Effect of Early Screening for Invasive Fungal Infections in U.S. Service Members with Explosive Blast Injuries

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Abstract

Background: An outbreak of invasive fungal infections (IFI) began in 2009 among United States servicemen who sustained blast injuries in Afghanistan. In response, the military trauma community sought a uniform approach to early diagnosis and treatment. Toward this goal, a local clinical practice guideline (CPG) was implemented at Landstuhl Regional Medical Center (LRMC) in early 2011 to screen for IFI in high-risk patients using tissue histopathology and fungal cultures.

Methods: We compared IFI cases identified after initiation of the CPG (February through August 2011) to cases from a pre-CPG period (June 2009 through January 2011).

Results: Sixty-one patients were screened in the CPG period, among whom 30 IFI cases were identified and compared with 44 pre-CPG IFI cases. Demographics between the two study periods were similar, although significantly higher transfusion requirements ($p < 0.05$) and non-significant trends in injury severity scores and early lower extremity amputation rates suggested more severe injuries in CPG-period cases. Pre-CPG IFI cases were more likely to be associated with angioinvasion on histopathology than CPG IFI cases (48% versus 17%; $p < 0.001$). Time to IFI diagnosis (three versus nine days) and to initiation of antifungal therapy (seven versus 14 days) were significantly decreased in the CPG period ($p < 0.001$). Additionally, more IFI patients received antifungal agent at LRMC during the CPG period (30%) versus pre-CPG period (5%; $p = 0.005$). The CPG IFI cases were also prescribed more commonly dual antifungal therapy (73% versus 36%; $p = 0.002$). There was no statistical difference in length of stay or mortality between pre-CPG and CPG IFI cases; although a non-significant reduction in crude mortality from 11.4% to 6.7% was observed.

Conclusions: Angioinvasive IFI as a percentage of total IFI cases decreased during the CPG period. Earlier diagnosis and commencement of more timely treatment was achieved. Despite these improvements, no difference in clinical outcomes was observed compared with the pre-CPG period.

TRAUMA-RELATED INVASIVE FUNGAL INFECTION (IFI) is an uncommon complication observed in patients who sustain traumatic injury (e.g., motor vehicle crashes, industrial and agricultural injuries, and natural disasters) [1–7]. The cause of trauma-related IFI is believed to be because of direct inoculation of fungal spores [2,3,8,9], impaired blood supply, tissue necrosis [2,8], immune suppression from chronic illnesses such as diabetes mellitus [2,6], massive blood transfusions [8,10,11], or severe trauma [12,13]. Although it is rare,

IFI is an important entity trauma clinicians need to be aware because of its high mortality rate (ranging from 25–38%) [3,7].

In recent years, trauma-related IFI in the military has garnered renewed attention because of the increased incidence of the disease among wounded personnel [8,9,14–16]. Increases in IFI were observed during the deployment surge in Iraq in 2007 [15] and again to a much greater extent in the third and fourth quarters of 2010 (following the surge in

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Afghanistan). Notably, in the last quarter of 2010, the IFI rate, as a function of trauma admissions to the Landstuhl Regional Medical Center (LRMC) in Germany, reached almost 10% [8].

Reports on injured United States (U.S.) and British Operation Enduring Freedom (OEF) personnel diagnosed with IFI describe common characteristics and patterns of injury [8,16]. Although not an exclusive set of circumstances, IFI occurred generally in servicemen who were injured severely by improvised explosive devices while on foot patrol. Injury patterns in these patients were characterized by traumatic amputation of at least one leg, severe injury to another extremity, and possible abdominal, pelvic, and/or urogenital damage [8]. Wound contamination with organic debris was also common in these injuries. Further analysis of IFI cases in U.S. servicemen from June 2009 to December 2010 revealed a median time of 10 d following injury to IFI diagnosis and four days after collection of diagnostic IFI specimens to initiation of antifungal therapy. Fungal etiology of IFI cases revealed that nearly 30% of the cases had more than one offending pathogen and often included fungi from the order *Mucorales* and non-*Mucorales* [8].

