Fungal Wound Infection (Not Colonization) Is Independently Associated With Mortality in Burn Patients

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Objective: To analyze the occurrence of fungal wound infection (FWI) after thermal injury and its relationship to mortality.

Background: FWI is an uncommon but potentially lethal complication of severe thermal injury.

Methods: The records of patients with thermal burns admitted to a single burn center (1991–2002) were reviewed. Analyses accounted for total burn size (TBS, percentage body surface area), full-thickness burn size (FTBS, percentage body surface area), age, inhalation injury, sex, and fungal-status category. Fungal colonization and infection were determined histopathologically.

Results: Criteria for inclusion were met by 2651 patients. Each patient's fungal-status category was defined according to the deepest level of fungal involvement observed during the hospital course: no fungus (2476 patients), fungal wound colonization (FWC, 121 patients), or fungal wound infection (FWI, 54 patients). Median TBS (9%, 47%, 64%, respectively) and mortality (5%, 27%, 76%, respectively) varied significantly among fungal-status groups. Logistic regression was used to detect significant independent associations. FWI was associated with higher TBS. Mortality was associated with TBS, FTBS, inhalation injury, FWI, and age. Unlike FWI, FWC was not independently related to mortality, the greater observed mortality in FWC being explained by other variables such as TBS. The odds ratio for FWI (8.16) suggested about the same mortality impact as augmenting TBS by 33%. A midrange TBS of 30% to 60% was required for most of the detectable association of FWI with mortality.

Conclusions: FWI accompanies larger burns and is associated with mortality in burn patients, particularly in those with TBS 30% to 60%. This association is independent of burn size, inhalation injury, and age.

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The views expressed herein are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.

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Despite major advances in the care of burned patients, infectious complications remain an important cause of morbidity and death. Furthermore, wound invasion still represents a major cause of infection in burn intensive care units.[1,2] While the broad acceptance of topical antibiotics, early excision, and patient isolation practices have resulted in a significant decline in bacterial wound infections, the incidence of fungal wound infections (FWIs) remains unchanged.[3,4] The ubiquity of fungi in the environment, combined with the suppression of normal bacterial flora (even with periodic and selective use of systemic antibiotics), promotes fungal superinfection. With the most severely burned patients providing ideal immunosuppressed hosts, it is no surprise that fungal infections in these persons are difficult to prevent or eradicate. Previous studies have shown a relationship between burn severity and the occurrence of fungal infection.[4] However, it is unclear whether specific clinical parameters exist that can predict FWI or what impact, if any, FWI has on mortality. The purpose of this study was to examine the incidence of FWI in our patient population, to determine what factors are predictive of FWI, and to address the effect of FWI on mortality.

METHODS

Patients

The U.S. Army Institute of Surgical Research (USAISR) Burn Center's electronic database was used to identify patients. The records of all patients admitted to the Institute from 1991 to 2002 were reviewed by hand to minimize errors in the data extracted for the study and to eliminate missing data among the variables to be analyzed. Besides dates of events (burn, biopsy, discharge, and death) and histopathologic reports, data extracted for analyses included total burn size as a percentage of the body surface area (TBS, percentage body surface area), full-thickness burn size (FTBS, percentage body surface area), age, inhalation injury, sex, and in-hospital mortality. Inclusion criteria for the study were admission within 7 days of injury and the presence of a thermal burn. Patients with electric or chemical injuries, or with nonburn skin diseases, were excluded. Admission burn sizes were estimated directly as a percentage of the body surface area in standard fashion with the use of Lund-Browder

charts. Inhalation injury was diagnosed by bronchoscopy or by xenon-133 scan. Stringent infection-control practices were in place. Full-thickness burns were excised by 7–10 days post-burn. Patients were treated topically with mafenide acetate (morning) alternating with silver sulfadiazine (evening). Surveillance cultures were obtained from wounds and other sites 3 times per week to give advance guidance for antimicrobial therapy. Patients were examined daily for signs of infection. Protein and calorie needs were supplied enterally as soon as possible after burn. Patients with fungal wound colonization or FWI were treated by aggressive surgical debridement and, in the case of FWI, with an intravenous antifungal drug, most commonly amphotericin B (although the recent availability of speciation and susceptibility testing now indicates that this drug is not suitable for all fungal infections[3]). This study was approved by the Institutional Review Board (IRB) of Brooke Army Medical Center and the USAISR.

Specimen Classification

The presence of fungal wound infection or fungal wound colonization (FWC) was defined histopathologically, according to previously published methods.[5] Tissue was obtained routinely at every excision and grafting operation, at the bedside when infection was suspected clinically, and at autopsy. Based upon histopathology, fungi were classified as one of the following: (a) *Aspergillus*-like morphology (presence of parallel-walled, branching, septate hyphae); (b) *Mucor*-like morphology (zygomycosis/mucormycosis: presence of wide, ribbon-like, rarely septate hyphae); or (c) yeast-like morphology (presence of budding yeasts or rounded yeast-like structures). FWI was defined as invasion of fungal elements into viable tissue; FWC as fungal elements in eschar (nonviable burned skin) or "neo-eschar" (a previously excised, now necrotic wound surface), but not viable tissue; and "no fungus" as absence of fungal elements.

