

# Experience with $\beta$ -D-Glucan Assay in the Management of Critically ill Patients with High Risk of Invasive Candidiasis: An Observational Study

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## Abstract

**Background:** The (1,3)- $\beta$ -D-glucan assay (BDG) is recommended for the early diagnosis of invasive candidiasis (IC). **Methods:** Records of 154 critically ill adults with suspected IC, on whom BDG was done, were analyzed. Patients were divided into three groups: Group A (confirmed IC), Group B (alternative diagnosis or cause of severe sepsis), and Group C (high candidal score and positive BDG [ $>80$  pg/mL] but without a confirmed diagnosis of IC). **Results:** Mean BDG levels were significantly higher in Group A ( $n = 32$ ) as compared to Group B ( $n = 60$ ) and Group C ( $n = 62$ ) ( $448.75 \pm 88.30$  vs.  $144.46 \pm 82.49$  vs.  $292.90 \pm 137.0$  pg/mL;  $P < 0.001$ ). Discontinuation of empiric antifungal therapy based on a value  $<80$  resulted in cost savings of 14,000 INR per day per patient. **Conclusion:** A BDG value of  $<80$  pg/ml facilitates early discontinuation of empirical antifungal therapy, with considerable cost savings.

**Keywords:** Antifungal stewardship, invasive candidiasis,  $\beta$ -D-glucan

## INTRODUCTION

*Candida* bloodstream infection (candidemia) or invasive candidiasis (IC) in Intensive Care Units (ICU) is associated with great morbidity and mortality.<sup>[1]</sup> The situation is no different in India: in a multicentric study, the 30-day crude and attributable mortality rates were 44.7 and 19.6%, respectively.<sup>[2]</sup> These high mortality rates highlight the fact that diagnosis of IC is often missed which leads to mortality.

Although blood culture is the gold standard for diagnosis of candidemia, it suffers from low sensitivity (50%) and longer turnaround time (2–4 days).<sup>[3]</sup> As blood cultures are insensitive and time-consuming, diagnosis of IC is often based on clinical suspicion, candidal score, and *Candida* colonization index, none of which are sensitive or specific; it has been estimated that up to 70% of patients who receive empiric antifungal agents do not have proven candidal infection.<sup>[4]</sup>

(1,3)- $\beta$ -D-glucan (BDG) is a cell wall polysaccharide found in almost all pathogenic fungi with exception of zygomycetes and *Cryptococcus*.<sup>[5]</sup> BDG assay has been utilized in the early diagnosis of IC/candidemia and also has a high negative

predictive value (NPV) and therefore can be utilized for stopping empirical antifungal therapy.<sup>[5]</sup>

The BDG assay has recently been introduced in India and the clinical efficacy data of BDG in Indian ICUs are lacking. We retrospectively analyzed BDG data to determine the effect of utilizing it in stopping or de-escalating antifungal therapy in Indian settings.

## METHODS

This was a retrospective study conducted in a 24-bed ICU of a tertiary care center in South India. Records of patients for which BDG assay (October 2016–August 2017) was requested were analyzed for basic parameters, candidal score, BDG value, duration and type of antifungal agent

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used, and outcome of the patient. Records were also looked at for factors which cause false-positive BDG which included concurrent bacteremia, IV albumin infusion, intravenous immunoglobulin (IVIG), beta-lactam (amoxicillin-clavulanate and piperacillin-tazobactam) antibiotics, and hemodialysis cellulose filters or membranes. The Food and Drug Administration-approved Fungitell® BDG assay was used. A value of <60 pg/mL is regarded as negative, >80 pg/mL is regarded as positive, and 60–80 pg/mL is taken as equivocal.<sup>[6]</sup> The Fungitell® assay has upper limit of 523 pg/mL, so values of >523 pg/mL were taken as 523 for ease of statistical analysis.<sup>[6]</sup>

Patients with age <18 years, absolute neutrophil count of <500 cells/mL, on immunosuppressive drugs, with diagnosis of other fungal infections, or those already on antifungal prophylaxis or therapy were excluded from the study.

Patients were divided into three groups. Group A comprised of patients in whom diagnosis of IC was confirmed, i.e., blood culture or culture from sterile site grew *Candida*. Group B comprised of patients in whom an alternative etiology of severe sepsis or septic shock was found and Group C had patients with high candidal score and positive BDG (>80 pg/mL) but without a confirmed diagnosis of IC or an alternative explanation for sepsis.

