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The Presence of (1→3)-β-D-Glucan as Prognostic Marker in Patients After Major Abdominal Surgery

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Background. While the serological detection of (1→3)-β-D-glucan (BDG) can indicate invasive fungal disease (IFD), false positivity occurs. Nevertheless, the presence of BDG can still be recognized by the host's innate immune system and persistent BDG antigenemia, in the absence of IFD, can result in deleterious proinflammatory immune responses.

Methods. During the XXX (INTENSE) study into the preemptive use of micafungin to prevent invasive candidiasis (IC) after abdominal surgery, the serum burden of BDG was determined to aid diagnosis of IC. Data from the INTENSE study were analyzed to determine whether BDG was associated with organ failure and patient mortality, while accounting for the influences of IC and antifungal therapy.

Results. A BDG concentration >100 pg/mL was associated with a significantly increased Sequential Organ Failure Assessment score (≤100 pg/mL: 2 vs >100 pg/mL: 5; $P < .0001$) and increased rates of mortality (≤100 pg/mL: 13.7% vs >100 pg/mL: 39.0%; $P = .0002$). Multiple (≥2) positive results >100 pg/mL or a BDG concentration increasing >100 pg/mL increased mortality (48.1%). The mortality rate in patients with IC and a BDG concentration >100 pg/mL and ≤100 pg/mL was 42.3% and 25.0%, respectively. The mortality rate in patients without IC but a BDG concentration >100 pg/mL was 37.3%. The use of micafungin did not affect the findings.

Conclusions. The presence of persistent or increasing BDG in the patient's circulation is associated with significant morbidity and mortality after abdominal surgery, irrespective of IC. The potential lack of a specific therapeutic focus has consequences when trying to manage these patients, and when designing clinical trials involving patients where host-associated BDG concentrations may be elevated.

Keywords. (1→3)-β-D-glucan; candidiasis; mortality, prognosis; abdominal surgery.

The (1→3)-β-D-glucans (BDGs) are naturally occurring polysaccharides, readily produced by a range of plants, algae, a few bacteria, and fungi, that are immunoreactive when present in the body. BDG, the major component of the fungal cell wall, is recognized by various immune receptors, typically C-type lectin receptors and most significantly dectin-1, present on a range of immune cells, including macrophages, monocytes, dendritic cells, and natural killer cells and confers a proinflammatory immune response, particularly T-helper 1 and T-helper 17 [1]. Exposure of BDG together with other pathogen-associated molecular patterns (PAMPs) with different receptor classes (Toll-like receptor and nucleotide-binding oligomerization domain–like receptors) has been demonstrated to elicit profound proinflammatory synergy [2–4]. BDG can be present in the body due to nonfungal infection sources and a range of clinical and nonclinical sources have been identified, including the gastrointestinal tract, where there may be an association with the translocation of fungal-derived inflammatory mediators, resulting in worsening sepsis and neurocognitive impairment [5–9].

During the XXX (INTENSE) study into the preemptive use of micafungin to prevent invasive candidiasis (IC) postsurgery for intra-abdominal infection, the serum burden of BDG was determined as part of the investigations for diagnosing IC [10]. The study showed that BDG positivity was significantly associated with IC (odds ratio [OR], 3.66 [95% confidence interval {CI}, 1.01–13.29]), but a large cohort of patients (72.2%) with positive BDG results did not have documented IC. The elevated BDG titer was likely associated with the abdominal surgery [11].

Given the potential association between abdominal translocation of BDG and worsening sepsis, it was decided to analyze the data from the INTENSE study to determine whether BDG positivity worsened the severity of disease and patient mortality, and how this related to a diagnosis of documented IC and the use of antifungal therapy (micafungin). This manuscript describes the findings and attempts to highlight parameters that predict mortality in BDG-positive patients after abdominal surgery, even in the absence of documented IC.

METHODS

Patients and Study Design

The INTENSE study (ClinicalTrials.gov identifier NCT01122368) was a double-blind randomized controlled trial to determine the

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efficacy of micafungin as a preemptive antifungal therapy for IC in adults requiring surgery and an intensive care unit admission for intra-abdominal infection [10]. IC was diagnosed by investigators at baseline, during therapy, at the end of therapy, and at the end of study, and by an independent data review board after completion of the trial, with diagnosis based on positive histology on biopsy, or positive culture from blood, tissue, peritoneal drain, biliary catheter, or intra-abdominal abscess. The performance of BDG was evaluated among other circulatory biomarker assays (*Candida* polymerase chain reaction [PCR], *Candida* antibody, and *Candida* [mannan] antigen), but played no role in defining IC.