Statistical Analysis

Each patient's fungal-status category was defined according to the deepest level of fungal involvement observed during the hospital course: no fungus, FWC, or FWI. This definition provided a 3-level categorical variable (fungal-status category) for initial analyses. While some patients in the no fungus category had no clinical indication for tissue excision or other tissue sampling, all FWC and FWI patients were categorized based on the histopathologic criteria. Because FWC had no detectable independent association with mortality, for subsequent analyses, a 2-level categorical variable (FWI category) was also recorded as FWI-Yes or FWI-No (FWI-No including both no fungus patients and FWC patients).

Continuous variables are reported as medians (with interquartile ranges [IQR] the 25th and 75th percentiles). Categorical variables are reported as numbers and percentages of patients in given categories. Significance was accepted at $P < 0.05$. The P values were Bonferroni-corrected for the appropriate number of multiple nonorthogonal comparisons, as needed. To aid in some analyses, patients were divided into 3 TBS groups, with TBS cutpoints chosen a priori at 30% and 60%. Univariate analyses and multivariate (logistic) analyses of outcomes (FWI or mortality) were performed with use of SPSS software (Chicago, IL). Receiver operating characteristic (ROC) curve analysis (DeLong empiric method) was performed with use of NCSS/PASS software (Kaysville, UT) to evaluate differences in discriminative ability of different logistic models.

In univariate analyses, Mann-Whitney U tests were used for continuous variables because of their nonnormal distributions. χ^2 tests were used for categorical data, unless otherwise noted.

For multivariate analyses, binary logistic regressions (backward-stepping likelihood-ratio method) evaluated dependent variables (mainly the occurrence of FWI or of mortality) according to the following potential independent variables: TBS, FTBS, age, inhalation injury, and sex. Age was entered as 3 variables (age, age-squared/100, and age-cubed/10,000) because of the previously documented curvilinear relationship between age and mortality in burn patients.[6] When mortality was the dependent variable, either no fungal information was entered as a potential independent variable, or fungal information was entered as the fungal-status category (3 levels) or as the FWI Category (2 levels) along with the other potential independent variables. For the calculation of sensitivity, specificity, and accuracy, the P cutpoint was chosen to generate equivalent sensitivity and specificity. Comparison of the discriminative ability of logistic models developed with and without accounting for FWI was made in the patients by empiric ROC analysis.

RESULTS

Descriptive Findings and Univariate Analyses

During the 12-year study period, 3466 persons were admitted to the Burn Center. Of these, 2651 patients met inclusion criteria for analysis. Among these 2651, overall data ranges included: TBS <1%–99% (median 10.3%), FTBS 0%–98% (median 0%), and age <1–101 years (median 28.8). Inhalation injury was diagnosed in 14.3%. Males comprised 78.2% of the patients. There were 206 deaths prior to discharge, for an overall raw mortality rate of 7.8%. Table 1A shows that 2476 patients (93.4%) did not have fungus identified on tissue histopathology, that the FWC category was assigned in 121 (4.6%) patients, and that 54 (2.0%) patients were categorized as FWI (on average, 4.5 patients per year). Table 1 summarizes patient observations in each of the 3 fungal-status categories. Table 2 summarizes mortality in the 2 FWI categories.

Fungal involvement (FWI or FWC in a tissue specimen) was first detected by means of surgical pathology obtained in the operating room in most cases (112, 64%). It was not possible to determine retrospectively whether such involvement was an incidental finding or directly correlated to clinical suspicion. In 59 patients (33.7%), the diagnosis was first made by means of biopsy performed outside the operating room, suggesting clinical suspicion for FWI. In only 4 cases (2.3%), it was made for the first time at autopsy. Of the 143 patients whose initial diagnosis was FWC, 22 (15.4%) progressed to FWI on subsequent biopsy (further analysis was based on FWI and not FWC). Stated another

TABLE 1. Numbers of Patients and Observed Values in Relation to Fungal-Status Category

A. Patient Counts (% of Column Total)	Fungal-Status Category			
TBS Group	**No Fungus**	**FWC**	**FWI**	**Row Total**
≤30%	2188 (88.4%)	34 (28.1%)	3 (5.6%)	2225 (83.9%)
>30–60%	230 (9.3%)	48 (39.7%)	20 (37.0%)	298 (11.2%)
>60%	58 (2.3%)	39 (32.2%)	31 (57.4%)	128 (4.8%)
Column Total	2476	121	54	2651
Fungal category (column total) as percent of all 2651 patients:	93.4%	4.6%	2.0%	