Armed with an awareness of the increased IFI rates, an understanding of key risk factors (i.e., severe injury resulting from dismounted blast incidents), and knowledge that early recognition is crucial to managing IFI, LRMC implemented a local process improvement initiative referred to as the "Blast" clinical practice guideline (CPG) in February 2011. Its aim was to decrease time to IFI diagnosis and increase uniformity in decision-making regarding the use of early antifungal therapy in high-risk patients.

Once a patient was determined to be at risk for IFI, the LRMC Blast CPG specified biopsy of each lower extremity amputation for histopathology and fungal/bacterial culture during the first debridement procedure at LRMC, which is generally the patient's second or third debridement overall following injury. Decisions about biopsies at LRMC following the first debridement were at the discretion of the surgeon, but were done routinely unless the wounds looked healthy and the patient was clinically improving. At the discretion of the attending surgeon, the CPG could be applied to other wounded service members (e.g., non-blast patients) or other wounds (e.g., upper extremity amputations) if there was clinical concern for IFI.

The guideline also sought to standardize utilization of antifungal therapy, which advocated liposomal amphotericin B and voriconazole if fungal elements were observed in tissue or if the trauma team believed the wounds were consistent with IFI pending further evaluation. Although not mandated in the CPG, growth of fungi from the order *Mucorales* often triggered antifungal therapy because of this pathogen's propensity to cause trauma-related IFI [1–7,14,15,20].

Work-up for IFI at LRMC was uncommon prior to initiation of the Blast CPG, as was the initiation of empiric antifungal agents prior to arrival at U.S. military treatment facilities (MTFs) where aggressive evaluation and empiric treatment for IFI were already the practice pattern. Therefore, the initial CPG period represented a substantial change in practice at a point early in the medical evacuation pipeline. The purpose of the current analysis is to determine if there are any outcome differences between patients with IFI diagnosed before or after the Blast CPG was implemented.

Patients and Methods

Pre-CPG and CPG populations, case identification, and definitions

The population for this analysis included any Department of Defense (DoD) beneficiary who was injured during deployment, evacuated to LRMC, and then transferred to a U.S. MTF participating in the Trauma Infectious Disease Outcomes Study (TIDOS). As described previously [17], TIDOS includes an infectious diseases module of the DoD Trauma Registry (DoDTR) and collects information on infections and their outcomes, microbiology, and treatment. The pre-CPG population included service members admitted to LRMC between June 1, 2009 (when TIDOS began) and January 31, 2011. The CPG population consisted of service members who had biopsies performed in accordance with the Blast CPG from February 1, 2011 through August 31, 2011. Table 1 outlines the criteria stipulated in the CPG to identify high-risk patients. Once identified as high-risk, specimens for histopathology and fungal/bacterial culture were collected according to the CPG (Table 2). After subsequent transfer to one of the participating U.S. MTFs, additional histopathology and culture specimens were collected for further evaluation at the discretion of the clinical team and not specifically as a result of the Blast CPG.

The TIDOS database was used to identify retrospectively pre-CPG and CPG cases that met the IFI case definition [8,18], which required tissue necrosis on ≥ 2 consecutive debridements along with culture and/or histopathology evidence of IFI from specimens collected at either LRMC or the U.S. MTFs. Cases were classified as proven, probable, or possible. Proven IFI cases required angioinvasion of tissue by fungal elements, although a probable case was defined by histopathology showing fungal elements in tissue without evidence of angioinvasion. Possible IFI included cases with positive

TABLE 1. LANDSTUHL REGIONAL MEDICAL CENTER (LRMC) BLAST CLINICAL PRACTICE GUIDELINE CRITERIA FOR THE SCREENING OF PATIENTS FOR INVASIVE FUNGAL INFECTIONS (IFI)

1. The trauma team should screen for IFI in all patients who sustained bilateral lower extremity amputation secondary to improvised explosive device or blast injury.
- OR
2. The trauma team should also strongly consider IFI screening in any patient with any one of the following conditions:
 - a. Large amputation burden: >1 Amputation, single amputation above mid-femur, or single amputation in conjunction with significant perineal soft tissue injury.
 - b. More than expected soft tissue necrosis (either by rate of progression or progression outside zone of injury) based on communication with downrange providers confirming the interval since last debridement and that prior debridement was to viable tissue.
 - c. Extensive wound contamination (as evidenced by continued gross contamination of wound at initial LRMC washout).
 - d. Significant revision of amputation level required at LRMC.

