Available online at www.sciencedirect.com
SciVerse ScienceDirect
journal homepage: www.elsevier.com/locate/burns

Clinical utility of fungal screening assays in adults with severe burns☆

Dana M. Blyth[a], Kevin K. Chung[b], Leopoldo C. Cancio[b], Booker T. King[b], Clinton K. Murray[a,c,*]

[a] Brooke Army Medical Center/San Antonio Military Medical Center, 3551 Roger Brooke Drive, Fort Sam, Houston, TX 78234, USA
[b] United States Army Institute of Surgical Research, 3400 Rawley E Chambers Ave, Fort Sam, Houston, TX 78234, USA
[c] Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

ARTICLE INFO

Article history:
Accepted 3 August 2012

Keywords:
Burn
Fungal infection
Serology
β Glucan
Galactomannan

ABSTRACT

Background: Fungal wound infection is a leading cause of burn wound infections, and diagnosis is often delayed as it conventionally requires culture and histopathology. Fungal screening assays have sped diagnosis of invasive fungal infections in other populations. Few studies have evaluated the performance of fungal screening assays outside of the hemato logic malignancy and hematopoietic stem cell transplant populations.

Methods: We performed a three year retrospective analysis of all fungal screening assays in burn patients in the ICU between 2008 and 2011. The primary goal was to evaluate the correlation between the two available fungal screening assays, (1 → 3) β D glucan (BG) and galactomannan (GM) assay, and fungal wound colonization (FWC) and infection (FWI). We also evaluated previously hypothesized causes of false positives and their associations with false positives in the burn population.

Results: We identified 53 patients [median 29% total body surface area burned (TBSA), IQR 17 51] with BG or GM serological tests available, of which 15 had a FWI or FWC. FWC/FWI was associated with higher TBSA ($p = 0.02$). BG and GM correlated with TBSA (BG 0.57, $p < 0.01$; GM 0.35, $p = 0.02$), but neither assay was associated with FWI/FWC or species of fungus involved when FWI/FWC was diagnosed.

Conclusions: Positive BG and GM fungal screening assays are not associated with FWI/FWC, or with species of fungus when FWC/FWI is present. BG false positives are common and associated with higher TBSA burns.

Published by Elsevier Ltd and ISBI

1. Introduction

Standardization of topical antibiotics, early surgical debride ment, and patient isolation have decreased bacterial wound infections and improved survival of burn patients over the last 50 years. However, the incidence of fungal wound infections has remained unchanged. These infections now represent one of the most common sources of burn wound infection [1,2]. Historically, invasive fungal infections have been diagnosed by correlation of signs and symptoms with recovery of the organism from histopathology or culture [3]. As fungal wound infection has been independently associated with mortality in

☆ *Disclaimer:* 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 the Air Force, Department of Defense, or the US Government. The authors are employees of the US government. This work was prepared as part of their official duties and, as such, there is no copyright to be transferred.

* *Corresponding author at:* Infectious Disease Service (MCHE MDI), Brooke Army Medical Center, 3551 Roger Brooke Drive, Fort Sam, Houston, TX 78234, USA. Tel.: +1 210 916 8752; fax: +1 210 916 0388.
E mail addresses: clinton.murray@us.army.mil, clinton.murray@amedd.army.mil (C.K. Murray).

0305 4179/$36.00. Published by Elsevier Ltd and ISBI
http://dx.doi.org/10.1016/j.burns.2012.08.009

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 MAY 2013	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Clinical utility of fungal screening assays in adults with severe burns.	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Blyth D. M., Chung K. K., Cancio L. C., King B. T., Murray C. K.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

burn patients [2], there has been interest in earlier diagnosis and intervention.