In the current study, the highest BDG concentration generated by each patient was recorded to determine any correlation between severity of illness, as indicated by the Sequential Organ Failure Assessment (SOFA) score and patient prognosis [12]. In addition, the impact of multiple (≥2) BDG-positive results, BDG concentrations persisting >100 pg/mL or increasing beyond this value, on patient prognosis was evaluated. Correlation between BDG and other biomarker positivity was assessed to identify any enhanced predictor of mortality. Finally, the comparison of mortality rates in patients diagnosed with and without IC and the BDG concentration were evaluated, along with the impact of micafungin therapy.

All data were generated as part of the INTENSE trial, performed following the ethical principles as defined in the Declaration of Helsinki, with written or witnessed informed consent obtained for all patients. The additional statistical analysis performed in this current study was performed anonymously and retrospectively and with the approval of Astellas Pharma EMEA.

Detection of (1→3)-β-D-Glucan and Other *Candida* Biomarker Testing

The presence of BDG was determined using the Associates of Cape Cod Fungitell assay, following the manufacturer's instructions and blinded to patients' diagnosis. Samples were tested in duplicate, using the following thresholds: negative, ≤60 pg/mL; indeterminate, 60–79 pg/mL; positive, ≥80 pg/mL. In addition to BDG, other *Candida*-specific biomarker testing (namely *Candida* PCR, *Candida* antibody, and mannan enzyme-linked immunosorbent assay [ELISA]) was performed in the study as previously described [13].

Statistical Analysis

Primary analysis involved the categorical comparison of BDG positivity (≥80 pg/mL) with mortality (yes/no) and median SOFA score on an individual patient basis. The highest BDG concentration recorded for each patient was used for primary analysis. However, for semiquantitative comparison with mortality and SOFA score, all available BDG concentrations were categorized into <100, 100–199, 200–299 and ≥300 pg/mL, and on the basis of this analysis a BDG concentration threshold of 100 pg/mL was identified as optimal for differentiating higher SOFA scores and increased likelihood of mortality. To enhance specificity for predicting mortality, further analyses using stricter thresholds requiring multiple BDG-positive results per patient and increasing BDG concentrations were applied. In addition, the added influence of positivity in BDG and other biomarker assays (namely *Candida* PCR, BioRad *Candida* antigen and antibody) on mortality was determined. To assess the impact of confounding factors that could influence the BDG concentration, mortality rates above and below the optimal BDG threshold of 100 pg/mL were calculated after adjustment for the presence of documented IC and the use of micafungin, both individually and combined.

When comparing pairs of median values, the Mann-Whitney *U* test was performed and Kruskal-Wallis 1-way analysis of variance with Dunn multiple comparisons test was used to compare multiple medians. For proportionate values, 95% CIs were calculated. When comparing mortality rates, 2 × 2 contingency tables were constructed to determine the sensitivity and specificity for predicting mortality and the OR and relative risk of mortality, using the Fisher exact test to determine the significance of any difference. GraphPad Prism version 5.02 software was used for all analyses, with significance determined by a *P* value ≤.05.

RESULTS

Study Population

Of the 252 patients randomized into the trial, 179 patients had samples tested for the presence of BDG; a total of 573 of 594 samples were successfully screened for BDG and 38 patients were diagnosed with IC, of whom 14 died. Patient demographics and BDG data for the overall population, categorized according to IC, are shown in Table 1. The median patient age was 63 years and the male-to-female ratio was 1.6:1. The median SOFA score and mortality rate across the entire population were 2 and 24.6%, respectively. The SOFA score and BDG concentration and positivity were significantly greater in patients diagnosed with IC, with a trend toward increased mortality (Table 1).

(1→3)-β-D-Glucan Concentration and SOFA Score

The median SOFA score increased with BDG concentration (Figure 1). In a patient with a BDG concentration consistently ≤100 pg/mL, the median SOFA score was 2, increasing to 5 for BDG concentrations between 100 and 299 pg/mL, and 7 when the BDG concentration was >300 pg/mL. The median SOFA score in patients with a BDG concentration consistently ≤100 pg/mL was significantly less than in those with a BDG concentration >100 pg/mL on at least 1 occasion (median, 2 vs 5, respectively; $P < .0001$). There was no significant difference in SOFA scores associated with the different categories of BDG concentrations that were >100 pg/mL ($P = .5337$).