TABLE 2. LANDSTUHL REGIONAL MEDICAL CENTER
BLAST CLINICAL PRACTICE GUIDANCE
FOR BIOPSY SPECIMEN COLLECTION

<i>Process of specimen collection</i>
Biopsy should be done at the conclusion of the first wound exploration at Landstuhl Regional Medical Center
Tissue sample will be taken from each lower extremity in patients with bilateral lower extremity amputations
Specimen should be taken from the junction of viable and necrotic tissue—basically the last piece of borderline-viable tissue removed.
For each site sampled, two tissue samples are needed, in separate containers.
A. One specimen (1 cm) in formalin for histological examination.
B. One specimen (1 cm) divided into two pieces and placed in a single sterile container for cultures.

wound tissue cultures; however, a histopathologic analysis was either not conducted or did not demonstrate evidence of fungal elements [18]. Probable and possible cases required evidence of progressive tissue necrosis on successive intraoperative evaluations at U.S. MTFs, as determined by review of intraoperative and case records. As their role in the pathogenesis of wound infections has not been determined definitively, *Candida* species were excluded from the analysis.

Data elements

Patient data, including trauma history, demographics, clinical parameters, and surgical management, were collected through utilization of the DoDTR (formerly called the Joint Theater Trauma Registry) [19]. Clinical outcomes included time to diagnosis, time to initiation of antifungal therapy, total hospitalization, length of stay in intensive care unit (ICU), number of operating room visits, and mortality. Time to diagnosis was defined as the earlier of time from injury to the date the first IFI culture or histopathology-positive specimen

was collected. All histopathology was reviewed by two surgical pathologists. This study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences, Bethesda, Maryland.

Statistical analysis

Because the purpose of our study was to compare IFI patients who underwent early diagnostic screening and consideration for empiric antifungals via the LRMC Blast CPG, only IFI cases during the CPG period that were assessed according to the CPG were included and evaluated as “CPG IFI” cases in the analysis. The IFI cases that occurred during the CPG period and did not undergo early screening at LRMC, either because of CPG non-compliance or because patients did not meet CPG screening criteria, were not included in the comparison between the two time periods.

Chi square and Fisher exact tests were used to test differences between the categorical parameters by time period (pre-CPG and CPG). These tests were also used to compare categorical parameters of CPG IFI cases and non-IFI subjects. Non-parametric tests were used to compare the overall distribution of continuous parameters among the groups. Statistical analysis was performed using SAS[®] version 9.3 (SAS, Cary, NC) and R[®] version 2.13.2 (R Project for Statistical Computing, Vienna, Austria). Statistical significance was defined as $p < 0.05$.

Results

Sixty-one U.S. combat casualties at LRMC were screened in accordance with the Blast CPG from February through August 2011. Of this early screening population, 30 had IFI (CPG IFI cases) and 31 did not (CPG non-IFI subjects) (Table 3). There were six IFI cases in the CPG period identified through TIDOS that did not undergo early screening at LRMC. Three met the screening criteria and should have had specimens collected, whereas three did not meet CPG criteria for early screening. As described in the Patients and Methods section, these six cases were not included in the CPG IFI

