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Prospective Survey of (1→3)-β-D-Glucan and Its Relationship to Invasive Candidiasis in the Surgical Intensive Care Unit Setting▽

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Non-culture-based diagnostic strategies are needed for diagnosing invasive candidiasis (IC). We evaluated serial serum (1→3)-β-D-glucan (BG) levels in patients in the surgical trauma intensive care unit (SICU) patients with clinical evidence of IC. Serum samples from patients admitted to the SICU for a minimum of 3 days were collected twice weekly and analyzed for BG by using a Fungitell kit with a positive cutoff of ≥80 pg/ml. Diagnosis of IC was done using a set of predefined and validated clinical practice-based criteria. A total of 57 patients consented to participate and were enrolled. The median ICU stay was 16 days (range, 3 to 51). A total of 14 of 57 (25%) false positives were observed in the first sample (ICU day 3) and, overall, 73% of the day 3 samples had higher BG levels than subsequent samples. On the date of clinical diagnosis of IC, the sensitivity of a positive BG for identifying invasive candidiasis was 87%, with a 73% specificity. In patients with evidence of IC, the median BG value was significantly higher than those without evidence of IC (171 versus 48 pg/ml, $P = 0.02$), respectively. In the three patients with proven IC, BG was detected 4 to 8 days prior to diagnosis. BG serum detection may be a useful tool to aid in the early diagnosis of IC in SICU patients, particularly after day 3 and in patients with at least two positive samples drawn several days apart. Elevated BG levels within the first 3 days need to be further characterized.

Invasive candidiasis is the most common serious fungal infection identified in non-neutropenic patients being cared for in the intensive care unit (20, 21). Although blood cultures have long been used as the principal diagnostic marker for invasive candidiasis, they have limited sensitivity (6). In addition to catheter-related candidemia, acute disseminated candidiasis frequently involves the bloodstream in its evolution, while chronic disseminated candidiasis and deep organ candidiasis are less frequently associated with candidemia.

As a consequence of the difficulties with diagnosis, significant effort has gone into developing non-culture-based diagnostic techniques for detecting invasive candidiasis. These have included detection of *Candida* enolase and antibodies to enolase (23), *Candida* mannoproteins (2, 25), (1→3)-β-D-glucan (BG) (11, 15, 16), the candidal metabolic produce D-arabinitol (5), and *Candida* DNA by PCR (3, 9, 22, 24). BG is a component of the cell wall of most fungi and is particularly found on the surface of all *Candida* spp. (4, 13, 14, 16, 17).

The purpose of the present study was to determine whether serial measurements of serum BG levels provide laboratory support for the clinical diagnosis of invasive candidiasis in high-risk surgical ICU patients.

(These data were presented in part at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, in 2006 September.)

MATERIALS AND METHODS

All patients in the Memorial Hermann–Texas Medical Center Surgical ICU for at least 48 h with an expected length of stay of at least 3 additional days were eligible for inclusion in the study. The study was conducted from April 2003 to February 2007. The study was approved by the Committee for the Protection of Human Subjects, which is the Institutional Review Board for the University of Texas Health Science Center at Houston. A signed informed consent was obtained from all patients.

Baseline demographics and patient characteristics were collected. When patients showed signs and symptoms of a presumed infection which included, but was not limited to, unexplained fever and leukocytosis, a thorough evaluation for the presumed infectious etiology was carried out based on the standardized protocols in the surgical ICU and included a laboratory evaluation; radiographic evaluation; microbiologic evaluation of blood, urine, respiratory secretions, and/or wounds, where appropriate; and an evaluation of sites with foreign body presence, including catheters, chest tubes, and orthopedic devices.

In addition to the clinical and laboratory evaluation for infection, serum was collected twice weekly during ICU stay and tested for BG using a Fungitell kit. Specimens were frozen at −70°C until testing was performed in triplicate and averaged according to the manufacturer's instructions at the Mycology Research Laboratory at the University of Texas Medical School, Houston, TX. According to the kit package insert, BG levels of ≥80 pg/ml were considered positive. Clinicians did not have access to BG data, since the testing was carried out retrospectively. The clinical course of the patients was monitored until 7 days after ICU discharge for evaluation of evidence of invasive candidiasis based on the criteria (1, 18) established in the ICU in which the present study was being conducted (Table 1). The sensitivity and specificity of the BG assay were determined based on the number of positive samples obtained over the ICU stay relative to the diagnostic criteria outlined in Table 1.