B. Patient Variables*	No Fungus	FWC	FWI	Overall
Median TBS (IQR)†	9% (4–18%)	47% (28–68%)	64% (49–76%)	10 (4–21%)
Median FTBS (IQR)‡	0% (0–3%)	30% (13–49%)	46% (29–69%)	0% (0–5%)
Median age (IQR)§	28 (14–43)	39 (26–55)	39 (27–48)	28 (15–44)
Inhalation injury (% with)¶	11.6	47.1	64.8	14.3
Sex (% male)**	78.2	76.9	83.3	78.2
Mortality (%)††	5.3	27.3	75.9	7.8

*Mann-Whitney U tests or χ^2 tests for comparisons among fungal-status categories:
†TBS: all $P < 0.001$. (All values are % body surface area.)
‡FTBS: all $P < 0.001$, except FWI versus FWC ($P < 0.01$). (Values: % body surface area.)
§Age: no fungus versus FWC ($P < 0.001$) and FWI ($P < 0.01$); FWI versus FWC (NS).
¶Inhalation Injury: no fungus versus FWC and FWI ($P < 0.001$); FWI versus FWC (NS).
**Sex: all NS.
††Mortality: all $P < 0.001$.
NS indicates not significant.

way, of the 54 patients finally categorized as FWI, 22 (40.7%) represented observed progression from FWC. Among the 143 patients having only colonization, or having colonization present prior to subsequent identification of invasion, progression to FWI was related only to TBS (positively, and without a strong relationship: $P < 0.05$, logistic regression).

TABLE 2. Observed Patient Counts and Mortality in Relation to TBS Group and FWI

TBS Group (n)	Pooled FWI-No/Yes	FWI-No*	FWI-Yes†
≤30 (2225)			
Survivors	2172	2170	2
Nonsurvivors	53 (2.4%)	52 (2.3%)	1 (33.3%)‡
>30–60 (298)			
Survivors	232	228	4
Nonsurvivors	66 (22.2%)	50 (18.0%)	16 (80.0%)§
>60 (128)			
Survivors	41	34	7
Nonsurvivors	87 (68.0%)	63 (64.9%)	24 (77.4%)¶

*FWI-No indicates the pool of no-fungus patients and FWC patients.
†Mortality comparisons of FWI-Yes versus FWI-No separately in the 3 TBS groups.
‡P not significant by Yates-corrected χ^2 or Fisher exact Test. (Minimal expected cell value: 0.07.)**
§$P < 0.001$ by Yates-corrected χ^2 and Fisher exact Test. (Minimal expected cell value: 4.4.)**
¶P NS by Yates-corrected χ^2 or Fisher exact Test (or by ordinary χ^2 test). (Minimal expected cell value: 9.9.)**
**Minimal expected cell values <5 suggest the need to rely on the Yates-corrected χ^2 or Fisher exact Test to interpret a positive result. The two very small observed cell counts (in the first comparison above‡, within the smallest-TBS group) suggest a need to avoid interpretation of that comparison.
NS indicates not significant.

Among the 175 patients with a primary fungal categorization as FWC or FWI, fungal wound involvement (specimen report as either FWI or FWC) was detected on median postburn day (PBD) 16 (IQR 9–24). Specifically, among the 121 FWC patients, FWC was first detected on median PBD 19 (IQR 11–27). Among the 54 FWI patients, FWI was first detected on median PBD 12.5 (IQR 9–23). Among these 54 FWI patients, the 41 nonsurvivors had a median lag of 8 days (IQR 2–20) from first detection of FWI to death. Table 3 indicates that, among the 175 patients in the FWC and FWI categories pooled, *Aspergillus*-like fungi were seen in the greatest fraction of patients (83%), *Mucor*-like in the smallest fraction (15%), and yeast-like in an intermediate fraction (36%). In addition, as seen in Table 3, *Aspergillus*-like fungi ($P < 0.01$) and *Mucor*-like fungi ($P < 0.001$) were more likely to be seen in FWI than in FWC patients, with no difference in frequency for yeast-like fungi.

Fungal-status category (deepest level of fungal involvement) correlated with TBS (Table 1; Figs. 1A, B), FTBS (Table 1, Fig. 1A), and mortality (Table 1). Each of these showed strong detectable differences between any 2 categories (Table 1, Figs. 1A and 2A). Age and inhalation injury were not detectably different between FWC and FWI, though they were higher in these categories than in the no fungus category (Table 1). There were no detectable differences in sex among the fungal-status categories. Figure 1B shows TBS distribution among the fungal-status categories. Low-TBS patients predominated in the no fungus category, high-TBS patients predominated in the FWI category, and FWC patients showed no obvious pattern of predominance with respect to TBS group. Figure 2A indicates increasing

TABLE 3. Fungal Morphology Identified Histopathologically in Sections of Burn Wound Tissue*

Descriptive Fungal Morphologic Class	FWC Patients (n = 121)	FWI Patients (n = 54)	FWC and FWI Patients Pooled (n = 175)
Aspergillus-like morphology: presence of parallel-walled, branching, septate hyphae	94 (77.7%)	51 (94.4%)†	145 (82.9%)
Mucor-like morphology (zygomycosis/mucormycosis): presence of wide, ribbon-like, rarely septate hyphae	11 (9.1%)	16 (29.6%)‡	27 (15.4%)
Yeast-like morphology: presence of budding yeasts or rounded yeast-like structure	43 (35.5%)	20 (37.0%)	63 (36.0%)
Any combination	24 (19.8%)	26 (48.1%)‡	50 (28.6%)

