Combat Trauma-Associated Invasive Fungal Wound Infections: Epidemiology and Clinical Classification

A. C. Weintrob[1,2], **A. B. Weisbrod**[1], **J. R. Dunne**[3], **C. J. Rodriguez**[1], **D. Malone**[1], **B. A. Lloyd**[4], **T. E. Warkentien**[1], **J. Wells**[1], **C. K. Murray**[5], **W. Bradley**[2], **F. Shaikh**[2], **J. Shah**[2], **D. Aggarwal**[2], **M. L. Carson**[2], **D. R. Tribble**[2], and **the Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group**

[1]Walter Reed National Military Medical Center, Bethesda, Maryland [2]Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences [3]Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland [4]Landstuhl Regional Medical Center, Landstuhl, Germany [current address is San Antonio Military Medical Center, Fort Sam Houston, Texas] [5]San Antonio Military Medical Center, Fort Sam Houston, Texas

Summary

The emergence of invasive fungal wound infections (IFI) among combat casualties led to development of a combat trauma-specific IFI case definition and classification. Prospective data were collected from 1133 United States military personnel injured in Afghanistan (June 2009 through August 2011). The IFI rates ranged from 0.2% to 11.7% among ward and intensive care unit admissions, respectively (6.8% overall). Seventy-seven IFI cases were classified as proven/probable (n=54) and possible/unclassifiable (n=23) and compared in a case-case analysis. There was no difference in clinical characteristics between the proven/probable and possible/unclassifiable cases. Possible IFI cases had shorter time to diagnosis (p=0.02) and initiation of antifungal therapy (p=0.05) and fewer operative visits (p=0.002) compared to proven/probable cases, but clinical outcomes were similar between the groups. Although the trauma-related IFI classification scheme did not provide prognostic information, it is an effective tool for clinical and epidemiological surveillance and research.

Corresponding author: David R. Tribble, MD, DrPH, Scientific Director, Infectious Disease Clinical Research Program, Preventive Medicine & Biometrics Department, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-5119. Phone: 301-816-8404; Fax: 301-295-1812; David.Tribble@usuhs.edu.
Author for requests for reprints: same as corresponding author

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INTRODUCTION

Invasive fungal infections (IFIs), such as mucormycosis or aspergillosis, generally develop in immunocompromised patients with the potential of resulting in substantial morbidity, but also considerable mortality (rates range as high as 80% and may exceed 90% with dissemination) [1-4]. Less is known about locally invasive IFI in immune competent individuals who sustain traumatic injuries as the available data are limited to a small number of case reports or series [5–13]. Even fewer studies are available related to this emerging trauma-related disease in wounded military personnel [14–17].

In early 2011, an IFI case investigation among combat-injured United States (U.S.) military personnel medically evacuated from Afghanistan (June 2009 through December 2010) to Landstuhl Regional Medical Center (LRMC), which is located in Germany and provides trauma care prior to transfer to the U.S., was conducted and reported an IFI rate of 3.5% in the fourth quarter of 2010 among trauma admissions [17]. Commonly cited IFI case definitions (proven, probable, and possible) from the European Organization for Research and Treatment of Cancer/IFI Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) Consensus Group [18] were reviewed for the case investigation. However, the EORTC/MSG IFI case definitions and diagnostic criteria were targeted for immunocompromised patients without traumatic injuries; thus, the case definitions were revised to reflect traumatic injury as the underlying risk factor for IFI. The result was a combat trauma-specific IFI classification scheme [17]. This scheme was used for the initial case investigation [17]; however, due to the small sample size (37 patients including only 4 probable cases), the previous report focused on a description of the clinical presentation and management of combat-related IFI and only a limited comparison of the various classes of IFI was briefly discussed. In order to better assess the utility of the modified IFI case definition and classification, we reviewed data from an expanded case series of medically evacuated U.S. combat casualties diagnosed with IFIs and conducted a case-case analysis (histopathology confirmed proven/probable versus possible/unclassifiable cases) with the objective of determining if there were any clinically significant differences in presentation or outcomes between the different IFI classes.

METHODS

Study Population

Prospective data were collected from U.S. military personnel with combat-related injuries (Figure 1; 1 June 2009 through 31 August 2011), medically evacuated from Afghanistan (Operation Enduring Freedom) to LRMC in Germany, then transferred to one of three U.S. tertiary care military treatment facilities (MTFs): Walter Reed Army Medical Center in Washington DC, National Naval Medical Center in Bethesda, MD, and San Antonio Military Medical Center in San Antonio, TX, as previously described [19]. Patient demographics, clinical and trauma history, injury patterns, surgical management, and treatment data were obtained through the Department of Defense Trauma Registry (formerly the Joint Theater Trauma Registry) [20]. Clinical outcome variables for evaluation of the classification scheme included total hospitalization, intensive care unit (ICU) length of stay, time to IFI diagnosis, time to administration of first antifungal agent, and mortality rate.

