because stepwise regression sometimes introduces bias due to multiple comparisons, here, we validate statistical significance of day-6 culture positivity by performing a permutation analysis and providing adjusted p-values. Limitations include the fact that all ulcers were enrolled in South Asia where infectious organisms may not be representative of other countries. For example the study did not include any non-filamentous fungal keratitis cases such as Candida, which is of particular importance for clinicians practicing in the northern United States, or parts of the world that are further from the equator. Most of the infections in this study were related to agricultural exposure rather than contact lens wear, as in developed countries. Our recruitment rate for eligible study participants was 26%. This was attributed to the fact that many patients travel long distances to obtain their eye care and were unable or unwilling to commit to hospitalization or follow up and did not appear to be related to severity of disease or fungal organism.

CONCLUSION

Here we identify a uniquely valuable clinical tool, day 6 culture results, for the treatment of severe fungal keratitis. Risk stratification based on repeat culture positivity is an objective way to assess response to medical therapy and identify patients who are at high risk of a poor clinical outcome. In addition to our previous findings in less severe ulcers, these studies establish a new standard of care for fungal keratitis management.[1]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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There are no 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.

Kathryn Ray and Jennifer Rose-Nussbaumer contributed to the data analysis and writing of this manuscript. Thomas Lietman, Stephen McLeod, and Nisha Acharya contributed to the design and implementation of this study and editing of the manuscript. Drs. N. Venkatesh Prajna, Prajna Lalitha, Tiruvengada Krishnan, Revathi Rajaraman, and Muthiah Srinivasan contributed to the study implementation and editing of this manuscript.

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Table 1

Baseline characteristic data for patients with a repeated fungal culture performed 6 days after enrollment and treatment in the MUTT-II trial (N=208). Continuous variables

Baseline Characteristic	Fungal Culture Positive on Day 6 (*N*=100)	Fungal Culture Negative on Day 6 (*N*=108)	*P*[5]
Sex, N			
Male	47	59	0.01
Female	44	43	
Age (years), mean (sd)	51.4 (12.8)	52.9 (12.8)	0.41
Occupation, N		55 (46, 60)	
Agriculture	38	35	0.37
Non-Agriculture[1]	55	67	
Medication use at enrollment[2], N			
Topical ocular antifungals	56	54	0.32
Other topical ocular drops[3]	47	50	0.89
Systemic antifungals	6	8	0.78
Other systemic	21	20	0.73
Trauma/Injury, N			
Vegetative Matter/Wood	26	39	0.14
Metal/Other[4]	27	24	0.52
Unknown Object	2	0	0.23
Contact Lens	0	0	1
Affected Eye, N			
Right	55	55	0.47
Left	38	47	
Visual Acuity (logMAR), mean (sd)	1.57 (0.36)	1.53 (0.37)	0.21
Infiltrate/Scar Size (mm[1]), mean (sd)	5.69 (1.49)	5.41 (1.67)	0.22
Presence of Hypopyon			
no	25	30	0.75
yes	67	70	
Depth			
>0–33%	19	33	
>33–67%	44	46	0.10
>67–100%	36	28	
Epithelial Defect (mm[1]), mean (sd)	4.92 (1.80)	4.44 (1.82)	0.06
Duration of Symptoms, days, median (25th, 75th percentiles)	10(5,15)	9 (7,15)	0.79
Day 6 Clinical Characteristics	**Fungal Culture Positive on Day 6 (*N*=91)**	**Fungal Culture Negative on Day 6 (*N*=97)**	***P*[5]**
Infiltrate/Scar Size (mm[1]), mean (sd)	5.72 (1.58)	5.21 (1.75)	0.04
Presence of Hypopyon			
no	27	48	0.004

Baseline Characteristic	Fungal Culture Positive on Day 6 (*N*=100)	Fungal Culture Negative on Day 6 (*N*=108)	*P*[5]
yes	64	47	
Depth			
>0–33%	16	33	
>33–67%	38	32	0.03
>67–100%	37	32	
Epithelial Defect (mm[1]), mean (sd)	4.45	3.8	0.02

[1]Includes unemployed, retired, etc.

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

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

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

[5]The P value for age was calculated using Wilcoxon rank sum test. All other continuous variables used t-test and categorical variables the Fisher's exact test.

Table 2

Results from MUTT-II patients who had isolates cultured at enrollment and 6-days after enrollment (N=208).

Fungal Culture Results		*Fusarium*		*Aspergillus*		Other Species**		All	
Enrollment	**6 Days After Enrollment**	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**
+	+	27	40%	41	69%	25	48%	93	45%
+	−	37	55%	16	27%	26	50%	79	38%
−	+	3	4%	2	3%	1	2%	6	3%
−	−	na	na	na	na	na	na	30	14%
Total		67	32.2%	59	28.4%	52	25.0%	208	100%

*Patients with negative culture at enrollment tested positive in a fungal smear required for enrollment.

**Other Species includes *Alternaria, Biopolaris, Curvularias, Exserohilum, Lasiodiplodia,* and unidentified.

Table 3

Backward stepwise elimination mixed effect models predicting MUTT-II pre-specified outcomes with day-6 culture positivity (yes/no), controlling for treatment arm, organism, enrollment clinical characteristics. Covariates listed are what remained in the best fit model. P_{adj} values for our variable of interest, "6-day culture positivity" was corrected using a permutation p-value and shown in last column

Covariate	Coefficient	95% Confidence Interval	*P*	P_{adj}
Cox proportional hazards model predicting perforation or need for TPK, *N* =190				
Culture positive at day 6 (vs. negative)	1.99	(1.28 to 3.08)	0.002	0.013
Enrollment Infiltrate/scar-size	1.31	(1.14 to 1.51)	<0.001	
Enrollment Depth	1.50	(1.09 to 2.05)	0.01	
Organism (Aspergillus vs. Fusarium)	0.63	(0.39 to 1.04)	0.07	
Enrollment Hypopyon (yes vs. no)	1.56	(0.92 to 2.64)	0.10	
Oral Voriconazole (vs. Oral Placebo)	0.75	(0.50 to 1.24)	0.16	33
Multiple linear regression predicting 3-month BSCVA (logMAR), *N*=162				
Culture positive at day 6 (vs. negative)	0.26	(0.10 to 0.42)	0.001	0.004
Enrollment BSCVA	0.56	(0.30 to 0.81)	<0.001	
Enrollment Infiltrate/scar-size	0.11	(0.06 to 0.17)	<0.001	
Enrollment Depth	−0.23	(−0.39 to −0.07)	0.01	
Enrollment Hypopyon (yes vs. no)	0.15	(−0.04 to 0.34)	0.12	
Multiple linear regression predicting 3-month infiltrate/scar (mm), *N*=170				
Culture positive at day 6 (vs. negative)	−0.24	(−0.86 to 0.38)	0.45	0.46
Enrollment Infiltrate/scar-size	0.73	(0.54 to 0.91)	<0.001	
Enrollment Hypopyon (yes vs. no)	0.74	(0.08 to 1.39)	0.03	
Oral Voriconazole (vs. Oral Placebo)	−0.57	(−1.15 to 0.01)	0.054	
Cox proportional hazards model predicting time to reepithelialization, *N*=190				
Culture positive at day 6 (vs. negative)	0.77	(0.46 to 1.28)	0.31	0.38
Baseline epithelial defect size	0.59	(0.50 to 0.71)	<0.001	
Enrollment Depth	0.73	(0.50 to 1.06)	0.10	
Organism (Other species vs. Fusarium)	0.67	(0.40 to 1.14)	0.14	