There is promising data regarding use of fungal screening assays in other immunocompromised populations to facilitate earlier diagnosis and thus earlier treatment [3 6]. A commercially available test, Fungitell™ Glucan Assay for (1,3) β D glucan (BG) in serum (Associates of Cape Cod Inc., East Falmouth, MA), is a qualitative, colorimetric assay that detects species of *Candida*, *Aspergillus*, *Fusarium*, *Paecilomyces*, *Trichosporon*, *Penicillium*, *Pneumocystis*, *Coccidioides*, *Histoplasma*, *Blastomyces*, and *Sporothrix* [7 10]. The company that produces this test cautions that *Cryptococcus* and the zygomycetes are not detected using the FDA approved assay due to the low cell wall BG content of these organisms. BG false positives have been associated with poor specimen handling, hemodialysis using certain cellulose membranes, exposure to certain types of gauze, mucositis, candida colonization, bacteremia, use of antimicrobials (especially cefepime), and recent administration of albumin or immunoglobulin products [3,11]. Galactomannan (GM) [Platelia® Aspergillus EIA (Bio Rad Laboratories)] is another serologic test that detects a polysaccharide component of the cell wall of all species of *Aspergillus* that is released during fungal growth [6,12]. GM is also a by product of the β lactam antibiotic fermentation process which is thought to be the reason for reports of cross reactivity with some antimicrobial agents including amoxicillin clavulanate and piperacillin tazobactam [3].

Only one study has previously looked at fungal screening assays in burn patients, which showed that BG false positives were common. BG was elevated at baseline, even prior to gauze coverage, in 50% of burn patients and was statistically associated with greater TBSA burn involvement [13]. No studies have evaluated the use of GM for fungal screening in the burn population. The purpose of this study is to describe the clinical utility of fungal screening assays in the diagnosis of fungal wound infection (FWI) and colonization (FWC) in the severely thermally burned patient population.

2. Methods

After institutional review board approval was obtained, we performed an electronic retrospective chart review in the U.S. Army Institute of Surgical Research (USAISR) Burn Center. The USAISR Burn Center is a 40 bed unit located within Brooke Army Medical Center that serves Department of Defense beneficiaries worldwide and the civilian population from Southern Texas. Standard practices for this institution have been previously described in detail, but include single rooms for all patients with aggressive infection control and environmental control processes to minimize nosocomial transmission and environmental inoculation of wounds; burn wound excision early after post injury; topical antimicrobial therapy based on silver or mafenide acetate; perioperative antimicrobials including vancomycin and aminoglycosides; daily examinations for signs of infection; enteral feedings as soon as possible; and FWC and FWI treated with surgical debridement and intravenous antifungals [2].

Using our electronic medical record system, we extracted all patients admitted to the burn center between 1 January 2008 and 1 January 2011 with burns of any size who had a fungal screening assay (either BG or GM) drawn sometime during their hospitalization. Patients were excluded if they were under age 18 or had injuries other than thermal burns that required care in the burn unit (including toxic epidermal necrosis and other desquamative processes not related to thermal burns). Fungal screening assays were drawn at the discretion of the primary team, based on perceived risk of fungal infection. Positive fungal assays were defined according to package inserts. A GM index (GMI) of $\geq$0.5 is considered a positive test in the United States and was used as a positive value in this study. A positive test is reported with a β D glucan concentration of $\geq$80 pg/ml and a negative test with a concentration $<$60 pg/ml. A retrospective chart review was performed to describe relevant demographic characteristics to include age, gender, % total body surface area (TBSA) burned, time since burn, positive bacterial and fungal cultures and their sites, confirmed fungal infection, date and cause of death (if applicable), other risk factors for fungal disease, medical comorbidities, co infections with their sources, ongoing antimicrobial therapy, and renal replacement therapy.