Table 1. Basic Demographics and (1→3)-β-D-Glucan According to the Diagnosis of Invasive Candidiasis

Parameter	Population			*P* Value (IC vs No IC)
	Overall (N = 179)	Documented IC (n = 38)	No IC (n = 141)	
Age, y, median	63	63	64	.4881
Male-to-female ratio	1.8:1	1.6:1	1.9:1	.8470
SOFA score, median	2	5	2	**<.0001**
Mortality rate, No. (%)	44/179 (24.6)	14/38 (36.8)	30/141 (21.3)	.0573
Samples per patient tested for BDG, median	3	3	3	.8763
BDG concentration, pg/mL, median	74.0	117.0	64.5	**<.0001**
BDG sample positivity rate, No. (%)	190/594 (32.0)	68/134 (50.7)	122/460 (26.5)	**<.0001**
BDG patient positivity rate, No. (%)	90/179 (50.3)	28/38 (73.7)	62/141 (44.0)	**.0017**
Type of IC	*Candida* peritonitis (n = 12) candidemia (n = 8), *Candida* intra-abdominal abscess (n = 8), *Candida* deep wound infection (n = 4), histological proven *Candida* infection (n = 2), *Candida* venous catheter infection (n = 2), not specified (n = 2)		NA	NA

Significant *P* values are highlighted in bold.

Abbreviations: BDG, (1→3)-β-D-glucan; IC, invasive candidiasis; NA, not applicable; SOFA, Sequential Organ Failure Assessment.

(1→3)-β-D-Glucan Concentration and Mortality

The mortality rate associated with BDG concentration is shown in Table 2. When the BDG concentration in a patient was consistently ≤100 pg/mL, the mortality rate was 13.7%, compared to 39.0% when the BDG concentration in a patient was >100 pg/mL on at least 1 occasion, resulting in a significant increase in mortality in patients with a BDG concentration >100 pg/mL on at least 1 occasion (difference, 25.3% [95% CI, 12.3%–37.6%]; $P < .001$, Fisher exact test). The OR associated with mortality in patients with a BDG concentration >100 pg/mL on at least 1 occasion compared to those with a BDG concentration consistently ≤100 pg/mL was 4.0 and relative risk was 2.8 (Table 3). The sensitivity and specificity for predicting mortality were 68.2% (30/44) and 65.2% (88/135), respectively.

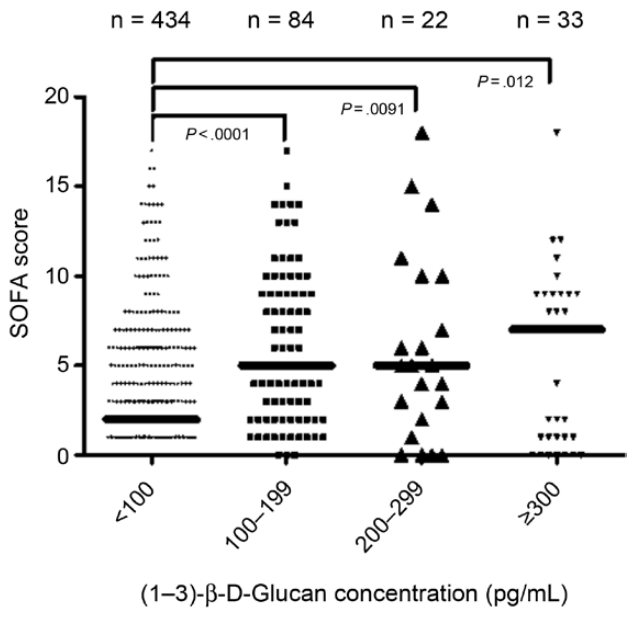

Figure 1. Sequential Organ Failure Assessment (SOFA) scores according to (1→3)-β-D-glucan concentration. The bold horizontal lines represents the median value for each category and the *P* values represent the significance of the difference between the medians as determined by the Mann-Whitney *U* test.

In an attempt to improve specificity for predicting mortality, further analysis comparing mortality in 60 patients with multiple (≥2) BDG-positive results (concentration ≥80 pg/mL) to patients with either a single sample BDG positive or consistently BDG negative (n = 119) was performed. The mortality rate for patients with multiple (≥2) BDG-positive results was 33.3%, but specificity was not significantly improved (95/135) (Tables 2 and 3). This was taken further by comparing mortality in 39 patients with multiple (≥2) BDG-positive results at a concentration >100 pg/mL to 140 patients with either a single BDG-positive sample at a concentration >100 pg/mL or with BDG concentrations consistently ≤100 pg/mL. The mortality rate was doubled in patients with multiple (≥2) BDG results >100 pg/mL, significantly improving the specificity (83.0%) for predicting mortality compared to a threshold incorporating a single BDG result >100 pg/mL (difference, 21.0% [95% CI, 5.2%–37.6%]; $P = .011$) (Tables 2 and 3). In 52 patients where BDG concentration remained >100 pg/mL or increased to a concentration >100 pg/mL, mortality was 48.1% and the specificity when predicting mortality was 80.0%; the odds and risk of death were significantly higher than 127 patients not meeting this parameter (Table 3).