*The numbers indicate the number of patients each having the given descriptive fungal morphologic class in at least 1 tissue specimen. Parentheses show the number of patients as the percentage of the number indicated in the column heading.
†$P < 0.01$; ‡$P < 0.001$ versus FWC patients (χ^2 test).

mortality across the fungal-status categories, and Figure 2B suggests a relatively large mortality for FWI in the TBS group of 30% to 60%.

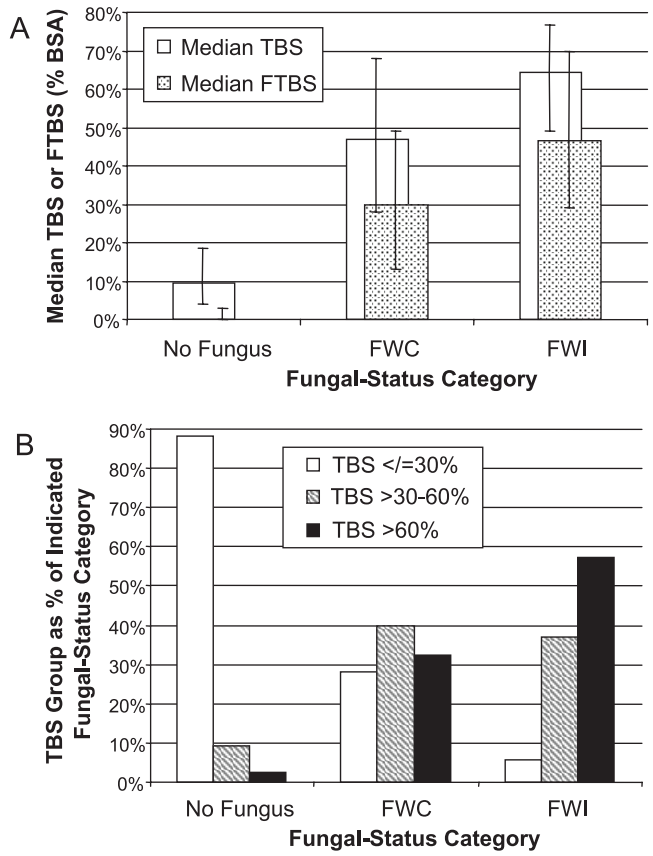


FIGURE 1. A, Correlation of burn size with histopathologically categorized deepest level of fungus during the hospital course. Vertical lines represent the interquartile range (IQR) for TBS, connecting the 25th and 75th percentiles. TBS, total burn size (percentage body surface area [BSA]); FTBS (% BSA); FWC, fungal wound colonization; FWI, fungal wound infection. TBS: $P < 0.001$ no fungus versus FWC, no fungus versus FWI, and FWC versus FWI. FTBS: $P < 0.001$ no fungus versus FWC, no fungus versus FWI; and $P < 0.01$ FWC versus FWI. B, TBS distribution according to the fungal category of each patient.

Because logistic regression analyses disclosed no detectable influence of FWC on mortality, no fungus and FWC categories were combined into 1 category (FWI-No) under the variable FWI Category. Table 2 shows observed mortality according to FWI and TBS group. The only clear FWI-related difference in raw mortality appeared in the TBS group with TBS 30% to 60%. Because the median TBS might vary