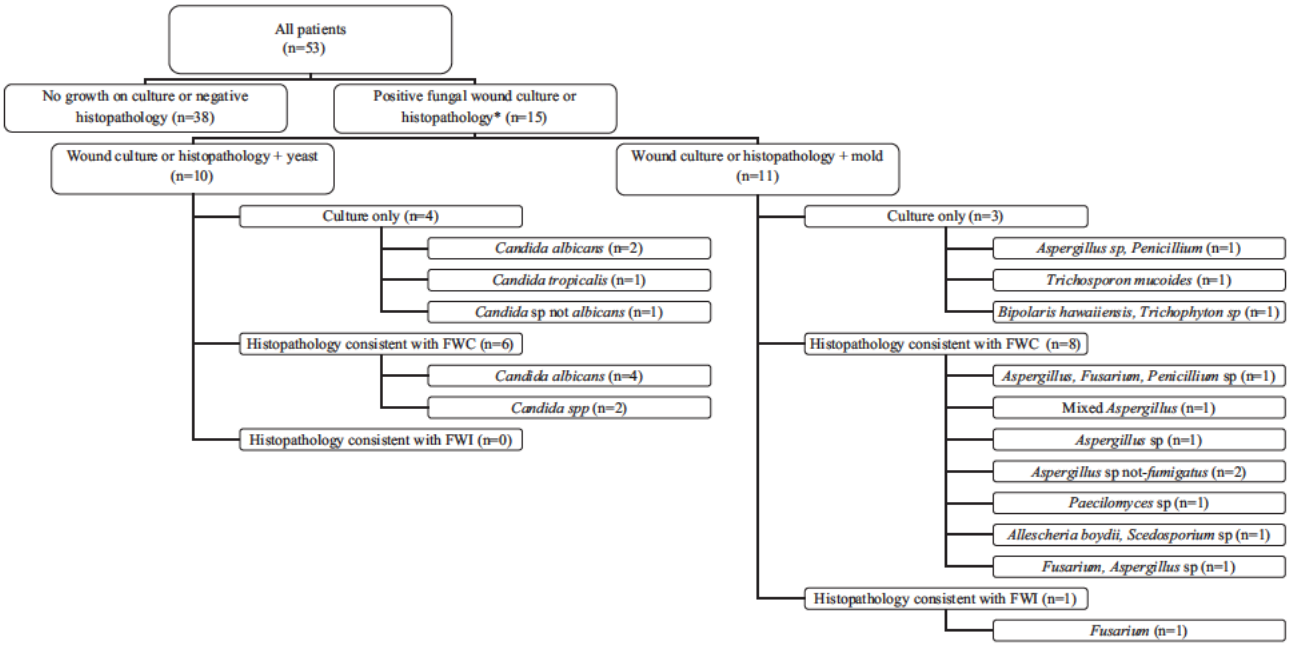
Patients were characterized according to FWI, FWC, or no evidence of either. FWI was defined as previously, that is, evidence of fungal invasion into viable tissue on histopathology [14]. FWC was defined as observation of fungal elements in the eschar (or in other nonviable tissue) without penetration into viable tissue. For this paper, patients without histopathologic results and with a positive fungal culture were defined as FWC. Patients with a positive culture only, patients with positive histopathology for colonization only, and patients with both positive culture and positive histopathology for colonization were all defined as FWC. Patients are defined as having FWI only if histopathology (indicating invasion into viable tissues) is present and these have been indicated in Fig. 1.

For statistical analyses, continuous variables are reported as medians with interquartile ranges (IQR). Categorical variables were assessed by Chi square test. Mann Whitney *U* and Kruskal Wallis tests were used for non normal continuous variables. Spearman's rho was performed for correlation. Significance was accepted at $p \leq 0.05$ and all tests were two tailed. Statistical analyses were performed with SPSS software (IBM® SPSS® Statistics Version 19, Chicago, IL).

3. Results

During the study period, 554 patients were admitted to the USAISR burn ICU due to burns with a median TBSA of 12% (IQR 5 25). Of these, 54 patients had fungal screening assays performed (either BG and/or GM) during their stay. Fungal screening assays were sent systematically for high risk patients [those with large TBSA (e.g., >20%), prolonged ICU stays, and/or wounds with high risk for fungal infection] from February 2009 to March 2010. Fungal screening assays were also sent based on clinical concern for fungal infection throughout that time period and for the remainder of the study period. One patient was excluded as he was under 18, and was transferred to a pediatric burn center.

Patients were predominantly male (68%) and median age was 50 years (IQR 35 63 years) (Table 1). Most patients were



* 6 patients with both yeast and mold FWC. 1 patient had FWC with mold diagnosed by both histopathology and culture only
** FWI is defined as invasion of fungi into the viable tissue below the eschar in a specimen [14]. FWC included specimens with positive fungal wound culture without associated histopathology or histopathology without evidence of invasion into viable tissue.

Fig. 1 – Identification of fungal wound colonization (FWC) and fungal wound infection (FWI). * 6 patients with both yeast and mold FWC. 1 patient had FWC with mold diagnosed by both histopathology and culture only ** FWI is defined as invasion of fungi into the viable tissue below the eschar in a specimen [14]. FWC included specimens with positive fungal wound culture without associated histopathology or histopathology without evidence of invasion into viable tissue.

admitted following burns occurring in our local region (*n* 44), while three were from combat operations in Iraq, three from combat operations in Afghanistan, and two from Mexico. Median TBSA was 29% (IQR 17 50%). In patients with no growth on fungal cultures and/or negative histopathology the median TBSA was 27% (IQR 15 41%), while patients with either yeast on culture or histopathology median TBSA was 48% (IQR 22 59%) and mold on culture or histopathology was 56% (IQR 45 64%) (*p* 0.05).