TABLE 3. STUDY POPULATION DEMOGRAPHIC CHARACTERISTICS AND INJURY CIRCUMSTANCES

<i>Characteristics</i>	<i>Pre-CPG IFI cases (n=44)</i>	<i>CPG IFI cases (n=30)</i>	<i>CPG non-IFI subjects (n=31)</i>	<i>p value 1</i>	<i>p value 2</i>
<i>IFI Classification, No. (%)</i>				<0.001	<0.001
Proven	21 (47.7)	5 (16.7)	-		
Probable	8 (18.2)	18 (60.0)	-		
Possible	15 (34.1)	7 (23.3)	-		
Culture-positive non-IFI	-	-	20 (64.5)		
Culture-negative non-IFI	-	-	11 (35.5)		
Age, median (IQR)	22.6 (21.35, 26.2)	24.2 (21.9, 26.8)	22.7 (21.8, 25.1)	0.590	0.315
Blast injury, No. (%)	41 (93.2)	30 (100)	29 (93.5)	0.267	0.492
Injured on foot patrol, No. (%)	38 (86.4)	29 (96.7)	28 (90.3)	0.328	0.745
LRMC ISS, median (IQR)	21 (15.5, 26)	24 (18, 29)	21 (18, 24)	0.131	0.365
<i>Amputations, No. (%)</i>					
Lower extremity	26 (59.1)	23 (76.7)	26 (83.9)	0.139	0.534
Upper extremity	1 (2.3)	0	1 (3.2)	1.0	1.0
Both upper/Lower extremities	6 (13.6)	4 (13.3)	1 (3.2)	1.0	0.195

P-value 1: compares pre-CPG IFI versus CPG IFI cases; p value 2: compares CPG IFI versus CPG non-IFI subjects.

CPG=clinical practice guideline; IFI=invasive fungal infection; IQR=interquartile range; ISS=injury severity score; LRMC=Landstuhl Regional Medical Center.

TABLE 4. MEDIAN (IQR) CLINICAL CHARACTERISTICS OF INVASIVE FUNGAL INFECTION (IFI) CASES AND NON-IFI SUBJECTS

<i>Clinical parameters</i>	<i>Pre-CPG IFI cases (n=44)</i>	<i>CPG IFI cases (n=30)</i>	<i>CPG non-IFI subjects (n=31)</i>	<i>p value 1</i>	<i>p value 2</i>
<i>Presentation to first theater hospital</i>					
Heart rate	126 (109, 144)	118 (100.75, 136)	122 (107.5, 144.75)	0.173	0.218
Systolic blood pressure	99.5 (80, 115.5)	99 (75, 136)	120 (106, 139)	0.562	0.193
Blood gas – base deficit	–7.5 (–11, –4)	–9.5 (–13.2, –5.75)	–5 (–8, –1)	0.214	0.004
Blood gas – pH	7.23 (7.15, 7.3)	7.19 (7, 7.29)	7.28 (7.23, 7.37)	0.449	0.022
<i>Blood product requirements in theater</i>					
Packed red blood cells + whole blood	29 (16, 37)	40 (23, 55)	19 (13.5, 30.25)	0.012	0.002
Fresh frozen plasma + whole blood	27 (16.5, 36)	34.5 (21.75, 51.25)	18 (13.5, 29)	0.035	0.002
<i>On admission to LPMC</i>					
White blood cell total (10 ⁹ cells/L)	7.9 (5.33, 9.85)	7.1 (6.12, 9.4)	8.3 (7.05, 9.9)	0.756	0.108
Absolute neutrophil count (10 ⁹ cells/L)	6.2 (4.14, 8.04)	5.86 (4.78, 7.42)	6.87 (5.4, 8.14)	0.894	0.376
Blood urea nitrogen (mg/dL)	12.5 (8, 17.25)	13 (10, 26)	10 (9, 12.5)	0.203	0.026
Creatinine (mg/dL)	0.7 (0.5, 0.8)	0.6 (0.5, 1.2)	0.6 (0.6, 0.7)	0.723	0.685
Aspartate aminotransferase (U/L)	102.5 (68.75, 141.25)	169 (116, 339)	125 (74.5, 206)	<0.001	0.02
Alanine aminotransferase (U/L)	37.5 (28, 51.25)	50 (38, 70)	45 (32, 62)	0.009	0.136
LRMC admission SOFA score	7 (4, 9.5)	8 (4, 11)	5 (4, 8)	0.469	0.086

P-value 1: compares pre-CPG IFI versus CPG IFI cases; p value 2: compares CPG IFI versus CPG non-IFI subjects.

CPG=clinical practice guideline; IQR=interquartile range; LRMC=Landstuhl Regional Medical Center; SOFA=sequential organ failure assessment.

group. Most CPG IFI cases (97%) were admitted initially to the LRMC ICU and represented 17% of LRMC trauma ICU admissions during the CPG study period.