Invasive Fungal Infection Case Identification, Definitions, and Investigation

Cases were identified and classified for analysis after reviewing the Trauma Infectious Disease Outcomes Study database [19] for positive fungal wound cultures, histology, and antifungal therapy during the period of interest. Case data from the infectious disease and trauma surgery services were also evaluated. The identified IFI cases include the 37 cases detailed in the original case series [17] as well as 40 additional cases. This study was approved by the Infectious Disease Institutional (Ethical) Review Board of the Uniformed Services University of the Health Sciences in Bethesda, Maryland.

The diagnostic criteria required a traumatic wound with recurrent tissue necrosis following at least two surgical debridements, with either evidence of tissue invasive mold infection on histopathology or mold growth from tissue. A proven IFI case was confirmed by angioinvasive fungal elements on histopathology, whereas, a probable IFI case had fungal elements identified on histopathology without angioinvasion (all histopathology specimens were reviewed by two surgical pathologists). While fungal cultures were performed for all suspected cases, classification as proven or probable was independent of culture results. A possible IFI described all cases in which wound tissue grew mold; however, histopathology was negative for fungal elements. Unclassifiable IFI cases had wound tissue which grew mold, meeting the definition of a possible case, but histopathology was not sent for evaluation; therefore, they could not be specifically classified.

Statistical Analysis

Proven/probable cases were compared to the possible/unclassifiable cases in a case-case analysis. Descriptive data from the negative histopathology possible cases (no evidence of fungal elements on histology) and absent histopathology unclassifiable cases (tissue specimens not sent for histopathological analysis) are also presented in tabular format for discussion purposes. Chi square and Fisher's exact tests were used to test the difference between the categorical variables by IFI classification. Non-parametric tests (Wilcoxon Rank Sum and t-tests) were used to compare the overall distribution of continuous variables between the groups. Statistical analysis was conducted using SAS® version 9.3 (SAS, Cary, NC) and R® version 2.13.2 (R Project for Statistical Computing, Vienna, Austria). Significance was defined as $p<0.05$.

RESULTS

Demographic Information and Injury Patterns

From 1133 eligible combat casualties (Figure 1), 77 patients met the IFI case definition leading to an IFI incidence rate of 6.8% with 27 categorized as proven (35%), 27 as probable (35%), and 23 as possible/unclassifiable (30%). Among patients admitted to the intensive care unit at LRMC, the IFI rate was 11.7%, while it was 0.2% for patients admitted to the ward at LRMC. Nearly all of the 77 IFI patients (99%) were injured in the Helmand or Kandahar Provinces of southern Afghanistan (Table 1). All cases were men with a median age of 23 for the proven/probable and 24 for the possible/unclassifiable group. Blast injury (>90%) sustained while dismounted (>78%) was the primary mode of injury. Among the 77

IFI cases, 65% sustained isolated lower extremity amputations, 1.3% had isolated upper extremity amputations, and 13% experienced both upper and lower extremity amputations.

Clinical Characteristics

Shock indices recorded on presentation to the first combat support hospital (i.e., heart rate, systolic blood pressure, and blood gas) and clinical characteristics at both LRMC and U.S. MTFs were not statistically different between the proven/probable and possible/unclassifiable groups (Table 2). Large volume blood product transfusions were required for all IFI cases during the first 24 hours post-injury and there were no statistical differences between the groups. Injury severity was high among both the proven/probable and possible/unclassifiable cases. There were no statistical differences among the median admission Sequential Organ Failure Assessment scores determined at LRMC or the U.S. MTFs for the possible/unclassifiable group when compared to proven/probable cases (Table 2). Excluding the unclassifiable cases from the possible group did not reveal any differences in the above clinical characteristics between the proven/probable cases and the possible cases (data not shown). Moreover, the possible/unclassifiable cases' maximal weekly temperature and white blood cell counts were not statistically different from the proven/probable group (Figure 2).

Invasive Fungal Infection Mycology

Per the IFI classification scheme, 100% of possible/unclassifiable cases had cultures with fungal growth in comparison to 76% of proven/probable cases (Table 3). *Mucorales*, *Aspergillus*, and *Fusarium* were the predominant molds isolated from wound cultures. Although the fungal growth distribution profiles were slightly different between the classification groups, the dissimilarities were not statistically significant.