Ten patients had wound culture and/or histopathology with yeast and eleven had wound culture and/or histopathol ogy with mold (Fig. 1). Ten patients had FWC with *Candida* recovered, five had mixed FWC with mold, one *Trichosporon mucoides* FWC, four *Aspergillus* spp. FWC, one *Paecilomyces* sp.

Table 1 – Demographics of all patients with (1 → 3)-β-D-glucan or galactomannan values drawn during hospitalization.

	All patients (*n* 53)	No growth on culture or histopathology (*n* 38)	FWC (*n* 14)	FWI (*n* 1)	P value
Age, years	50 (35 63)	48 (36 57)	55 (35 67)	49	0.66
Gender (male, %)	70	70	64	100	0.22
History of DM (%)	19	16	29	0	0.55
TBSA	29 (17 51)	26.6 (15 41)	53 (30 64)	41	0.02
FTB	9 (2 33)	6 (1 15)	32 (11 52)	11	0.05
Burn location					0.01
Domestic	44	34	9	1	
Iraq	3	3	0	0	
Afghanistan	3	0	3	0	
Mexico	2	1	1	0	
Survival to discharge[a]	68%	79%	43%	0%	0.14
Hospital days	36 (23 66)	35 (18 56)	69 (35 99)	32	0.11
ICU days	27 (8 40)	14 (6 36)	45 (35 78)	32	0.11
Ventilator days	17 (1 38)	3 (0 29)	54 (35 54)	32	0.06

Data are medians (IQRs) or frequencies. DM, diabetes mellitus; TBSA, total body surface area burned, percent; FTB, full thickness burn size, percent; ICU, Intensive Care Unit.
[a] Only two patients with death attributable to mold fungal wound colonization (FWC), 1 patient with mold fungal wound infection (FWI) with death attributable to sepsis.

Table 2 – Patients with fungal wound colonization (FWC) and fungal wound infection (FWI) based upon culture or histopathological results within 7 days of (1 → 3)-β-D-glucan (BG) and galactomannan (GM) results.

Labs drawn	Negative culture or histopathology[a] (*n* 12)		All FWC/FWI (*n* 10)		Yeast FWC (*n* 4)		Mold FWC (*n* 5)		Mold FWI (*n* 1)	
	GM	BG	GM	BG	GM	BG	GM	BG	GM	BG
Patients tested (# of samples)	11 (34)	11 (38)	5 (10)	8 (15)	2 (4)	4 (5)	3 (6)	4 (9)	0 (0)	1 (1)
Positive patients (% positive patients)	5 (45)	11(100)	2 (40)	8 (100)	0 (0)	4 (100)	2 (67)	4 (100)	n/a	0 (0)
Positive samples (% positive samples)	10 (29)	38 (100)	3 (30)	14 (93)	0 (0)	5 (100)	3 (50)	9 (100)	n/a	0 (0)
BG+/GM+ patients (# of samples)	3 (8)		2 (3)		0		2 (3)		0	
BG+/GM patients (# of samples)	8 (21)		4 (6)		2 (3)		2 (3)		0	
BG /GM+	0		0		0		0		0	
BG /GM	0		0		0		0		0	

[a] Samples are 7 days pre or post tissue culture excluding urine culture, BAL/respiratory cultures and blood cultures. Only negative histopathology included (excluded if patients with only fungal wound culture).

FWC, and one patient had FWI with *Fusarium*. Patients with FWC with mold or mixed mold and yeast had greater TBSA (45% and 49%, respectively) than patients with no FWC or involvement of yeast (25% and 24%, respectively) (p 0.02). Patients without FWI/FWC had 6% full thickness burns (FTB), while patients with FWC and FWI had 32% and 11% FTB, respectively (p 0.05).

Median hospital days for patients with no culture growth or negative histopathology were 35 days (IQR 18 56), 43 (IQR 30 97) in patients with yeast FWC, and 95 (IQR 40 110) in patients with mold FWC (p 0.02). The one patient with mold FWI died after 32 days of hospitalization. There was no statistical difference between days spent on mechanical ventilation or in the intensive care unit between patients with FWI/FWC/no growth. Seventy nine percent of patients with no growth on fungal culture and/or negative histopathology survived to discharge, while 30% of patients with yeast FWC survived to discharge, and 50% of mold FWC survived to discharge. The only patient with mold FWI died during hospitalization. Two patients had death attributable to mold FWC, one patient with mold FWI with death attributable to "sepsis" according to death summaries.