Evaluation of the TIDOS database found 44 IFI cases prior to the initiation of the Blast CPG (June 2009 through January 2011). As shown in Table 3, among these pre-CPG IFI cases, significantly more were classified as proven (48% versus 17%) and possible (34% versus 23%) and significantly less as probable (18% versus 60%) compared with the CPG period ($p < 0.001$).

Patient demographics and injury patterns

All patients included in the analysis were male, median age of 22.6 to 24.2 y, served in OEF, and were predominantly enlisted in the U.S. Marines. An explosive blast was the causal mechanism for the majority of injuries ($\geq 93\%$) and

injury severity was uniformly high. The median injury severity score (ISS) was 21 among pre-CPG IFI cases and 24 among CPG IFI cases (Table 3), although the difference was not statistically significant. Pattern of injury was also comparable with early amputation of lower extremities being predominant. A non-significant trend toward higher rates of lower extremity amputations prior to admission to LRMC in CPG IFI versus pre-CPG IFI cases was observed (Table 3).

Clinical parameters

Shock indices recorded upon presentation to first theater combat support hospital and the median Sequential Organ Failure Assessment on presentation to LRMC were not statistically different between pre-CPG and CPG IFI cases (Table 4). However, CPG IFI cases had greater first 24-h

TABLE 5. INVASIVE FUNGAL INFECTION (IFI) HISTOPATHOLOGY, CULTURES, AND TIMELINE

	<i>Pre-CPG IFI cases (n=44)</i>	<i>CPG IFI cases (n=30)</i>	<i>CPG non-IFI subjects (n=31)</i>	<i>p value 1</i>	<i>p value 2</i>
LRMC positive histopathology, No. (%)	3 (6.8)	12 (40)	0	<0.001	<0.001
LRMC positive mold culture, No. (%)	17 (38.6)	21 (70)	18 (58.1)	0.01	0.426
<i>IFI-Related Timeline, median days (IQR)</i>					
Injury to first positive mold culture	8.5 (5, 12.25)	3 (2, 5)	3 (2, 3.75)	<0.001	0.542
Injury to first positive histopathology	9 (5.75, 11.25)	5 (3, 7)	NA	0.018	NA
Injury to IFI diagnosis	9 (5, 12.25)	3 (2, 5)	NA	<0.001	NA
Injury to antifungal treatment	14.5 (8.75, 31.25)	7 (5, 10)	7 (5.5, 12)	<0.001	0.961

P-value 1: compares pre-CPG IFI versus CPG IFI cases; p value 2: compares CPG IFI versus CPG non-IFI subjects.

CPG=clinical practice guideline; IQR=interquartile range; LRMC=Landstuhl Regional Medical Center; NA=not applicable.

blood product transfusion requirements and higher markers of tissue damage such as aspartate aminotransferase (AST) on presentation to LRMC compared with pre-CPG IFI cases (Table 4).

Invasive fungal infections mycology

Among IFI cases (Table 5), 70% of CPG IFI cases had at least one positive LRMC fungal culture compared with 39% of pre-CPG IFI cases ($p=0.01$). However, no difference was observed when combining overall findings (LRMC and U.S. MTFs) between the number of pre-CPG IFI (33 patients, 75%) and CPG IFI (25 patients, 83%) cases with positive fungal cultures. It is noteworthy that growth of a mold from a screening culture obtained at LRMC did not predict subsequent IFI.

Invasive fungal infection diagnosis

Decreases were observed in the time to IFI diagnosis and initiation of antifungal treatment when comparing CPG and pre-CPG IFI patients. Correspondingly, the time (d) from injury to date of collection of a specimen that yielded an IFI-related positive mold culture (8.5 d to 3 d; $p<0.001$) and first positive histopathology result (9 d to 5 d; $p=0.018$) decreased from the pre-CPG to the CPG period (Table 5). Additionally, at least one positive histopathology result was observed at LRMC in 6.8% of pre-CPG IFI versus 40% of CPG IFI cases ($p<0.001$). Notably, positive histopathology findings at LRMC were predictive of meeting the case definition for IFI.