Invasive Fungal Infection Management

Overall, 16% of the 77 IFI cases did not receive any antifungal treatment (Tables 4, 5), of which the possible/unclassifiable group contributed a larger proportion (26%) compared to the proven/probable cases (11%), but the difference was not statistically significant (Table 4). Among the 12 IFI cases that did not receive antifungal treatment (Table 5), 58% experienced lower extremity amputations with a median injury severity score (ISS) of 23, which was comparable to the overall classification groups. Of those who did receive antifungal therapy, there was no difference between proven/probable and possible/unclassifiable cases in time to IFI diagnosis or initiation of therapy. Excluding the unclassifiable cases, however, there was a significantly shorter duration between injury and IFI diagnosis (p=0.02) and between injury and initiation of antifungal therapy (p=0.05) for the possible cases compared to proven/probable ones.

Use of a single antifungal agent (monotherapy) during the treatment regimen was roughly 9% for both groups (Table 4). Approximately 59% of cases in the proven/probable group were prescribed dual antifungal agents compared to 44% among the possible/unclassifiable group (p=0.31). For both of the groups, amphotericin B (liposomal) and voriconazole were the primary antifungal agents utilized; however, the possible/unclassifiable cases had significantly shorter duration of amphotericin B use (p=0.01). Considering the possible cases alone, however, there was no significant difference in duration of amphotericin B use

between the possible and proven/probable cases (p=0.16). Patients in the possible/ unclassifiable group had a median duration of total antifungal therapy of 11 days compared to 22 days for the proven probable cases; however, the difference was not statistically significant (p=0.06; Table 4). Possible/unclassifiable cases also had a statistically reduced number of operating room visits compared to the proven/probable group (p=0.03). When the unclassifiable cases were excluded, the possible cases continued to have fewer operating room visits (p=0.002).

Clinical Outcomes

An evaluation of clinical outcomes observed few differences between the groups (Table 4). Total hospitalization among the proven/probable cases ranged (interquartile) from 36 to 71 days, while it was 29 to 57 days among the patients in the possible/unclassifiable group. Although 22% of proven/probable cases resulted in high-level amputations (i.e., total hip disarticulation or hemipelvectomy) compared to 13% in the possible/unclassifiable group, the difference was not statistically significant (p=0.53). Overall, six patients with IFI died (one possible/unclassifiable [4.3%], five proven/probable [9.3%]; p=0.66) yielding a crude mortality rate of 7.8% for the cohort.

Unclassifiable Cases

All 13 unclassifiable cases occurred prior to December 2010. The unclassifiable cases received significantly fewer blood transfusions (p=0.04) and had lower injury severity scores (p=0.05) compared to the proven/probable cases, but their clinical characteristics were otherwise similar. They received shorter durations of amphotericin B (p=0.03) compared to proven/probable cases; however, their outcomes were not different. Three cases (two with an ISS of 21 and the third, an ISS of 29) required high-level amputations. The two surviving high-level amputation cases received antifungal treatment for 10 and 16 days and visited the operating room 11 and 14 times, respectively. Additionally, the surviving cases respectively reported 9 and 25 days length of stay in the ICU and 33 and 40 days of total hospitalization. The third case was not prescribed any antifungals because the IFI was diagnosed port-mortem.

DISCUSSION

Combat-related IFI has emerged as an important cause of morbidity and mortality among U.S. service members during recent military conflicts [15-17]. This is the largest case series of trauma-related IFI reported to date and determined an overall incidence of 6.8% IFI cases among combat casualties. An earlier case investigation of IFIs in U.S. combat casualties [17] proposed modified IFI case definitions in order to identify groups of patients with similar degrees of certainty of IFI diagnoses to allow for clinical and epidemiological research on trauma-related IFI. The goal of the current report was to apply those definitions to a larger series of patients to investigate the similarities and differences between the groups which may affect management and/or prognosis of future cases.

Overall, this investigation found few differences between the IFI classification groups. The possible cases were similar to the proven/probable ones in their presenting clinical

characteristics and management. Although they had fewer operating room visits, their clinical outcomes did not differ. This suggests that possible cases with negative histopathology either require fewer debridement efforts because of less extensive tissue invasion or that some cases of wound contamination with environmental molds were included as true cases. The requirement of recurrent tissue necrosis after at least two debridements makes the latter less likely; however, there are other reasons for recurrent necrosis, such as poor vascular supply due to the traumatic injury. Another possibility is that the earlier initiation of antifungal therapy (7 versus 10 days) led to equivalent outcomes with the proven/probable group despite fewer operative procedures.

The unclassifiable cases occurred early in the investigation period (prior to December 2010) when sending tissue for histopathology from wounds with recurrent necrosis was not standard practice. Compared to the proven/probable cases, the unclassifiable ones had less severe injuries, required fewer blood transfusions, and received less amphotericin B, but had similar overall outcomes. Despite this, there were several patients in the unclassifiable group who required high level amputations because of IFI. It is likely that the unclassifiable group represents both possible cases along with cases which would have been classified as proven/ probable if tissue had been sent for histopathology.