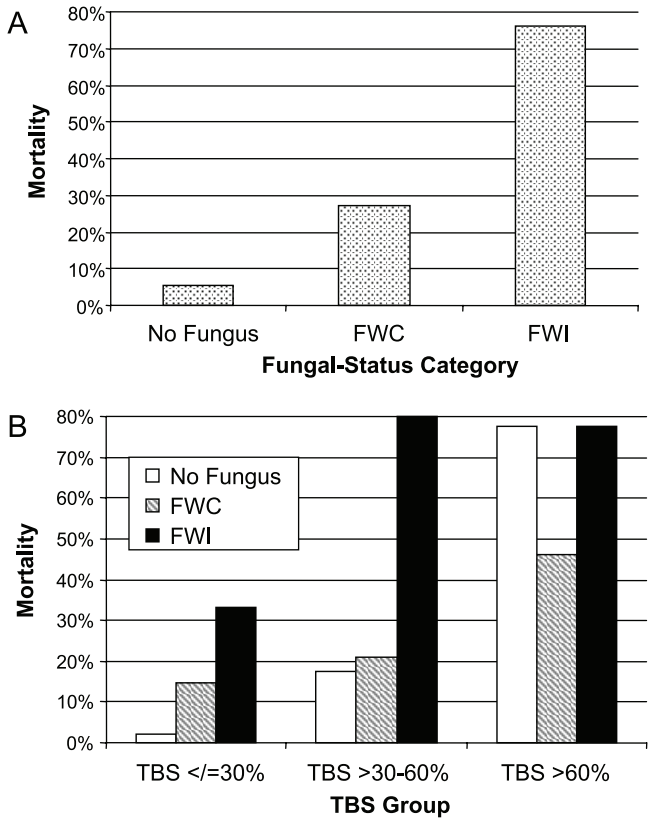


FIGURE 2. A, Higher observed mortality with deeper fungal involvement. FWC, fungal wound colonization; FWI, fungal wound infection (invasion). Each category has significantly different ($P < 0.001$) mortality from each of the others. B, Observed mortality in each fungal-status category within each burn-size group. TBS, total burn size (percentage body surface area).

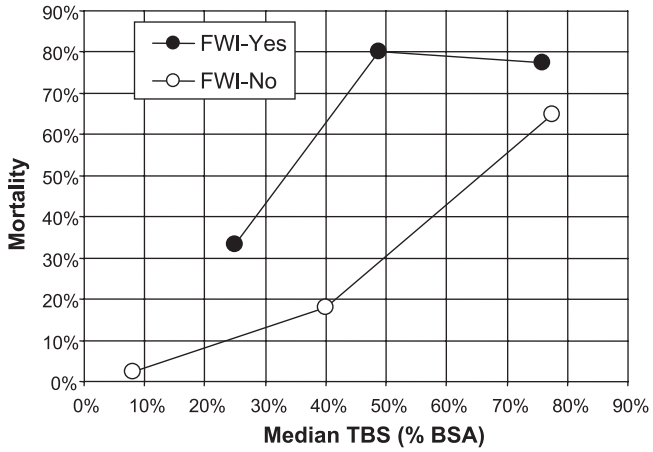


FIGURE 3. Observed mortality in relation to total burn size (TBS, percentage body surface area [BSA]) and fungal wound infection (FWI). FWI-No includes FWC and no fungus. TBS groups are represented in the following way: the first (left-most) symbol for FWI-Yes and for FWI-No represents TBS ≤30%, the second symbol TBS >30%–60%, and the third (right-most) symbol TBS >60%. For the second (middle) point in each curve, mortality is different between FWI-Yes and FWI-No ($P < 0.001$), although not significantly different (FWI-Yes vs. FWI-No) at the first points or at the third points.

within a defined TBS range, the observed mortality was plotted against the median TBS. Raw mortality was higher in the presence of FWI, which was significant only in the middle range of TBS (Fig. 3).

Multivariate (Multiple Logistic Regression) Analyses

In logistic models, the binary dependent variable was either FWI (yes = 1 or no = 0) or observed mortality (in-hospital death yes =1 or no = 0). When mortality was the dependent variable, FWI was entered as a potential independent variable. For the equations given below, each contributing independent variable's level of significance of the contribution is noted within the equation, as follows: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. This notation is used also for 2 of the performance indices. TBS, FTBS, age, age^2/100, and age^3/10,000 were continuous variables, and inhalation injury, sex, and fungal information were categorical. For all the cited regressions, the Hosmer-Lemeshow test was negative, indicating good agreement between observed and predicted outcome.

Predicted Probability of FWI

Equation 1. Because of lack of a demonstrable relationship of FWC to mortality (see below), it was mainly of interest to find contributors to the probability of FWI. The dependent variable (FWI) was coded 0 = no (ie, comprising FWC and no fungus) or 1 = yes. Logistic regression yielded the following equation for predicted probability of FWI:

$$P \text{ (invasion)} = 1/(1 + 1/e^k),$$

where $k = -6.742 + 0.0626$ (TBS***) $+ 0.124$ (Age^2/100*) $- 0.158$ (Age^3/10,000*).

Performance indices were N 2651, model χ^2***, Nagelkerke r^2 0.368, and area under the ROC curve (ROC AUC) 0.951***. Sensitivity was 90.7%, specificity 88.9%, and accuracy 88.9% (at a P cutpoint of 0.02). FTBS, age, inhalation injury, and sex were rejected as independent contributors to the probability (P) of invasion. Thus, the main contributor to the likelihood of FWI was TBS, with a highly significant association. Any association of FWI with age had low detectability and involved only 2 cubic elements.

Predicted Probability of Mortality

Logistic analyses confirmed strong independent positive relationships of TBS and inhalation injury to mortality, and a less strong but consistent positive independent relationship of FTBS with mortality. The relationship of mortality to age was consistently curvilinear (coefficients negative for age, positive for the square, and negative for the cube; see below). No logistic regression indicated any relationship of mortality to sex in these burn patients. These relationships to mortality were seen by developing the logistic model either with or without accounting for fungal information.

Equation 2. A logistic regression without use of fungal information gave the following model for the predicted probability of mortality:

$$P \text{ (mortality)} = 1/(1 + 1/e^k),$$

where $k = -5.712 + 0.0664$ (TBS***) $+ 0.019$ (FTBS*) $- 0.0916$ (Age*) $+ 0.295$ (Age^2/100**) $- 0.157$ (Age^3/10,000*) $+ 0.809$ (Inhalation Injury***).