Impact of Other Positive Biomarker Assays

A BDG concentration of >100 pg/mL was a good indication that other biomarkers would also be positive, irrespective of patient prognosis. Sixty-five of 77 (84%) patients with at least a single sample with a BDG concentration of >100 pg/mL were positive

Table 2. Mortality Rates According to (1→3)-β-D-Glucan Concentration and Confounding Factors

Factor	Population (No.)	Mortality Rate, % (95% CI)	Survived/Died (Ratio)
Total population	All patients (179)	24.6 (18.9–31.4)	135/44 (3.1:1)
Varying BDG thresholds	BDG <100 pg/mL (102)	13.7 (8.4–21.7)	88/14 (6.3:1)
	BDG 100–199 pg/mL (42)	42.9 (29.1–57.8)	24/18 (1.3:1)
	BDG 200–299 pg/mL (11)	27.3 (9.8–56.6)	8/3 (2.7:1)
	BDG ≥300 pg/mL (24)	37.5 (21.2–57.3)	15/9 (1.7:1)
	BDG >100 pg/mL (77)	39.0 (28.8–50.1)	47/30 (1.6:1)
	Multiple (≥2) BDG-positive samples (≥80 pg/mL) (60)	33.3 (22.7–45.9)	40/20 (2.0:1)
	Single BDG-positive sample (≥80 pg/mL) or BDG consistently <80 pg/mL (119)	20.2 (13.9–28.3)	95/24 (3.9:1)
	Multiple (≥2) BDG-positive samples (>100 pg/mL) (39)	41.0 (27.1–56.6)	23/16 (1.4:1)
	Single BDG-positive sample (>100 pg/mL) or BDG consistently ≤100 pg/mL (140)	20.0 (14.2–27.4)	112/28 (4.0:1)
	Multiple (≥2) BDG-positive samples (≥100 pg/mL) or BDG concentration increasing to >100 pg/mL (52)	48.1 (35.1–61.3)	27/25 (1.1:1)
	BDG concentration decreasing to ≤100 pg/mL or BDG consistently ≤100 pg/mL (127)	15.0 (9.8–22.2)	108/19 (5.7:1)
BDG plus other biomarkers	BDG >100 pg/mL plus 1 other *Candida* biomarker assay positive (65)	36.9 (26.2–49.1)	41/24 (1.7:1)
	BDG >100 pg/mL with no other *Candida* biomarker assay positive and BDG ≤100 pg/mL (114)	17.5 (11.7–25.6)	94/20 (4.7:1)
	BDG >100 pg/mL plus all other *Candida* biomarker assays positive (12)	50 (25.4–74.6)	6/6 (1:1)
	BDG >100 pg/mL with <3 other *Candida* biomarker assays positive and BDG ≤100 pg/mL (167)	22.8 (17.1–29.7)	129/38 (3.4:1)
BDG with IC	BDG >100 pg/mL with documented IC (26)	42.3 (25.5–61.1)	15/11 (1.4:1)
	BDG ≤100 pg/mL with documented IC (12)	25.0 (8.9–53.2)	9/3 (3.0:1)
	BDG >100 pg/mL without documented IC (51)	37.3 (25.3–50.9)	32/19 (1.7:1)
	BDG ≤100 pg/mL without documented IC (90)	12.2 (7.0–20.6)	79/11 (7.2:1)
BDG with AFT	Micafungin (93)	22.6 (15.3–32.1)	72/21 (3.4:1)
	Placebo (86)	25.6 (17.5–35.7)	64/22 (2.9:1)
	BDG >100 pg/mL with micafungin (37)	35.1 (21.8–51.2)	24/13 (1.8:1)
	BDG ≤100 pg/mL with micafungin (56)	14.3 (7.4–25.7)	48/8 (6:1)
	BDG >100 pg/mL with placebo (40)	40.0 (26.4–55.4)	24/16 (1.5:1)
	BDG ≤100 pg/mL with placebo (46)	13.0 (6.1–25.7)	40/6 (6.7:1)
	BDG >100 pg/mL with documented IC, receiving micafungin (14)	35.7 (16.3–61.2)	9/5 (1.8:1)
	BDG >100 pg/mL with documented IC, receiving placebo (12)	50.0 (25.4–74.6)	6/6 (1:1)
	BDG ≤100 pg/mL with documented IC, receiving micafungin (8)	37.5 (13.7–69.4)	5/3 (1.7:1)
	BDG ≤100 pg/mL with documented IC, receiving placebo (4)	0.0 (.0–4.9)	4/0 (NA)
	BDG >100 pg/mL without documented IC, receiving micafungin (23)	34.7 (18.8–55.1)	15/8 (1.9:1)
	BDG >100 pg/mL without documented IC, receiving placebo (28)	39.2 (23.6–57.6)	17/11 (1.5:1)
	BDG ≤100 pg/mL without documented IC, receiving micafungin (47)	10.6 (4.6–22.6)	42/5 (8.4:1)
	BDG ≤100 pg/mL without documented IC, receiving placebo (43)	14.0 (6.6–27.3)	37/6 (6.2:1)

Abbreviations: AFT, antifungal therapy; BDG, (1→3)-β-D-glucan; CI, confidence interval; IC, invasive candidiasis; NA, not applicable.