Due to the broad surveillance of laboratory and pathological data, antifungal use, and case records from the infectious disease and surgical services, the potential for failure in detecting IFI cases in this investigation was low. If IFI cases were not recognized, these would presumably have been less severe; thus, not triggering the collection of diagnostic specimens or initiation of antifungal therapy. It is possible that less severe cases exist, which are cured with surgical therapy alone.

If there were any inclusion of false-positives (or over-diagnosing), it would most likely occur among the cases in the possible and unclassifiable groups where there is a lack of histopathological evidence of fungal invasion. Despite fewer operating room visits (possible cases) and shorter durations of amphotericin B (unclassifiable cases), the clinical outcomes of these cases were similar to the proven/probable cases. As mentioned earlier, this may suggest some misclassification of non-IFI cases as cases; however, given the similarities in clinical characteristics, as well as durations of hospitalization and ICU stay, it is not certain how often this may have occurred. There were also 12 patients who did not receive any antifungal agents. Half of these patients were in the probable group and, therefore, had fungal elements seen on histopathology. It is possible that the fungal elements were contamination and not truly invasive. The outcomes of these patients were not worse than those who received antifungals, therefore, it is uncertain if these patients were misclassified or if they were successfully treated with surgery alone.

Our data indicate that the combat trauma-specific IFI case definition and classification has utility for epidemiological surveillance and clinical research. While our IFI case definition relies on culture and histopathology results, molecular diagnostic assays (i.e., polymerase chain reaction-based) also exist for fungal identification [21, 22]. However, they are not readily available at most clinical laboratories and, therefore, not yet an effective tool for early diagnosis. Classification across the case definition does not provide prognostic value;

although this may in part be limited by number of cases across each definition category. In addition to utility within combat casualty care, the IFI case definition may prove to be applicable to civilian IFI cases resulting from agricultural accidents or natural disasters, such as the tornado in Joplin, Missouri [11], and allow comparison to other outbreaks and future analysis of clinical outcomes relative to therapeutic strategies. Ongoing surveillance coupled with continued clinical investigation is needed to support early interventions to prevent IFI, enhance timely identification and diagnosis, and improve treatment strategies.