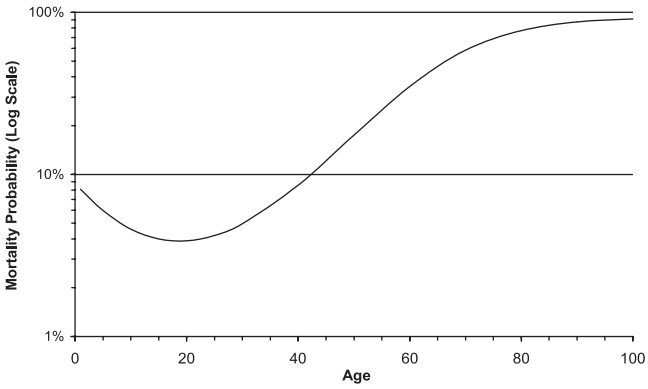
Performance indices were N 2651, model χ^2***, Nagelkerke r^2 0.608, and ROC AUC 0.955***. Sensitivity was 88.8%, specificity 88.1%, and accuracy 88.1% (at P cutpoint 0.07). Sex was the only variable rejected as noncontributory. TBS (positively), FTBS (positively), age (curvilinearly), and inhalation injury (positively) contributed independently to the probability of death.

When fungal-status category (3-level variable: no fungus, FWC, FWI) was added as an independent variable, there was no significant association of FWC with mortality but there was a strong association of FWI. Finally, after excluding patients with FWI, regression for mortality rejected FWC (and sex). The significance of TBS, all 3 age terms, FTBS, and inhalation injury was stable over all the regressions. Because of this lack of association of FWC with mortality, the role of FWI as its own variable (coded 1 for yes and 0 for no), with both FWC and no fungus considered as FWI-No, was tested further.

Equation 3. A logistic regression including the use of fungal information (as the variable FWI Category) gave the following model for the predicted probability of mortality:

$$P \text{ (mortality)} = 1/(1 + 1/e^k),$$

where $k = -5.69 + 0.0636$ (TBS***) $+ 0.0166$ (FTBS*) $- 0.100$ (Age*) $+ 0.316$ (Age^2/100***) $- 0.169$ (Age^3/10,000**) $+ 0.781$ (Inhalation Injury**) $+ 2.10$ (FWI***).



	Age ≤ 10	Age >10-25	Age >25-40	Age >40-60	Age >60-80	Age >80
N	542	596	706	523	223	61
Obs Deaths	10 (1.9%)	16 (2.7%)	47 (6.7%)	51 (9.8%)	51 (22.9%)	31 (50.8%)
Pred Deaths	9.3 (1.7%)	19.4 (3.3%)	41.1 (5.8%)	54.5 (10.4%)	51.5 (23.1%)	30.4 (49.9%)
FWI-Yes	2 (0.4%)	11 (1.9%)	16 (2.3%)	19 (3.6%)	6 (2.7%)	0 (0%)
Median TBS	8.5%	10%	11%	11.5%	12.5%	17%
Inhalation Inj	27 (5.0%)	73 (12.3%)	119 (16.9%)	92 (17.6%)	53 (23.8%)	16 (26.2%)

FIGURE 4. Predicted mortality probability from the logistic model of Equation 3 developed with inclusion of fungal information as FWI. The curve has FWI set to "no," TBS set to 45%, FTBS set to 30%, inhalation injury set to absent, and age points varying across the abscissa. The variables other than age must be held constant over the full range of age, to see only the effect of age in the predicted curve. Entries below the plot represent actual patient data in age groups chosen to represent various parts of the curve. Obs, observed deaths (mortality). Pred, predicted deaths (mortality); pred deaths as the sum of the probabilities calculated with Equation 3 (and actual patient data). Obs and pred mortality are in good agreement across age groups. Age is in years. TBS and FTBS are in percentage body surface area.

Performance indices were N 2951, model χ^2***, Nagelkerke r^2 0.626, and ROC AUC 0.958***. Sensitivity was 89.3%, specificity 89.1%, and accuracy 89.1% (at *P* cutpoint 0.07). Sex was the only variable rejected as nonsignificant. FWI was strongly associated independently and positively with greater mortality, even after accounting for the strong associations of TBS, age variables, and inhalation injury.

The persistent curvilinear relationship of mortality to age is exemplified in Figure 4, in which Equation 3 was used to predict the mortality probability over the age span of the study, calculated without simultaneous variation in the other independent variables. The latter were set to be constant, so that only the influence of age is seen. Below the plot, actual patient characteristics are listed for strategically chosen age groups.