Table 3. Predicting Mortality in Patients According to Single, Multiple, and Increasing (1→3)-β-D-Glucan Concentrations, and When Combined With Positivity in Other *Candida* Biomarker Assays

Population (No.)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Odds Ratio (95% CI)	Relative Risk (95% CI)	*P* Value
BDG ≤100 pg/mL (102) vs BDG >100 pg/mL (77)	68.2 (54.3–79.9)	65.2 (60.7–69.0)	4.01 (1.94–8.30)	2.84 (1.58–5.28)	**<.001**
Multiple (≥2) BDG-positive samples (≥80 pg/mL) (60) vs single BDG-positive sample (≥80 pg/mL) or BDG consistently <80 pg/mL (119)	45.5 (32.3–58.8)	70.4 (66.1–74.7)	1.98 (.98–3.98)	1.65 (.95–2.83)	.066
Multiple (≥2) BDG-positive samples (>100 pg/mL) (39) vs single BDG-positive sample (>100 pg/mL) or BDG consistently ≤100 pg/mL (140)	36.4 (24.4–48.8)	83.0 (79.1–87.0)	2.78 (1.3–5.95)	2.05 (1.16–3.42)	**.011**
Multiple (≥2) BDG-positive samples (≥100 pg/mL) and BDG concentration increasing to >100 pg/mL (52) vs BDG concentration decreasing to ≤100 pg/mL or BDG consistently ≤100 pg/mL (127)	56.8 (43.5–69.0)	80.0 (75.6–84.0)	5.26 (2.54–10.93)	3.21 (1.88–5.45)	**<.001**
BDG >100 pg/mL plus 1 other *Candida* biomarker assay positive (65) vs BDG >100 pg/mL with no other *Candida* biomarker assay positive and BDG ≤100 pg/mL (114)	54.5 (40.9–67.5)	69.9 (65.2–73.9)	2.75 (1.37–5.23)	2.11 (1.21–3.64)	**.006**
BDG >100 pg/mL plus all other *Candida* biomarker assay positive (12) vs BDG >100 pg/mL with discordant positivity in other *Candida* biomarker assays and BDG ≤100 pg/mL (167)	13.6 (6.2–21.0)	95.6 (93.1–98.0)	3.40 (1.04–11.14)	2.20 (.93–3.70)	.074

Significant *P* values are highlighted in bold.

Abbreviations: BDG, (1→3)-β-D-glucan; CI, confidence interval.

by 1 of the other tests (OR, 2.3 [95% CI, 1.1–4.8]; *P* = .0337). Sixteen percent (12/77) of patients with at least a single sample with a BDG concentration of >100 pg/mL were positive by all other tests (OR, 3.0 [95% CI, 1.1–8.3]; *P* = .044). Combining a BDG concentration of >100 pg/mL with positivity in any other individual biomarker assay did not improve the specificity for predicting mortality (Table 3). In patients with a BDG concentration of >100 pg/mL plus all other biomarkers being positive on at least 1 occasion, the mortality rate was 50% and specificity for predicting mortality was 95.6%.

Influence of Invasive Candidiasis

The overall mortality rate in patients with documented IC was 36.8% compared with 21.3% in patients without documented IC, and the median BDG concentration was significantly greater in documented cases (Table 1). A BDG concentration of >100 pg/mL was noted in 26 cases of IC, of which the mortality rate was 42.3% compared with 25% in the 12 cases of IC where the BDG concentration was ≤100 pg/mL (Tables 2 and 4). Mortality was similar in patients with a BDG concentration of >100 pg/mL on at least 1 occasion, whether or not the patient had IC documented. In patients without documented IC and a BDG concentration of >100 pg/mL on at least one occasion, the mortality rate was comparable to that in cases of documented IC, and was significantly greater than the mortality rate in patients without documented IC and a BDG concentration consistently ≤100 pg/mL (difference, 25.1%; *P* = .001) (Table 4). The mortality rate in patients with documented IC and a BDG concentration consistently ≤100 pg/mL was not significantly greater than patients without documented IC and a BDG concentration consistently ≤100 pg/mL.