Acknowledgments

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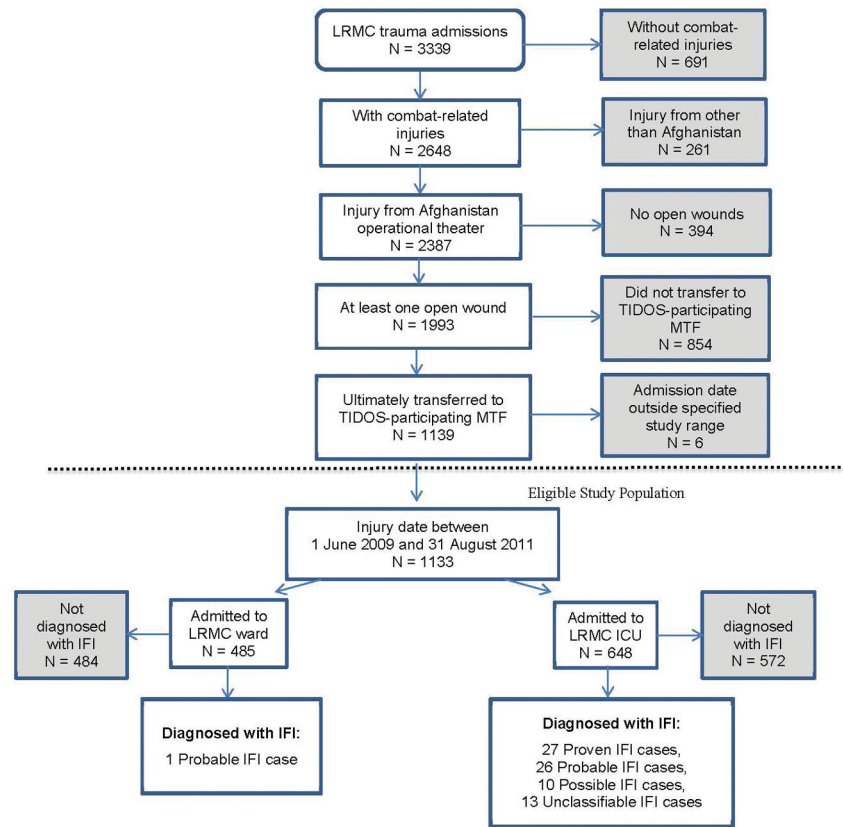
Figure 1.
(Colour online) Flow diagram showing of the eligible study population and resulting invasive fungal infection (IFI) cases from the total number of Landstuhl Regional Medical Center (LRMC) trauma admissions. Factors triggering review of data for IFI diagnosis was wound culture with fungal growth, pathology with fungal elements, or administration of two or more days of antifungal agents. Confirmation of IFI diagnosis required recurrent wound tissue necrosis following two or more surgical debridements in addition to the presence of tissue invasion with fungal hyphae angioinvasion (proven classification), histopathological fungal elements (probable classification), and/or fungal growth on culture (possible classification). Unclassifiable IFIs are cases with fungal culture evidence, but histopathology was not sent for evaluation; therefore, they are not able to be specified to a specific classification on the basis of the available evidence. ICU – intensive care unit; MTF – military treatment facility; TIDOS – Trauma Infectious Disease Outcomes Study
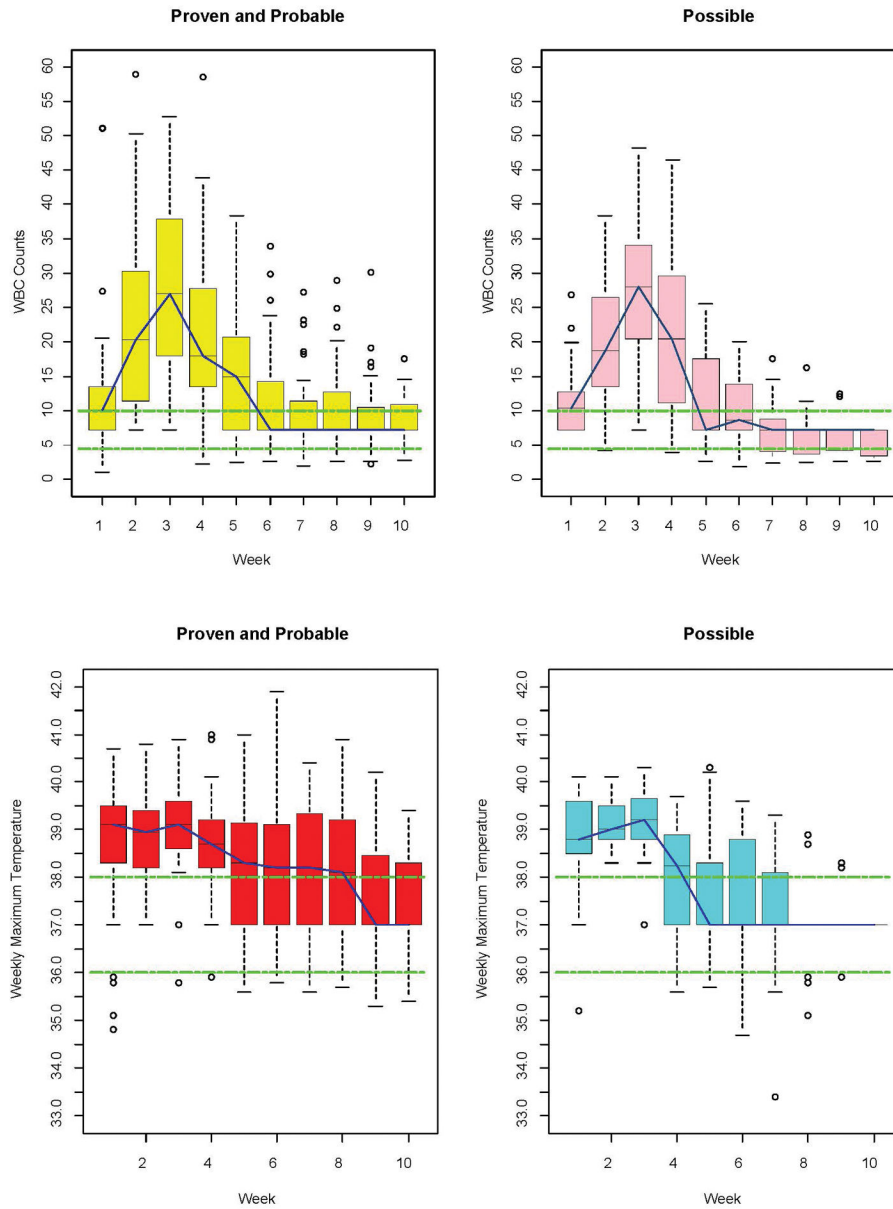
Figure 2.
(Colour online) Boxplots of (a) maximum white blood cell (WBC, 10^9 cells/L) counts and (b) maximum weekly temperatures (°C) among U.S. military personnel injured in combat (2009–2011). Weekly data combined from Landstuhl Regional Medical Center and U.S. military treatment facilities.