RESULTS

A total of 57 sequential patients met the criteria for enrollment and provided informed consent. The baseline characteristics for these patients are shown in Table 2. Based on the clinical diagnostic criteria for the present study, 15/57 (26%)

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TABLE 1. Unit clinical criteria for establishing a diagnosis of proven, possible, or probable invasive candidiasis

Type of candidiasis	Criteria
I. Proven invasive candidiasis	1. Candidemia with temporally related clinical signs and symptoms compatible with the relevant organism 2. *Candida* spp. from sterile site other than (i) urine or (ii) peritoneal fluid in a setting of gastrointestinal perforation
II. Probable invasive candidiasis	1. Candidemia without the clinical findings of criteria I.1 2. *Candida* spp. from ≥2 nonsterile sites in association with all of the following within the preceding or subsequent 3 days (day of positive cultures ± 3 days). If the nonsterile site cultures are obtained on different days, then the time window for the listed supporting factors extends from 3 days before the first positive culture for *Candida* spp. to 3 days after the last positive culture for *Candida* spp.: (a) Temperature ≥ 38.5°C (101.3°F) on at least on occasion (b) WBC[c] ≥ 12,000/mm^3 on at least one occasion (c) No bacterial pathogens at any possibly infected site, with the exception of coagulase-negative staphylococci in the blood or on a catheter tip 3. *Candida* spp. from urine at ≥100,000 CFU/ml plus other criteria as in II.2 4. *Candida* spp. from a central venous catheter tip at ≥15 CFU in association with all of the following within the preceding or subsequent 3 days (day of positive culture ± 3 days): (a) Temp ≥ 38.5°C (101.3°F) OR WBC ≥ 12,000/mm^3 (b) No bacterial pathogens at any possibly infected site, with the exception of coagulase-negative staphylococci in the blood or on a catheter tip 5. Empirical treatment with systemic antifungal agents initiated due to a persistent temp of ≥38.5°C (101.3°F) OR WBC ≥ 12,000/mm^3 despite ≥3 days of broad-spectrum antibiotics in association with one of the following clinical scenarios: Scenario A—the patient shows both of the following: (a) *Candida* spp. from at least one nonsterile site OR at least one central venous catheter tip at <15 CFU (b) No bacterial pathogens at any possibly infected site within the preceding or subsequent 3 days (day of initiation of therapy ± 3 days) with the exception of coagulase-negative staphylococci in the blood or on a catheter tip Scenario B[a]—the patient has *Candida* spp. at ≥2 of the following: (a) Any nonsterile site (b) A central venous catheter tip at <15 CFU
III. Possible invasive candidiasis	1. *Candida* from a central venous catheter tip at ≥15 CFU not satisfying criteria as in II.4[b] 2. Empirical treatment as in II.5, but without data satisfying either clinical scenario

[a] Note that this scenario does not exclude concomitant bacterial infections at other sites. This is the weakest of the probable forms.
[b] Note that growth of <15 CFU from a catheter tip without findings as in II.5 does not even meet the definition of possible and is not coded as a candidal infection at all.
[c] WBC, white blood cell count.

patients developed invasive candidiasis during their ICU stay. A total of 3/15 (20%) of the invasive fungal infections were proven, 6/15 (40%) were probable, and 6/15 (40%) were possible. The three patients with proven invasive candidiasis all had a positive blood culture for *C. albicans*, and the patients with probable invasive candidiasis had a variety on nonsterile sites, such as urine, peritoneal fluid, and intravenous catheter tips, that were positive for a variety of *Candida* species with concurrent clinical signs and symptoms.

TABLE 2. Patient demographics and risk factors for invasive candidiasis at study entry

Parameter	Finding
Sex (no. male/no. female)	40/17
Median age in yrs (range)	39 (18–76)
Median ICU LOS[a] (range)	16 (3–51)
No. (%) of invasive candidiasis risk factors[b]	
Presence of central venous catheter	54 (95)
Received any antibiotics	41 (72)
Any surgery under general anesthesia	17 (30)
Intra-abdominal surgery	8 (14)
Pancreatitis	2 (4)
Neutropenia (<500 WBC/mm^3)	1 (2)
Steroids (>20 mg prednisone equivalent)	5 (9)
Dialysis	2 (4)
≥4 risk factors	40 (70)
≥5 risk factors	18 (32)

[a] LOS, length of stay in days.
[b] That is, the number detected during the first 48 h of ICU admission.