In examining BG, 38 out of 47 patients (81%) had a positive value (≥80 pg/ml) at one point during the hospitalization of which 11 (23%) had negative culture and/or histopathology drawn within 7 days of the assay. Four had histopathology and culture indicating yeast FWC and five revealed mold FWC (Table 2). A positive BG was not associated with FWC or FWI (p 0.22) or involvement of yeast, mold, or mixed fungus (p 0.75). In examining the only patient with mold FWI during the study period, he had a BG in the indeterminate range (69 pg/ml) 5 days after culture and histopathology was performed revealing *Fusarium* FWI. The sensitivity of BG for FWC (yeast and mold) was 100%, but specificity was 0% for FWC (yeast and mold) as well as mold FWI (Table 3). Positive predictive value for BG was 27% and negative predictive value was 0%. Considering the upper limit of the BG test is 500 pg/ml, the median BG value for positive results was 239 (IQR 169 320). Overall BG values only correlated with %TBSA burn (0.57, $p < 0.01$) and hospital days (0.29, p 0.05).

In examining GM, four of five patients with a GM drawn within 7 days of *Aspergillus* FWC were positive (index ≥ 0.5). All of these patients were on antifungals (four on voriconazole, one on amphotericin) at the time of assay. Only one patient had a GM drawn six days prior to diagnosis of *Aspergillus* FWC, and this value was negative (GMI 0.3). No samples were drawn within 7 days of FWI. GM was not associated with FWC or FWI (p 0.68) or involvement with yeast, mold, or mixed fungus (p 0.30). The specificity of GM for mold FWC/FWI was 71%. Sensitivity for mold FWC was 50%. No sensitivity or specificity for mold FWI could be calculated from our data set. Positive predictive value for GM was 23% and negative predictive value was 69%. The median GM value for positive results was 0.8 (IQR 0.6 1.1). Overall GM values only correlated with %TBSA burn (0.35, p 0.02) and hospital days (0.61, $p < 0.01$).

An analysis of patients who had both BG and GM drawn revealed that positive BG and GM were associated with hospital days (p 0.02) and TBSA (p 0.04) but not with FWC/FWI (p 0.62). Negative BG and GM in combination were associated with lower TBSA (p 0.02). No association was found with FWI/FWC (p 0.26).

Table 3 – Sensitivity and specificity of (1→3)-β-D-glucan and galactomannan for diagnosing fungal wound colonization (FWC) and fungal wound infection (FWI) by patient and by sample.

	specificity for mold FWC	Sensitivity for yeast FWC	Sensitivity for mold FWC	Sensitivity for mold FWI
Fungal screening assay by patient				
(1 → 3) β D glucan	0%	100%	100%	0%
Galactomannan	55%	0%	67%	n/a
Fungal screening assay by sample				
(1 → 3) β D glucan	0%	100%	100%	0%
Galactomannan	71%	0%	50%	n/a

Due to the USAISR's antibiogram, β lactam antibiotics are less frequently used as empiric coverage. During the study period, only seven patients were exposed to cefepime. Cefepime was not significantly associated with positive BG or GM. No patients received amoxicillin clavulanate during the study period. Piperacillin tazobactam was used in seven patients and was not significantly associated with positive BG or GM. In examining carbapenem exposures, two patients were exposed to meropenem, five were exposed to imipenem/cilastatin, and one patient exposed to ertapenem. Patients with FWC were significantly more likely than those without FWC to have been exposed to a carbapenem during their hospitalization (p 0.02), but there was no significant association with positive BG or GM.

Thirteen patients underwent continuous renal replacement therapy (CRRT) within 12 h of BG/GM assays and had no association with positive BG or GM. Patients with FWC were also significantly more likely to be exposed to CRRT during their hospitalization than those without FWC (p 0.03).