Influence of Antifungal Therapy

The impact of micafungin on BDG concentration was minimal, with no significant difference in the rate of patients with a BDG concentration of >100 pg/mL on at least 1 occasion whether they received micafungin (37/93; 39.8% [95% CI, 30.4%–50.0%]) or a placebo (40/86; 46.5% [95% CI, 36.4%–57.0%]) (Table 2). Furthermore, mortality rates in patients with a BDG concentration of >100 pg/mL on at least 1 occasion were similar whether they received micafungin (35.1% [95% CI, 21.8%–51.2%]) or placebo (40.0% [95% CI, 26.4%–55.4%]), but significantly greater than in patients with a BDG concentration ≤100 pg/mL, irrespective of antifungal treatment (Table 4). The administration of micafungin or a placebo did not alter mortality in patients with a BDG concentration of >100 pg/mL, irrespective of a diagnosis of IC, with mortality rates comparable in patients with or without IC (Table 2). Mortality rates were comparable in patients without IC and BDG ≤100 pg/mL, irrespective of antifungal therapy, and were lower than patients diagnosed with IC, or with a BDG concentration >100 pg/mL in the absence of an IC diagnosis (Table 4).

Table 4. Influence of Confounding Factors (Documented Invasive Candidiasis and Micafungin Therapy) on the Mortality of Patients With (1→3)-β-D-Glucan Concentrations >100 and ≤100 pg/mL

Population (No.)	Mortality Rate, %	Difference in Mortality, % (95% CI)	Odds Ratio (95% CI)	Relative Risk (95% CI)	*P* Value
Documented IC with BDG >100 pg/mL (26) vs documented IC with BDG ≤100 pg/mL (12)	42.3 vs 25.0	17.3 (−15.5 to 42.0)	2.20 (.48–10.07)	1.69 (.58–7.11)	.472
Documented IC with BDG >100 pg/mL (26) vs BDG >100 pg/mL without documented IC (51)	42.3 vs 37.3	5.0 (−16.6 to 27.3)	1.24 (.47–3.24)	1.14 (.57–2.06)	.805
BDG >100 pg/mL without documented IC (51) vs documented IC with BDG ≤100 pg/mL (12)	37.3 vs 25.0	12.3 (−18.4 to 33.4)	1.78 (.43–7.40)	1.49 (.56–6.01)	.516
BDG >100 pg/mL without documented IC (51) vs documented IC (38)	37.3 vs 36.8	0.5 (−19.5 to 19.6)	0.98 (.41–2.35)	0.99 (.53–1.79)	1.000
Documented IC with BDG >100 pg/mL (26) vs BDG ≤100 pg/mL without documented IC (90)	42.3 vs 12.2	30.1 (11.4–49.6)	5.27 (1.93–14.34)	3.4 (1.54–7.43)	**.001**
Documented IC with BDG ≤100 pg/mL (12) vs BDG ≤100 pg/mL without documented IC (90)	25.0 vs 12.2	12.8 (−5.4 to 41.5)	2.39 (.56–10.22)	2.05 (.47–6.20)	.364
BDG >100 pg/mL without documented IC (51) vs BDG ≤100 pg/mL without documented IC (90)	37.3 vs 12.2	25.1 (10.5–39.7)	4.26 (1.83–9.96)	3.05 (1.51–6.34)	**.001**
BDG >100 pg/mL receiving micafungin (37) vs BDG >100 pg/mL receiving placebo (40)	35.1 vs 40.0	4.9 (-16.3 to 25.2)	0.81 (.32–2.05)	0.88 (.46–1.66)	.814
BDG >100 pg/mL receiving micafungin (37) vs BDG ≤100 pg/mL receiving micafungin (56)	35.1 vs 14.3	20.8 (3.3–38.4)	3.25 (1.19–8.90)	2.46 (1.05–5.97)	**.024**
BDG >100 pg/mL receiving micafungin (37) vs BDG ≤100 pg/mL receiving placebo (46)	35.1 vs 13.0	22.1 (3.8–39.6)	3.61 (1.21–10.76)	2.69 (1.06–7.42)	**.021**
BDG >100 pg/mL receiving placebo (40) vs BDG ≤100 pg/mL, receiving micafungin (56)	40.0 vs 14.3	25.7 (7.9–42.6)	4.00 (1.50–10.66)	2.80 (1.26–657)	**.008**
BDG >100 pg/mL receiving placebo (40) vs BDG ≤100 pg/mL, receiving placebo (46)	40.0 vs 13.0	27.0 (8.4–43.9)	4.44 (1.53–12.91)	3.07 (1.27–8.20)	**.006**
BDG ≤100 pg/mL receiving micafungin (56) vs BDG ≤100 pg/mL, receiving placebo (46)	14.3 vs 13.0	1.3 (−13.1 to 14.6)	1.11 (.36–3.47)	1.10 (.37–3.39)	1.000
BDG >100 pg/mL without IC, receiving micafungin (23) vs BDG ≤100 pg/mL without IC, receiving micafungin (47)	34.7 vs 10.6	24.1 (4.2–45.3)	4.48 (1.27–15.85)	3.27 (1.07–10.56)	**.022**
BDG >100 pg/mL without IC receiving a placebo (28) vs BDG ≤100 pg/mL without IC, receiving placebo (43)	39.2 vs 14.0	25.3 (4.7–45.1)	3.99 (1.27–12.58)	2.56 (1.09–7.80)	**.022**