Table 1

Demographic Characteristics and Injury Circumstances of Combat-Injured U.S. Service Members (2009–2011) by Invasive Fungal Infection Case Classification

Characteristics	Proven (N=27)	Probable (N=27)	Proven/ Probable (N=54)	Possible/ Unclassifiable (N=23)	Possible: Negative Histopathology[1] (N=10)	Unclassifiable: No Histopathology[2] (N=13)
Demographics						
Age, median (IQR)	22.6 (21.6, 28.7)	22.4 (21.7, 26.6)	22.6 (21.7, 26.9)	24.2 (21.9, 25.4)	24.3 (23.4, 25.1)	22.7 (20.7, 26.5)
Enlisted, No. (%)	27 (100)	24 (88.9)	51 (94.4)	21 (91.3)	9 (90.0)	12 (92.3)
Marine, No. (%)	20 (74.1)	23 (85.2)	43 (79.6)	15 (65.2)	9 (90.0)	6 (46.2)
Army, No. (%)	6 (22.2)	2 (7.4)	8 (14.8)	5 (21.7)	0	5 (38.5)
Injury Circumstances/Severity, No. (%)						
Blast Injury	27 (100)	26 (96.3)	53 (98.1)	21 (91.3)	9 (90.0)	12 (92.3)
Injured on Foot Patrol	26 (96.3)	25 (92.6)	51 (94.4)	18 (78.3)	9 (90.0)	9 (69.2)
Amputations, No. (%)						
Lower Extremity	21 (77.8)	15 (55.6)	36 (66.7)	14 (60.9)	8 (80.0)	6 (46.2)
Upper Extremity	1 (3.7)	0	1 (1.9)	0	0	0
Both Upper/Lower Extremities	2 (7.4)	5 (18.5)	7 (13.0)	3 (13.0)	1 (10)	2 (15.4)

IQR - Interquartile Range

[1] Tissue specimens sent for histopathological analysis and no fungal elements were reported

[2] Tissue specimens were not sent for histopathological analysis

Table 2

Median (IQR) Clinical Characteristics by Invasive Fungal Infection Case Classification among U.S. Military Personnel Injured in Combat (2009–2011)

Clinical Parameters	Proven (N=27)	Probable (N=27)	Proven/ Probable (N=54)	Possible/ Unclassifiable (N=23)	Possible: Negative Histopathology (N=10)[1]	Unclassifiable: No Histopathology (N=13)[2]	p-value
In-Theater Shock Indices (combat support hospital)							
Heart Rate	134 (118, 152)	114 (97, 136)	127 (101, 140)	119 (107, 132)	121 (104, 135)	119 (109, 127)	0.54
Systolic blood pressure	100 (80, 120)	99 (75, 132)	100 (80, 128)	97 (65, 117)	96 (65, 132)	97 (81, 112)	0.63
Blood gas – base deficit	11 (14, 8)	6 (10, 3)	9 (13, 4)	7 (9, 4)	6 (11, 2)	7 (9, 5)	0.40
Blood gas – pH	7.2 (7.0, 7.3)	7.3 (7.2, 7.3)	7.3 (7.1, 7.3)	7.2 (7.1, 7.3)	7.3 (7.2, 7.3)	7.2 (7.1, 7.3)	0.66
Blood Product Requirements In-Theater							
PRBC	31 (26, 42)	30 (15, 44)	31 (18, 42)	22 (13, 33)	33 (22, 37)	18 (13, 24)	0.17
Plasma[3]	31 (26, 40)	24 (15, 42)	30 (18, 40)	22 (13, 36)	30 (20, 36)	18 (12, 31)	0.11
LRMC							
White blood cell total (10^9 cells/L)	7.5 (5.0, 10.0)	8.1 (6.3, 9.6)	7.9 (5.6, 9.8)	7.6 (6.1, 9.3)	7.1 (5.4, 10.9)	7.9 (7.1, 8.4)	0.89
Blood urea nitrogen (mg/dl)	14 (9, 21)	12 (8, 15)	13 (8, 18)	12 (10, 19)	16 (12, 29)	10 (6, 13)	0.93
Aspartate aminotransferase (U/L)	133 (93, 210)	101 (69, 218)	116 (75, 217)	136 (104, 234)	178 (128, 301)	122 (103, 142)	0.28
Alanine aminotransferase (U/L)	43 (36, 67)	38 (28, 63)	40 (30, 65)	50 (36, 75)	51 (42, 82)	46 (30, 63)	0.31
U.S. MTF							
White blood cell total (10^9 cells/L)	11.4 (7.8, 20.1)	10.9 (8.8, 13.0)	11.1 (8.4, 13.5)	11.3 (9.4, 14.9)	12.6 (9.5, 17.9)	11.1 (9.4, 12.1)	0.80
Blood urea nitrogen (mg/dl)	15 (10, 23)	15 (12, 18)	15 (11, 20)	12 (9, 18)	16 (11, 40)	11 (9, 15)	0.27
Aspartate aminotransferase (U/L)	78 (60, 122)	55 (45, 110)	72 (50, 115)	97 (68, 126)	106 (73, 125)	89 (66, 126)	0.46
Alanine aminotransferase (U/L)	45 (32, 64)	38 (26, 53)	43 (28, 63)	44 (36, 54)	41 (35, 52)	44 (40, 59)	0.93
LRMC Injury severity score	21 (18, 28)	22 (18, 25)	21 (17, 26)	21 (15, 26)	24 (21, 28)	18 (14, 21)	0.41
LRMC SOFA Score	8 (5, 11)	8 (3, 9)	8 (4, 9)	7 (4, 11)	9 (5, 12)	6 (4, 8)	0.88
U.S. MTF SOFA Score	6 (4, 8)	5 (0, 7)	5 (2, 8)	2 (1, 8)	4 (1, 12)	2 (1, 7)	0.49