One output of the logistic program is the odds ratio ([OR], with its 95% confidence limits [CL]) for each contributing independent variable: here, OR = odds of death at 1 value (associated with greater mortality) of the independent variable divided by the odds of death at another value (associated with less mortality) of the same independent variable. Related to Equation 3, the OR (95% CL) for FWI on mortality was 8.16 (3.56, 18.69) and for inhalation injury was 2.18 (1.38, 3.46). TBS required a span of 33% for its OR to equal that (8.16) of FWI. Thus, FWI had more than 3 times the impact on the odds of death than did inhalation injury, and as much impact as an augmentation of TBS by 33%.

Despite the biologic information (strong association of injury extent and fungal-infection status with mortality) inherent in those observations, fungal information did not translate into much discriminative advantage when *P* (mortality) from Equation 3 (with fungal information as the FWI status) was compared with *P* (mortality) from Equation 2 (developed without fungal information) in 2 respective ROC curves applied over the entire sample of patients. There were many patients with small calculated *P* (mortality) and without observed mortality who contributed a large number of correct discriminations, depending mainly on such factors as small burn size and/or absence of inhalation injury, rather than on absent FWI. In comparing these Equations (3 vs. 2) with respect to criteria related to the whole set of patients, indeed, the differences in Nagelkerke r^2, ROC AUC (0.958 vs. 0.955, $P = 0.026$), sensitivity, specificity, and total accuracy were small, limiting our ability to apply Equation 3 to all burn patients.

It appeared that infection might allow an advantage in discriminating mortality outcome within a subgroup of patients having TBS of 30% to 60%. The plot of observed mortality as a function of TBS group, each split according to fungal-status category (Fig. 2B), suggested that the influence of FWI on increasing mortality was most evident in the middle TBS range (30%–60%). The same is suggested by consideration of FWI-Yes versus FWI-No (Table 2 and Fig. 3). Therefore, we applied Equations 3 and 2 to the 298 patients with TBS >30% and ≤60%, for ROC analysis in these selected patients. In this case, Figure 5 shows that the

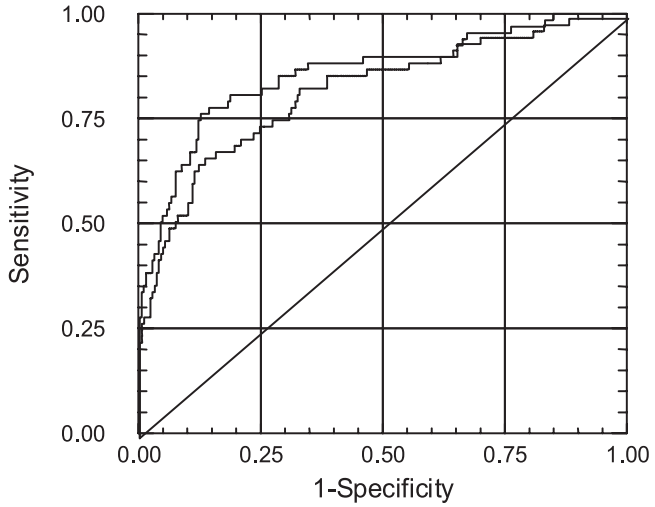


FIGURE 5. Receiver operator characteristic (ROC) curves comparing the ability of the mortality model including FWI as an independent variable (Equation 3) and the model without fungal information (Equation 2) to discriminate between observed survival and nonsurvival. For this comparison and these curves, the 2 models (each developed in all the patients) were applied only to the 298 patients with TBS >30%–60%. The area under the curve (AUC) from application of Equation 3 (AUC = 0.867) was significantly greater ($P = 0.005$) than that from application of Equation 2 (AUC = 0.826). The straight diagonal line would indicate no discrimination (AUC = 0.5).

curves appeared separated, and the AUCs differed ($P = 0.005$) between the curves for Equation 3 (accounting for fungal infection, AUC = 0.867) and Equation 2 (not accounting for fungal information, AUC = 0.826). No difference between the AUCs was detected if patients were selected for TBS ≤30% or for TBS >60% for application of the models (not shown). The apparent exertion of a maximal additive effect of FWI on mortality in patients with burns in the midrange of TBS suggests an interaction between TBS and FWI. Indeed, a logistic regression similar to that for Equation 3, but including the interaction for TBS × FWI, excluded only sex but included the interaction term ($P < 0.01$). A regression substituting TBS group (categorical variable, 3 levels) for TBS gave essentially the same result as in Equation 3, excluding only sex, with TBS group contributing at $P < 0.001$. Then, after adding the interaction for TBS group × FWI, the effect of the FWI interaction with TBS group 30% to 60% on mortality was significantly greater than that with TBS group ≤30% ($P < 0.001$) and greater than that with TBS group >60% ($P < 0.001$). It appears that FWI influences mortality more in the TBS 30% to 60% range than in the TBS ranges lower or higher than this, when the contributions of other variables are taken into consideration.