Significant *P* values are highlighted in bold.

Abbreviations: BDG, (1→3)-β-D-glucan; CI, confidence interval; IC, invasive candidiasis.

DISCUSSION

This study shows that elevated BDG concentrations >100 pg/mL are associated with increased organ failure and mortality in patients after abdominal surgery. A summary of the SOFA scores and mortality rates associated with varying BDG concentrations are shown in Figure 2. While BDG is commonly used as a diagnostic test for invasive fungal disease, including IC, there is more evidence emerging linking it to deleterious proinflammatory responses within the host [5–9]. Obviously, the cohort in the INTENSE study is at high risk of developing IC, so data were adjusted to assess for the mortality in patients with and without documented IC. Subsequently, a BDG concentration >100 pg/mL was associated with a significant increase in mortality, even in the absence of documented IC (37.3%), and the mortality rate in patients with or without IC and a BDG concentration >100 pg/mL was numerically higher than patients with IC where the BDG concentration was ≤100 pg/mL, but numbers were limited and a large-scale clinical trial is required (Tables 2 and 4). The elevated BDG concentration could be associated with undiagnosed IC (or other invasive fungal disease [IFD]), a diagnostic limitation that is well documented or alternatively could be associated with dietary regimen or clinical sources (eg, administration antibiotics or surgical gauze) of BDG [14]. However, the mortality rate in patients without IC, but with a BDG concentration >100 pg/mL, was similar whether they received micafungin (34.7%) or not (39.2%) and was higher than patients with a BDG concentration ≤100 pg/mL irrespective of treatment, indicating that the elevated BDG concentration did not necessarily originate from IC (Tables 2 and 4). No significant alternative IFDs were diagnosed in these patients, but other than BDG and blood culture, diagnostics were focused on the detection of IC and other IFDs may have been overlooked, albeit unlikely in this specific cohort. In the 12 cases of IC where the BDG concentration was ≤100 pg/mL, the mortality rate was consistent with previous studies [15, 16]. The mortality rates increased if BDG concentrations persisted or increased >100 pg/mL, with the risk of dying increased ≥3-fold, and if a patient was positive by all 4 biomarker tests, mortality was 50% (Tables 2 and 3), with the latter, given the specificity of the other tests, likely indicating severe IC. The similar mortality in patients with and without IC and a BDG concentration of >100 pg/mL on at least 1 occasion indicates that the presence of BDG may be an important driver of mortality, irrespective of fungal infection. Independent studies of the association of BDG titers with the course of disease severity and mortality, with or without suspected/proven IFD, observed similar results [15–17].

The presence of BDG resulting in systemic inflammatory response syndrome without documented fungal disease, leading to increased likelihood of death, represents a very difficult clinical management scenario. If fungal sepsis is documented, antifungals can be administered in an attempt to reduce the fungal burden and enhance recovery. However, if the source of BDG is not fungal disease per se, then the success of this strategy will be limited and