Antifungal therapy

An increase ($p=0.005$) was observed in the number of IFI patients prescribed antifungal therapy at LRMC during the CPG period (30%) compared with pre-CPG period (4.5%). As shown in Table 6, CPG IFI cases were more likely to receive dual therapy during their duration of treatment (73%) than pre-CPG IFI cases (36%; $p=0.002$), but less likely to be prescribed combination therapy (7% versus 30%, respectively; $p=0.019$). Duration of antifungal therapy for pre-CPG and CPG IFI cases was similar, except that voriconazole

duration was longer in CPG IFI cases ($p=0.051$). Of the four CPG IFI cases that did not receive antifungal therapy, three were classified as probable and one as possible. When antifungal therapy was initiated at LRMC during the CPG period, 100% of the time it consisted of at least dual therapy.

Clinical outcomes

Total hospitalization, length of stay in the ICU, number of high-level amputations (i.e., total hip disarticulation or hemipelvectomy) performed at U.S. MTFs, and number of operating room visits were comparable among the IFI cases (pre-CPG and CPG). The crude mortality rate in CPG IFI cases decreased, although not significantly, to 6.7% from 11.4% in the pre-CPG IFI cases (Table 7).

Comparison of CPG IFI cases and non-IFI subjects

When the CPG IFI cases were compared with non-IFI subjects screened during the CPG period, some differences were observed. First, the base deficit was significantly decreased and blood pH increased on presentation among non-IFI subjects ($p=0.004$ and $p=0.022$, respectively). Additionally, non-IFI subjects required fewer blood products within the first 24 h ($p=0.002$) and had decreased AST concentrations at presentation at LRMC ($p=0.02$; Table 4). Although differences for non-*Mucorales* molds were not observed, 10 CPG IFI cases had growth of *Mucorales* on LRMC screening cultures, which was higher compared with the two CPG non-IFI subjects with *Mucorales* growth ($p=0.011$). Growth of mold from the order *Mucorales* from tissue collected at LRMC or an U.S. MTF also predicted IFI and was observed in nine (30%) CPG IFI cases and only two (6.5%) of the non-IFI subjects ($p=0.022$).

Differences were also noted with antifungal treatment. The CPG non-IFI subjects were less likely to have antifungal therapy initiated at LRMC (9.7%) than CPG IFI cases, although this did not reach statistical significance. Some non-IFI subjects (32%) also received dual therapy, but this was

TABLE 6. INVASIVE FUNGAL INFECTION (IFI) MANAGEMENT

	Pre-CPG IFI cases (n=44)	CPG IFI cases (n=30)	CPG non-IFI subjects (n=31)	p value 1	p value 2
<i>Systemic antifungal agents, No. (%)</i>					
Amphotericin B (liposomal)	32 (72.7)	25 (83.3)	10 (32.3)	0.401	<0.001
Voriconazole	30 (68.2)	25 (83.3)	11 (35.5)	0.181	<0.001
Posaconazole	15 (34.1)	3 (10)	0	0.026	0.113
Caspofungin	13 (29.5)	2 (6.7)	0	0.019	0.238
Micafungin	3 (6.8)	0	0	0.267	NA
<i>Antifungal duration, median days (IQR)</i>					
Amphotericin B (liposomal)	21.5 (8.75, 34.25)	19 (16, 29)	8 (4.75, 14.75)	0.864	0.036
Voriconazole	10 (3.5, 18.75)	17 (10, 30)	12 (6.5, 19.5)	0.051	0.204
<i>IFI antifungal treatment, No. (%)</i>					
No treatment	8 (18.2)	4 (13.3)	20 (64.5)	0.751	<0.001
Single Agent	6 (13.6)	2 (6.7)	1 (3.2)	0.461	0.612
Dual (Amphotericin B + triazole)	16 (36.4)	22 (73.3)	10 (32.3)	0.002	0.002
Echinocandin	1 (2.3)	0	0	1.0	NA
Combination (Dual + echinocandin)	13 (29.5)	2 (6.7)	0	0.019	0.238

P-value 1: compares pre-CPG IFI versus CPG IFI cases; p value 2: compares CPG IFI versus CPG non-IFI subjects. CPG=clinical practice guideline; IQR=interquartile range; NA=not applicable.