Eighteen patients were bacteremic within 3 days before or after fungal screening assays. Of these, no patients had a negative BG value. BG values were positive in relation to bacteremia with *Pseudomonas aeruginosa*, *Mycobacterium abscessus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, *Enterococcus faecium*, and coagulase negative *Staphylococcus* (p 0.02). Eight patients had positive GM within 3 days of bacteremia with *P. aeruginosa*, *Enterobacter cloacae*, *E. coli*, *S. marcescens*, and *Streptococcus viridans* (one patient with multiple bacteremic episodes) (p 1.0). Eleven patients had negative GM within 3 days of bacteremia with *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, *Candida* non *albicans*, *Bacillus* non *anthracis*, *S. marcescens*, *S. maltophilia*, *Corynebacterium* species, *E. aerogenes*, *S. aureus*, and coagulase negative *Staphylococcus* (two patients with multiple episodes of bacteremia).

4. Discussion

Fungal wound infections lead to significant morbidity and mortality in burn patients. Diagnosis continues to be made with culture and histopathology, leading to delays in diagnosis and treatment. Only one study has examined fungal screening assays in burn patients, but previous literature has shown that in patients with a high prevalence of fungal infection [i.e. hematopoetic stem cell transplant patients (HSCT)], fungal screening assays can be of use in diagnosis [3,4,6]. Of the patients included in this study, the majority had TBSA >20%, with 8 patients having greater than 60% TBSA. One in 53 patients had FWI (2%) and 14/53 patients had FWC (26%). This is similar to prevalence noted in prior studies at this institution which found FWI in 6% of patients with <30% TBSA and >57% in patients with >60% TBSA [2]. Rates in our study are higher than other studies such as a multicenter study that revealed 6% of burn patients had positive fungal cultures; however, these were primarily associated with Candida [15].

Fungal serologies have also been studied as markers for response to therapy and predictors of outcome in HSCT and hematologic malignancy patients with invasive fungal infections [5,16]. As multiple possible etiologies for false positives are common in burn patients including bacteremia, hemodialysis, gauze covering, β lactam antibiotics, and *Candida* colonization we sought to evaluate the utility of GM and BG assays in facilitating diagnosis of FWI/FWC in this population. Our data revealed 100% sensitivity of BG in diagnosing FWC. However the only patient with FWI during this study had an indeterminate BG prior to diagnosis. GM had a specificity of 71% and a positive predictive value of 23% while sensitivity for mold FWC was very low at 67%. Most importantly, our study showed that BG and GM were not significantly associated with FWI, FWC, or species of fungal involvement; but did correlate with TBSA.

Only one study has previously examined BG values in burn patients. Because gauze is associated with false positive BG levels, this study examined the role of gauze dressings. It revealed that BG was positive at baseline in 50% of burn patients prior to application of a gauze dressing. Elevated BG was statistically associated with greater TBSA rather than with gauze dressings [13]. Our data is consistent with these findings, as we found that a positive BG was statistically associated with higher TBSA. Both studies are in contrast with numerous other reports in other patient populations. A multicenter study of non trauma patients with a variety of risk factors for fungal infections revealed a sensitivity of 64% and a specificity of 92% using BG [17]. Among patients with acute myeloid leukemia and myelodysplastic syndrome using a BG $\geq$ 60 pg/ml, the absence of a positive test result had a 100% negative predictive value, and the specificity of the test was 90% for a single positive test result [4]. In a study of HSCT patients, potential etiologies of false positive BG were identified in 25/38 cases including mucositis, candida colonization, bacteremia, use of antimicrobials (especially cefepime), erythrocyte and thrombocyte filtered blood products, collecting tubes or sampling via venous catheters [11]. Only one trauma study has evaluated BG, which revealed that the sensitivity of a positive BG for identifying invasive candidiasis was 87%, with 73% specificity on the day of suspicion for invasive candidiasis. However, this study also showed that in trauma ICU patients elevated BG was common early in their stay [18]. Overall, the sensitivity of BG for detection of yeast and mold FWC in our study was very high at 100%, but it had 0% specificity. Most concerning was the fact that the single patient with *Fusarium* FWI, who subsequently died as a result of this, was one of the minority of patients who did not have a positive BG. This patient had an indeterminate BG (69 pg/ml) during this episode. Decreasing the BG threshold in burn patients to <60 pg/ml (eliminating the indeterminate range) could increase sensitivity, and as the test has such low specificity at baseline could potentially provide some usefulness as a negative predictor in this patient population.