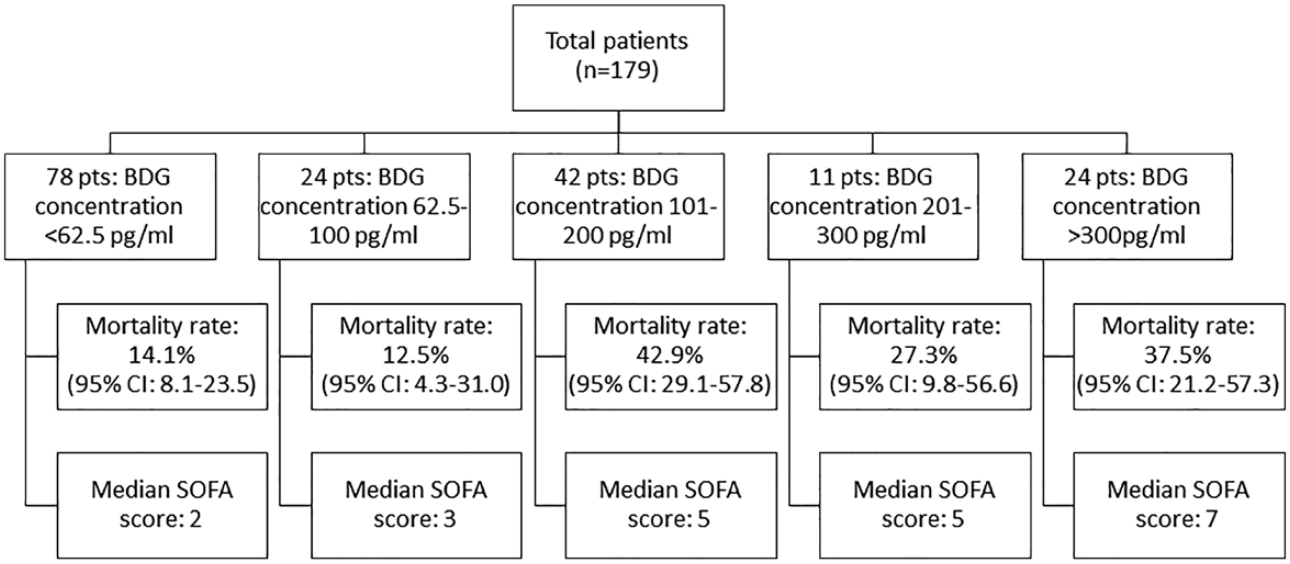

Figure 2. The distribution of (1→3)-β-D-glucan (BDG) concentrations among the 179 patients screened by the Associates of Cape Cod Fungitell assay, along with association with mortality and Sequential Organ Failure Assessment (SOFA) score as part of the Invasive Candidiasis – Pre-Emptive Treatment in High Risk Surgical Subjects INTENSE study. Abbreviation: CI, confidence interval.

attempts to find and restrict the source of BDG are required. Intestinal mucosal barrier injury is 1 potential source of elevated serum BDG titers, due to translocation of luminal contents to the circulation. Recently, research studies have utilized biomarkers of mucosal barrier injury, such as intestinal fatty acid binding protein, occludin, zona occludens-1 protein, and L-citrulline, along with elevated circulating microbial response markers, to identify a context suggestive of microbial PAMP translocation due to a damaged intestinal barrier [18–21]. In the interim period it may be possible to diminish an individual's inflammatory immune response (eg, through the administration of corticosteroids), but in doing so the risk of opportunistic infection, including fungal disease, will be increased. This association between increased mortality and BDG that is not necessarily associated with fungal disease also has implications when designing clinical trials. Studies involving patients with potentially increased concentrations of "host-associated" BDG (eg, abdominal surgery) may complicate the interpretation of mortality endpoints and could even undermine other endpoints (eg, drug efficacy) if not accounted for.

For pharmaceutical trials, the specificity of documented cases is key when determining therapeutic efficacy. In the INTENSE study, cases of IC were documented by the individual centers and by the independent data review board, cases were defined using conventional culture-based diagnosis (ie, blood culture or culture from a sterile site). It is likely that some cases of IC would have gone undiagnosed, due to the limitations of conventional diagnostic approaches that lack sensitivity. Some of the "false positive" BDG results, and indeed other biomarker results, could reflect the limitations of conventional diagnostics [14]. A further limitation is that as part of the trial design, patients with documented IC would have received appropriate antifungal therapy, irrespective of whether they initially received micafungin or a placebo. Unfortunately, the data were not available to assess the impact of this on mortality.

To conclude, BDG positivity after abdominal surgery occurs in a significant proportion of patients (50.6%), likely a result of the anatomically disruptive clinical intervention, and while not necessarily linked to IC, limiting the primary diagnostic application of the test, it has prognostic value. The results of this study indicate that the presence of BDG at a concentration >100 pg/mL is an important indicator of poor prognosis after abdominal surgery, even in the absence of documented IC. If BDG concentrations increase or persist >100 pg/mL, then probability of death is significantly elevated (approximately 50%). It is unclear whether the presence of other positive biomarkers (*Candida* PCR, antigen/antibody) also increases relative risk, but specificity is improved and likely indicates the involvement of IC. Persistent or increasing BDG antigenemia, even in the absence of additional evidence of fungal infection, is cause for concern in both the clinic and clinical trial settings.

Notes

Disclaimer. Fungitell is cleared for use as an adjunct test in the diagnosis of invasive fungal disease. The data presented herein are for research purposes only.

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treasurer, and steering committee member of the Fungal PCR Initiative. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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