TABLE 7. INVASIVE FUNGAL INFECTION (IFI) CLINICAL OUTCOMES

	<i>Pre-CPG IFI cases (n=44)</i>	<i>CPG IFI cases (n=30)</i>	<i>CPG non-IFI subjects (n=31)</i>	<i>p value 1</i>	<i>p value 2</i>
<i>Hospitalization, median days (IQR)</i>					
LRMC	2 (2, 3)	2 (2, 4)	3 (2, 4.5)	0.369	0.272
U.S. MTF	48 (27.5, 67)	47 (29, 74)	40 (27.5, 62.5)	0.578	0.486
Total hospitalization	48.5 (27.75, 68.5)	49.5 (30.75, 75.5)	44 (33.5, 64)	0.633	0.754
<i>ICU Length of Stay, median days (IQR)</i>					
LRMC	2 (1.5, 3)	2 (2, 3.75)	3 (2, 4)	0.508	0.705
U.S. MTF	10 (4, 19)	7 (4, 18)	3 (1, 12)	0.908	0.049
Total ICU	11 (4.5, 20)	9.5 (6, 20)	7 (3, 14.5)	0.847	0.054
<i>OR Visits, median (IQR)</i>					
LRMC	1 (1, 2)	1 (1, 2.75)	1 (1, 2)	0.548	0.91
U.S. MTF	13 (6.75, 18)	12.5 (6.5, 17)	8 (5, 11)	0.846	0.04
Total OR visits	15 (8, 19)	13.5 (9.25, 18)	10 (6.5, 12)	0.809	0.034
<i>Overall Endpoints, No. (%)</i>					
High-level amputations ¹	9 (20.5)	7 (23.3)	1 (3.2)	0.781	0.026
Deaths	5 (11.4)	2 (6.7)	0	0.694	0.238

P-value 1: compares pre-CPG IFI versus CPG IFI cases; p value 2: compares CPG IFI versus CPG non-IFI subjects.

CPG=clinical practice guideline; ICU=intensive care unit; IQR=interquartile range; LRMC=Landstuhl Regional Medical Center; MTF=military treatment facility; OR=operating room.

¹High-level amputations are defined as total hip disarticulation or hemipelvectomy, which are performed at U.S. MTFs.

less compared with CPG IFI cases (73%, $p < 0.002$). The non-IFI subjects also received a shorter duration of amphotericin B compared with CPG IFI cases ($p = 0.036$; Table 6). Lastly, non-IFI subjects had fewer operating room visits ($p = 0.034$), high-level amputations ($p = 0.026$), and shorter U.S. MTF ICU stays ($p = 0.049$; Table 7).

Discussion

Since late 2009, the incidence of IFI has increased dramatically in service members with traumatic injuries [8]. This change coincided with intensifying combat operations in Afghanistan and increasing rates of dismounted complex blast injuries. Our analysis of the Blast CPG period (February through August 2011) shows an even higher IFI incidence rate, with 17% of OEF trauma casualties admitted to the LRMC ICU and 50% of patients screened according to the LRMC CPG eventually meeting IFI diagnostic criteria.

Our assessment indicates that early screening based on risk factors produces a considerable decrease in time to diagnosis and treatment in patients who develop IFI ultimately. It also demonstrates that heightened awareness of IFI risk factors can help focus resources on the highest-risk patients as evidenced by the fact that more than 90% of IFI cases in the CPG evaluation period met Blast CPG screening criteria at admission to LRMC.

After implementation of the CPG, the number of days following injury to first positive culture/histopathology, IFI diagnosis, and antifungal treatment decreased significantly ($p < 0.001$). In addition, utilization of antifungal therapy at LRMC significantly increased during the CPG period ($p = 0.002$). These results are not surprising because prior to the implementation of the CPG, evaluation for IFI and initiation of early empiric antifungal therapy at LRMC was uncommon.