In a multicenter study of 179 patients with HSCT or hematologic malignancies with a 31% incidence of proven or probable invasive aspergillosis (IA), studies using GM showed an 81% sensitivity and 89% specificity for diagnosis of IA. Approximately two thirds of patients have detectable circulating GM antigen an average of eight days prior to diagnosis by other means [6]. Few studies have evaluated GM in patients without HSCT or hematologic malignancies and none have

previously looked at the burn population. Studies evaluating GM in solid organ transplant recipients had a sensitivity and specificity ranging between 22 30% and 84 90%, respectively [3,6]. The authors recommend that GM assay should only be used in patients with high pretest probability of IA, such as patients with neutropenia or patients who have undergone transplantation as increasing the prevalence of IA increases the positive predictive value [6]. This recommendation is directly applicable to burn patients as fungal infections have a low prevalence in the burn population. In a prior study at this institution, of 2651 burn patients admitted between 1991 and 2002, FWC was identified in 4.6% and FWI in 2% compared with the prevalence of approximately 30% in the patient population in which GM has previously been studied most extensively [2]. In our study, only one patient had a GM drawn within 7 days prior to *Aspergillus* FWC which was negative 6 days prior to diagnosis. This patient had multiple specimens showing *Aspergillus* FWC, and had one positive and one negative GM drawn with these cultures. In patients with GM drawn within 7 days after diagnosis of *Aspergillus* FWC, five of seven were positive. Positive GM was associated with longer hospitaliza tions ($p < 0.01$) but not FWI, FWC, or yeast, mold, or mixed fungal wound involvement.

A study designed to assess the benefit of combined screening with BG and GM showed improved specificity and positive predictive value to 100% for the diagnosis of IA without detriment to sensitivity or negative predictive value. In this study, BG tended to become positive prior to GM [3]. This fits with the data that we have from our study as the strength of BG lies in its high sensitivity, while GM has a low sensitivity but higher specificity. We found that combining BG and GM did not increase their performance in the burn population. This makes the utility of BG and GM in burn patients very limited. There may be value in the setting of a negative BG or positive GM, which would further narrow pre test probability while awaiting confirmatory culture results. However, in our small study no association between GM or BG and FWC, FWI, or species of fungus was evident.

Our study has several limitations first of which is that this was a retrospective study of BG and GM screening assays which were drawn for a variety of clinical reasons. The small number of study patients also limits the conclusions that can be drawn from our study. Our study also may have under estimated the prevalence of FWI, as not all patients had histopathology corresponding with fungal cultures and any patients without histopathology were considered to be FWC. Despite evaluating three years of data, there was only one FWI (*Fusarium*). There was only 1 BG value drawn within 7 days, and this was 5 days after the culture and histopathology showing FWI. There were no cases of FWI with *Aspergillus* to further evaluate the utility of GM in diagnosing invasive *Aspergillus* infections. In a prior study at this institution, of the patients with FWI, 41% had observed progression from FWC on histopathology [2], emphasizing the importance of aggressive treatment of FWC. Using the predicted values of the GM assay for diagnosis of IA based on prevalence from prior studies and the prevalence of FWC in our institution, the positive predictive value of GM would be predicted to be 31% (CI 28 35%) for proven or possible IA in burn patients at our institution [6], however the positive predictive value is only 23% according to our data. Finally, since our study focused on higher risk patients further study into broader ranges of %TBSA or degree of illness are required to ensure the findings of this study are applicable to a larger burn population.

In burn patients, the utility of fungal screening assays remains unclear. In some patients, FWC can be followed by invasion of microorganisms, giving rise to burn wound infection [2,19]. Therefore, diagnosis of FWC is clinically important. It can be argued that with the high sensitivity of BG, a negative test could be reassuring that there is not FWC present, with the caveat that this was indeterminate in the only case of *Fusarium* FWI. Decreasing the threshold to BG < 60 pg/ml would lead to 100% sensitivity of BG for diagnosis of FWC/FWI in this very small study without changing specificity, as this was 0%. GM had a specificity of 71% and a positive predictive value of 23% which is lower than predicted based on the test's performance in other patient populations. It could be argued that a positive GM or negative BG could be useful in further estimating pre test probability while awaiting cultures, however we found no statistical association between BG or GM and FWI, FWC, or species of fungal organisms identified from culture. Larger, multi center prospective studies with BG and GM assays in burn patients correlated with culture and histopathology are needed to further clarify the utility of these assays in this specific population.