IQR- Interquartile Range; LRMC - Landstuhl Regional Medical Center; MTF - Military Treatment Facility; PRBC – Packed Red Blood Cells plus whole blood; SOFA - Sequential Organ Failure Assessment p-value: represents comparison between proven/probable and possible/unclassifiable groups

[1] Tissue specimens sent for histopathological analysis and no fungal elements were reported

[2] Tissue specimens were not sent for histopathological analysis

[3] Plasma is fresh frozen plasma plus whole blood

Table 3

Wound Culture Results by Cases, No. (%), Following Combat-Related Injuries, 2009–2011

	Proven (N=27)	Probable (N=27)	Proven/Probable (N=54)	Possible/Unclassifiable (N=23)	Possible: Negative Histopathology (N=10)[1]	Unclassifiable: No Histopathology (N=13)[2]	p-value
Cases with positive wound cultures	22 (81.5)	19 (70.4)	41 (75.9)	23 (100)	10 (100)	13 (100)	0.01
Cases with Wound Cultures Growing[3]:							
Mucorales	14 (51.9)	7 (25.9)	21 (38.9)	5 (21.7)	1 (10.0)	4 (30.8)	0.19
Aspergillus	8 (29.6)	5 (18.5)	13 (24.1)	11 (47.8)	5 (50.0)	6 (46.2)	0.07
Fusarium	8 (29.6)	5 (18.5)	13 (24.1)	4 (17.4)	2 (20.0)	2 (15.4)	0.77
Other non-*Mucorales* organisms[4]	11 (40.7)	11 (40.7)	22 (40.7)	9 (39.1)	4 (40.0)	5 (38.5)	1.0
Mucorales and non-*Mucorales* organisms	11 (40.7)	3 (11.1)	14 (25.9)	2 (8.7)	1 (10.0)	1 (7.7)	0.13

p-value: represents comparison between proven/probable and possible/unclassifiable groups

[1] Tissue specimens sent for histopathological analysis and no fungal elements were reported

[2] Tissue specimens were not sent for histopathological analysis

[3] Wound cultures commonly grew more than one organism; therefore, the column data total more than the number of cases with positive wound cultures

[4] Growth of non-*Mucorales* organisms other than *Aspergillus* and *Fusarium*

Table 4

Invasive Fungal Infection (IFI) Management and Clinical Outcomes among Military Personnel Injured in Combat (2009–2011)