There were 239 samples obtained from the 57 patients. The median number of samples obtained from each patient was 4 (range, 1 to 11). Diagnostic performance according to the different diagnostic categories based on the total number of positive samples obtained was analyzed (Table 3). On the date of clinical diagnosis of IC, the sensitivity of a positive BG for identifying IC was 87%, with a 73% specificity.

Of the 35 patients without any clinical evidence of invasive candidiasis and with more than 1 sample obtained, 9 had a positive BG at baseline. In 8 patients, the BG level decreased by an average of 237 pg/ml (range, 10 to 983 pg/ml), and 3 returned to normal with the subsequent sample, despite the lack of antifungal therapy. When eliminating the sample obtained in the first 72 h, the sensitivities and specificities of two consecutive positive BG levels for identifying proven, proven-plus-probable, or proven-plus probable plus-possible IC were 100 and 72%, 90 and 80%, and 78 and 86%, respectively.

The median (range) BG level in patients with proven, prob-

TABLE 3. Sensitivity and specificity of positive (1→3)-β-D-glucan assay for invasive candidiasis in surgical ICU patients based on all samples obtained

No. of positive BG samples	Sensitivity and specificity (%)					
	Proven (n = 3)		Proven plus probable (n = 9)		Proven plus probable plus possible (n = 15)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
1	100	50	91	57	93	61
2	100	59	66	73	73	80
≥3	100	67	63	73	71	80

able, or possible IC was 171 (5 to 490), and it was 48 (3 to 388) in patients without any evidence of invasive candidiasis (P = 0.02) (Student t test). In patients with proven and proven-plus-probable IC, the first positive BG was detected an average of 6 and 4 days prior to the clinical diagnosis being made, respectively, based on the date in which the B-glucan demonstrated a positive result and the initial culture results grew yeast.

DISCUSSION

This study presents a systematic survey of BG levels in surgical ICU patients. Our survey found a sensitivity and a specificity of 100 and 50%, respectively, for proven IC. When the number of positive samples required to make a diagnosis was increased to two or three, the specificities increased to 59 and 67%, respectively, without experiencing a decrease in sensitivity. Furthermore, when adding probable and possible cases using clinically relevant definitions (recognizing that the gold standard—blood culture—only has a sensitivity of 50 to 70% in autopsy studies), we documented modest decreases in sensitivity and corresponding slight increases in specificity. Our diagnostic performance findings are similar to those of Pazos et al. (19) in the critical care setting and are in general agreement with large recent surveys of BG in other patient populations and autopsy studies (4, 10, 13, 16, 17, 19). As previously reported, BG levels were able to discern between patients that were ultimately diagnosed with IC, and the BG levels tended to be elevated several days before a positive culture or diagnosis of IC was made (16).

An interesting finding is the frequency of positive BG early in the IC admission and the subsequent decrease in levels. It is unknown whether these represent subclinical infection early in the ICU admission or whether this is related to iatrogenic causes such as translocation/leaching, or introduction, of BG into the bloodstream, such as has been described with surgical gauze, transfusions, hemodialysis, and certain drugs (7, 8, 10, 12). The reason for high BG levels on day 3 of ICU stay and the subsequent decrease remains to be studied in detail.

Aside from a limited sample size, our study is limited by the low frequency of cases of proven IC in this data set, as well as by the use of the local clinical definitions of IC. However, we believe it is important to share this information as it represents performance of BG outside of a clinical trial setting with the typical incidence of IC and practice parameters one would see in a surgical ICU.

The present study confirms the diagnostic value of BG to detect invasive fungal infection (IFI) earlier that waiting for culture results in the surgical critical care setting and describes elevated BG levels in patients without a documented IFI early in the ICU admission. The significance of this finding remains to be explored, until then BG levels should be approached with caution in the first 3 days of ICU admission.

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