5. Conclusion

Our results are consistent with the only prior study of fungal screening assays in burn patients, demonstrating that BG positivity is statistically associated with extent of burn (TBSA). No prior studies have evaluated GM in burn patients, but we found that GM was associated with hospital length of stay and TBSA but not fungal infection. Overall, we showed that neither BG nor GM fungal screening assays used separately or in combination are associated with FWI, FWC, species of fungus isolated from patients with FWC or FWI, or mortality. However, the number of FWI was too small to make a meaningful conclusion. Despite 100% sensitivity of BG for diagnosis of FWC, its 0% specificity, and elevation in the setting of higher TBSA, makes utility of this test in burn patients very limited. GM had neither high sensitivity nor specificity. These fungal screening assays should be used with extreme caution in the burn population.

Conflict of interests

The authors have no conflict of interests to report.

REFERENCES

[1] Becker WK, Cioffi Jr WG, McManus AT, Kim SH, McManus WF, Mason AD, et al. Fungal burn wound infection. A 10 year experience. Arch Surg 1991;126:44 8.
[2] Horvath EE, Murray CK, Vaughan GM, Hospenthal DR, Wade CE, Holcomb JB, et al. Fungal wound infection (not

colonization) is independently associated with mortality in burn patients. Ann Surg 2007;245:978 85.
[3] Alexander BD, Pfaller MA. Contemporary tools for the diagnosis and management of invasive mycoses. Clin Infect Dis 2006;43:S15 27.
[4] Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, et al. β D Glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin Infect Dis 2004;39:199 205.
[5] Miceli MH, Grazziutti ML, Woods G, Zhao W, Kocoglu MH, Barlogie B, et al. Strong correlation between serum *Aspergillus* galactomannan index and outcome of *Aspergillosis* in patients with hematological cancer: clinical and research implications. Clin Infect Dis 2008;46:1412 22.
[6] Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive *Aspergillosis* using a galactomannan assay: a meta analysis. Clin Infect Dis 2006;42:1417 27.
[7] Associates of Cape Cod Incorporated, Fungitell™ Instructions, 2007.
[8] Mennik Kersten MASH, Verweij PE. Non culture based diagnostics for opportunistic fungi. Infect Dis Clin North Am 2006;20:711 27.
[9] Persat F, Ranque S, Derouin F, Michel Nguyen A, Picot S, Sulahian A. Contribution of the 1,3 β D glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol 2008;46:1009 13.
[10] Yoshida M. Infections in patients with hematological diseases: recent advances in serological diagnosis and empiric therapy. Int J Hematol 1997;66:279 89.
[11] Racil Z, Kocmanova I, Lengerova M, Weinbergerova B, Buresova L, Toskova M, et al. Difficulties in using (1 → 3) β D glucan as the screening test for the early diagnosis of invasive fungal infections in patients with haematological malignancies high frequency of false positive results and their analysis. J Med Microbiol 2010;59:1016 22.
[12] Wheat LJ, Walsh TJ. Diagnosis of invasive *Aspergillosis* by galactomannan antigenemia detection using an enzyme immunoassay. Eur J Clin Microbiol Infect Dis 2008;27:245 51.
[13] Shupp JW, Petraitiene R, Jaskille AD, Pavlovich AR, Matt SE, Nguyen DT, et al. Early serum (1 → 3) β D glucan levels in patients with burn injury. Mycoses 2011;49:58 61.
[14] Schofield CM, Murray CK, Horvath EE, Cancio LC, Kim SH, Wolf SE, et al. Correlation of culture with histopathology in fungal burn wound colonization and infection. Burns 2007;33:341 6.
[15] Ballard J, Edelman L, Mphil JS, Sheridan R, Kagan R, Bracco D, et al. Positive fungal cultures in burn patients: a multicenter review. J Burn Care Rehabil 2008;29:213 21.
[16] Boutboul F, Alberti C, Leblanc T, Sulahian A, Gluckman E, Derouin F, et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. Clin Infect Dis 2002;34:939 43.
[17] Ostrosky Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, et al. Multicenter clinical evaluation of the (1 → 3) β D glucan assay as an aid to diagnosis of fungal infections in humans. Clin Infect Dis 2005;41:654 9.
[18] Mohr JF, Sims C, Paetznick V, Rodriguez J, Finkelman MA, Rex JH, et al. A prospective survey of (1 → 3) β D glucan and its relationship to invasive candidiasis in the surgical ICU setting. J Clin Microbiol 2011;49:58 61.
[19] Mayhall GC. The epidemiology of burn wound infections: then and now. Clin Infect Dis 2003;37:543 50.