	Proven (N=27)	Probable (N=27)	Proven/ Probable (N=54)	Possible/ Unclassifiable (N=23)	Possible: Negative Histopathology (N=10)[1]	Unclassifiable: No Histopathology (N=13)[2]	p- value
IFI Antifungal Treatment Regimen, No. (%)							
No treatment	0	6 (22.2)	6 (11.1)	6 (26.1)	2 (20.0)	4 (30.8)	0.17
Single Agent	1 (3.7)	4 (14.8)	5 (9.3)	2 (8.7)	1 (10.0)	1 (7.7)	1.0
Dual (amphotericin B + triazole)	16 (59.3)	16 (59.3)	32 (59.3)	10 (43.5)	6 (60.0)	4 (30.8)	0.31
Combination (dual + echinocandin)	10 (37.0)	1 (3.7)	11 (20.4)	4 (17.4)	1 (10.0)	3 (23.1)	1.0
Systemic Antifungal Agents, No. (%)							
Amphotericin B (liposomal)	26 (96.3)	19 (70.4)	45 (83.3)	15 (65.2)	7 (70.0)	8 (61.5)	0.13
Voriconazole	25 (92.6)	19 (70.4)	44 (81.5)	15 (65.2)	8 (80.0)	7 (53.8)	0.15
Posaconazole	12 (44.4)	2 (7.4)	14 (25.9)	3 (13.0)	1 (10.0)	2 (15.4)	0.25
Caspofungin	10 (37.0)	1 (3.7)	11 (20.4)	4 (17.4)	1 (10.0)	3 (23.1)	1.0
Micafungin	1 (3.7)	0	1 (1.9)	2 (8.7)	0	2 (15.4)	0.21
Antifungal Duration, median days (IQR)							
Amphotericin B (liposomal)	25 (17, 42)	19 (11, 32)	23 (13, 38)	17 (8, 21)	18 (15, 20)	11 (6, 22)	0.01*
Voriconazole	14 (5, 26)	18 (14, 28)	18 (9, 27)	16 (9, 20)	16 (10, 23)	15 (6, 19)	0.54
Total antifungal treatment	26 (13, 41)	18 (4, 30)	22 (9, 34)	11 (6, 22)	21 (8, 23)	10 (6, 16)	0.06
Hospitalization, median (IQR)							
U.S. MTF Hospitalization (days)	59 (38, 73)	46 (30, 61)	51 (35, 70)	37 (27, 53)	47 (32, 50)	31 (26, 56)	0.08
Total Hospitalization (days)[3]	61 (40, 76)	48 (34, 63)	53 (36, 71)	40 (29, 57)	50 (37, 54)	33 (27, 59)	0.12
U.S. MTF Duration in ICU (days)	14 (5, 23)	6 (3, 17)	9 (4, 19)	8 (4, 11)	9 (4, 11)	7 (4, 10)	0.41
Total Duration in ICU (days)[3]	16 (7, 24)	9 (5, 20)	11 (5, 20)	9 (6, 16)	11 (7, 16)	9 (6, 14)	0.60
Overall OR Visits	17 (12, 24)	14 (9, 18)	16 (11, 21)	13 (8, 16)	8 (7, 12)	14 (8, 15)	0.03*
IFI-Related Timeline, median days (IQR)							
Injury to 1st Positive Mold Culture	9 (5, 12)	4 (3, 7)	6 (3, 10)	7 (3, 12)	4 (2, 7)	8 (5, 12)	0.84
Injury to IFI Diagnosis	9 (5, 11)	5 (3, 9)	7 (3, 9)	6 (3, 12)	3 (2, 6)	8 (5, 12)	0.70
Injury to Antifungal Treatment	9 (8, 12)	10 (6, 14)	10 (7, 13)	8 (7, 11)	7 (6, 7)	11 (8, 12)	0.28
Overall Outcome, No. (%)							

	Proven (N=27)	Probable (N=27)	Proven/ Probable (N=54)	Possible/ Unclassifiable (N=23)	Possible: Negative Histopathology (N=10)[1]	Unclassifiable: No Histopathology (N=13)[2]	p-value
High-level Amputations[4]	7 (25.9)	5 (18.5)	12 (22.2)	3 (13.0)	0	3 (23.1)	0.53
Deaths	4 (14.8)	1 (3.7)	5 (9.3)	1 (4.3)	0	1 (7.7)	0.66

ICU - Intensive Care Unit; IQR - Interquartile Range; MTF - Military Treatment Facility; OR - Operating Room p-value: represents comparison between proven/probable and possible/unclassifiable groups

[1] Tissue specimens sent for histopathological analysis and no fungal elements were reported

[2] Tissue specimens were not sent for histopathological analysis

[3] Total hospitalization/duration in ICU combines data from Landstuhl Regional Medical Center and U.S. MTFs

[4] High-level amputations are defined as total hip disarticulation or hemipelvectomy

*
indicates statistical significance

Table 5

Case Series of Invasive Fungal Infection (IFI) Cases Not Treated with Antifungals among Military Personnel Injured in Combat (2009–2011)

Case	Lower Extremity Amputation	High-level Amputation[I]	Injury Severity Score	PRBC transfusion in-theater (units)	Maximum WBC count at U.S. MTFs (10^9 cells/L)	Total Hospitalization (days)	Total ICU Duration (days)	Number of OR Visits	Death
Probable IFI Cases									
1	No	No	14	5	6.4	16	2	6	No
2	Yes	No	34	16	12.6	71	6	11	No
3	No	No	50	30	9.2	64	20	18	No
4	Yes	No	21	12	9.2	20	4	3	No
5	No	No	10	4	8.8	15	6	5	No
6	Yes	No	24	17	NA	48	3	1	No
Possible IFI Cases									
7	Yes	No	11	NA	5.0	29	2	7	No
8	Yes	No	24	33	7.4	38	13	8	No
Unclassifiable IFI Cases									
9	No	No	14	10	16.2	59	8	15	No
10	Yes	No	26	13	11.1	28	6	19	No
11	No	No	10	2	20.3	32	7	8	No
12	Yes	Yes	29	22	7.4	12	11	7	Yes

ICU – Intensive Care Unit; MTFs - Military Treatment Facilities; OR - Operating Room; PRBC – Packed Red Blood Cells; WBC – White Blood Cell

[I] High-level amputations are defined as total hip disarticulation or hemipelvectomy

NA – not applicable due to missing data