Unexpectedly, a shift in IFI case classification was also observed with relatively more probable and fewer proven cases. This finding could be because of many factors. One

explanation is that early diagnosis and treatment aborted the progression to angioinvasive (i.e., proven) disease. An alternative theory is that because the IFI diagnosis was made earlier in the patient's course, as occurred more frequently during the CPG period, further diagnostic procedures that could have shown angioinvasion were not pursued. Another possibility is that angioinvasion was not prevented or underdiagnosed. Instead, some of the CPG IFI cases may have had wound fungal colonization in conjunction with progressive necrosis because of severe blast injury and were misclassified as IFI.

Despite timelier diagnosis, earlier initiation of antifungal therapy, and a shift in IFI case classification, no difference in clinical outcomes was observed. The crude mortality rate was lower among the CPG IFI cases compared with the pre-CPG IFI cases. However, the reduction was not statistically significant, possibly because of an inadequate number of IFI cases in the CPG period.

Other study limitations may have also affected our ability to detect differences in clinical outcomes. First, although the LRMC CPG was implemented in February 2011, practice patterns did not change overnight. In reality, practices were in evolution starting in late 2010 through the early winter of 2011. This limitation could affect several results. For example, it could have blunted the observed improvement in time to diagnosis and treatment and resulted in decreasing differences in clinical outcomes which showed trends, but did not reach statistical significance. Overall, we believe this limitation had minimal effect on the results as there were only a few patients included in the pre-CPG IFI group who received dual antifungal therapy at LRMC, indicating that even though some patients were evaluated for IFI at LRMC in the pre-CPG period, few diagnoses were recognized. Furthermore, if there was any effect, it would influence our study results conservatively (favoring not showing an effect when one may exist).

Our method of IFI classification and diagnosis also has limits. Specifically, it is a schema for research purposes.

Although angioinvasion is agreed upon as definitive IFI from a clinical perspective, there is progressively less clinical certainty about probable and possible cases. Our classification relies on determination of recurrent necrosis via retrospective chart and operative note review. We acknowledge that some cases are likely mis-classified as IFI cases (false-positives) and vice versa (false-negatives). The fact that several CPG non-IFI subjects received dual antifungal therapy and some IFI cases in both periods did not receive antifungal medication, demonstrates the inherent difficulty in diagnosing clinically, and as a result, classifying IFI.

Moreover, it is important to recognize that antifungal therapy was started empirically in many cases while the results of confirmatory testing were pending. This was true at LRMC as well as at U.S. MTFs. This resulted in many patients, who did not meet our IFI case definition, receiving antifungal therapy. We do not see this as a limitation of our study and advocate consideration for empiric antifungals at the earliest sign of post-traumatic IFI.

Other factors may have limited our ability to show a difference in outcomes. Although clinical characteristics between the pre-CPG and CPG IFI cases were comparable generally, significant differences and non-significant trends were observed. Specifically, ISS, rate of lower extremity amputation, blood product requirements, and markers of tissue destruction (e.g., AST) were higher in CPG versus pre-CPG IFI cases. The clinical relevance of these differences is uncertain, but we believe they indicate that CPG IFI case patients were more severely injured. Future evaluations of IFI interventions may need to utilize outcome metrics that adjust for injury severity.

Although not the primary intent of this analysis, comparisons of CPG IFI cases with non-IFI subjects suggest that patients with eventual IFI diagnoses are likely to present with acidosis and have higher blood product requirements within the first 24 h. Markers of injury severity also suggest that patients who develop IFI are injured more severely (although not all indices reached statistical significance). However, it is unclear how to implement these factors into a screening program, and we recommend further study.

Of the fungi that grew from cultures at LRMC, *Mucorales* was the most predictive of IFI compared with other molds. Positive histopathology at LRMC was also predictive of IFI development. As a result, we believe that empiric therapy with antifungal agents is warranted in these circumstances, especially when risk factors indicate high possibility for IFI.

In summary, despite earlier diagnosis and treatment, there was no statistical difference identified in our measured clinical outcomes. Crude mortality did decrease (although not statistically significant), even though the CPG IFI cases were assessed to be more severely injured than IFI cases from the pre-CPG time period. Future evaluations should consider additional outcome metrics that may be more sensitive in measuring differences in these severely injured patients.

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