DISCUSSION

The near eradication of invasive gram-negative burn wound infection following the introduction of effective topical antimicrobial agents in 1964 highlighted, in subsequent years, the importance of fungal infections both of the burn wound and of open, previously excised wounds.[4,7] As demonstrated by this study, FWI is associated with an unacceptably high mortality that is essentially unchanged since a previous report from this Institute for 1979–1989.[4] Odds ratio analysis (OR = 8.16 for fungal infection on the odds of death) was performed by use of the equation accounting for fungal infection (Equation 3) for predicted probability of mortality, and suggested that fungal infection has more than 3 times the impact of inhalation injury on mortality odds and about the same impact as augmenting the TBS by 33%. Whether fungal infection itself contributes causally to mortality, or just represents a marker for other contributors, has not yet been definitively assessed in these patients. The relationship between fungal infection and the anatomic cause of death in burn patients remains to be determined.

In our study, FWI but not FWC was independently associated with higher mortality, along with TBS, FTBS, inhalation injury, and age. An advantage of the FWI-containing model in discriminating mortality outcome was detectable by ROC AUC by applying the models (Equations 3 vs. 2) in the 30% to 60% TBS group, but not in the other 2 TBS groups. This suggests an interaction between FWI and categorical burn group as a variable and that FWI is more effective in augmenting mortality in the TBS range of 30% to 60%. Such an interaction was confirmed when sought specifically in further logistic regressions and was expressed in the middle TBS group more strongly than in the other 2 TBS groups, after simultaneously accounting for the other independent contributors to mortality. These findings are similar to the previously reported maximal mortality impact of inhalation injury (and inhalation injury complicated by pneumonia) in those patients in the midrange of burn size.[8] Another similarity in this regard is the finding that bacteremia's augmentation of the occurrence of death occurred mainly in patients without maximally severe injuries and with expected mortality probability <60%.[9] In our study, it is probable that the lowest TBS group (≤30%) had too few patients with FWI to adequately evaluate an interaction, whereas the highest TBS group (>60%) was reaching a ceiling of mortality such that any greater mortality due to FWI would have diminished detectability.

This TBS-FWI interaction suggests that special attention should be given to the aggressive prevention and early diagnosis of these infections especially in the TBS range of 30% to 60%, including investigation of early prophylactic or empiric treatment of fungal invasion. With the advent of newer diagnostic strategies such as serology and molecular techniques and newer antifungal therapies with less toxicity than standard treatments like intravenous amphotericin B, the importance of accurate prediction models of fungal burn wounds is paramount. Empiric antifungal therapy is applied in other immunosuppressed states, such as neutropenic fevers, but its role in burn patients is unclear. Although the mortality was higher in patients categorized as FWC than in those without fungus, this was accounted for, not by FWC, but by other factors such as burn size, since FWC had no detectable independent association with mortality. However, FWI progressed from FWC in 22 (15.4%) of 143 patients with FWC, and these 22 were 40.7% of the 54 patients ultimately categorized as FWI. Thus, colonization itself should not be considered lightly by the burn team. Certainly, a study to determine the role of prophylaxis or empiric therapy might have its highest yield in patients with colonization.

It is not evident from this study why some patients with large burns are at increased risk for FWI and/or FWC. We suspect, but could not establish retrospectively, that more aggressive excision and timely wound closure may benefit patients at risk for FWI. Fungal wound invasion in burns is certainly more likely to occur at larger TBS, and perhaps less likely to occur in the young. After accounting for these factors, there was no detectable independent effect of inhalation injury or sex on the probability of FWI. Additional factors such as immune status and the timing of wound closure should be investigated in further studies.

Performing a prospective study of FWI at any burn center would be difficult because of the low annual rate of such infections. In our study, the annual rate of FWI was 4.5 patients per year or 2% of the number of burn admissions qualified for consideration over the 12-year study period. If one includes FWC, there were 14.6 patients per year or 6.6% of the total number studied, a rate that did not significantly change from year to year over the 12-year study period. In the patients in whom detection of invasive fungal infection might have the greatest impact on mortality (TBS 30%–60%), there were 1.7 patients per year with FWI and 4.0 patients per year with FWC. In a previous 10-year retrospective study from our

institution (1979–1989) the annual fungal burn wound infection rate ranged from 2.6% to 10.2% of the yearly total admissions, with the median being 6.3%, and without a systematic change over the observation interval.[4] In the present study, there was no detectable change in the proportion of patients with FWI or in the mortality rate over the study years, with simultaneous logistic accounting for the other contributing variables. Without more definitive data, multicenter, prospective randomized controlled trials should be considered. Logistic models such as those presented in this paper should be validated prospectively and, if adequate, applied to identify patients at risk in such trials.

It might be noted that our results confirm the curvilinear relationship of burn mortality to age expressed as a cubic polynomial, as reported previously.[6,8,9]

In conclusion, we showed that fungal invasion is associated with higher mortality independent of age, burn size, or inhalation injury. Fungal invasion was detected on average 16 days after injury. Whether fungal invasion itself contributes causally to mortality or just represents a marker for other contributors has not yet been definitely assessed in these patients. Attention should be paid to the development and evaluation of prophylactic or empiric therapy strategies in this group of patients, such that rapid treatment might be employed.

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