Intergroup analysis for continuous data was performed using Student's *t*-test. Categorical data were analyzed using Kruskal–Wallis test and Chi-square test. *P* < 0.05 was considered statistically significant. Statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS) Statistics 21.0. Institutional ethical committee clearance was taken for the study.

## RESULTS

The medical records of 154 patients were analyzed. The mean age of the study participants was 53.19 years and 88 (57.14%) were male. Mean age across the three groups was comparable. Various clinical features are described in Table 1. Distribution of comorbid conditions was comparable in all the three groups. Surgical conditions were significantly higher in Groups A and C (proven or probable candidemia).

Mean candidal scores were  $3.06 \pm 0.85$ ,  $2.63 \pm 0.71$ , and  $2.90 \pm 0.87$  in Groups A, B, and C, respectively, which was not significantly different [Figure 1]. Distribution of individual risk factors for candidiasis across the three groups is summarized in Table 1. Mean central line days were  $3.56 \pm 0.92$ ,  $3.57 \pm 0.85$ , and  $6.09 \pm 1.2$  days in Groups A, B, and C, respectively, wherein, mean central line

**Table 1: Characteristics of the study population**

Variables	Group A (n=32)*	Group B (n=60)*	Group C (n=62)*	P
Age (mean±2SD) years	51.87±14.80	55.85±15.83	53.87±12.76	0.948
Male (n)	18	34	36	0.991
Medical diagnosis (n)	14	40	31	0.710
Surgical diagnosis (n)	16	20	31	<0.001
Diabetes mellitus (n)	22	39	40	0.547
Hypertension (n)	17	36	37	0.412
Chronic kidney disease (n)	10	16	19	0.984
Chronic liver disease (n)	5	7	8	0.234
Mortality (n)	8	14	12	0.138
BDG (mean±2SD) pg/mL	448.75±88.30	144.46±82.49	292.90±137.0	<0.001
BDG ≥523 (n)	10	0	4	<0.001
BDG 80-523 (n)	22	50	56	0.945
BDG <80 (n)	0	10	2	<0.001
Use of $\beta$ -lactams (n)	4	22	18	0.002
Surgical mesh (n)	2	2	4	0.204
Dialysis by cellulose membranes (n)	0	0	0	-
IVIG (n)	2	6	4	0.848
Bacteremia (n)	0	12	0	0.007
IV albumin (n)	10	34	20	0.066
Risk factors for invasive candidiasis				
Candidal score (mean±2SD)	3.06±0.85	2.63±0.71	2.90±0.87	0.217
Use of ≥2 antibiotics for ≥5 days (n)	18	26	28	0.699
Days of central line (mean±2SD)	3.56±0.92	3.57±0.85	6.09±1.2	<0.001
TPN (n)	10	10	8	0.414
Surgery (n)	10	20	22	0.957
Ventilator (n)	28	50	58	0.855

\*Group A (proven candidemia/IC) comprised of patients in whom diagnosis of IC was confirmed. Group B comprised of patients in whom an alternative etiology of severe sepsis or septic shock was found and Group C (probable IC) had patients with high candidal score and positive BDG (>80 pg/mL) but without a confirmed diagnosis of IC or an alternative explanation for sepsis. BDG:  $\beta$ -D-glucan; IVIG: Intravenous immunoglobulin; IC: Invasive candidiasis; SD: Standard deviation; TPN: Total parenteral nutrition

days were significantly higher in Group C. All the other risk factors were comparable among the three groups.

The mean BDG values were  $448.75 \pm 88.30$ ,  $144.46 \pm 82.49$ , and  $292.90 \pm 137.0$  pg/mL in Groups A, B, and C [Figure 2], respectively, and were significantly higher in Groups A and C as compared to Group B ( $P < 0.001$ ). None of the patients with proven candidemia or IC had values  $<80$  pg/mL, whereas it was  $>80$  pg/mL in 50 (83.3%) patients of Group B. BDG value between 60 and 80 pg/mL was seen in two patients but none with  $<60$  pg/mL among those belonging to Group C. No patient in Group B had a value of  $>523$  and no patient in Group A had a value of  $<80$ . Fourteen patients had BDG of  $\geq 523$  in Groups A and C combined, but none in Group B. Sensitivity, specificity, NPV, and positive predictive value (PPV) of IC/candidemia with BDG of  $\geq 523$  pg/mL were 14.8%, 100%, 42.8%, and 100%, respectively. Specificity of ruling out IC/candidemia with BDG of  $<80$  pg/mL was 97.8%.

Use of  $\beta$ -lactam antibiotics, bacteremia, and IV albumin was significantly higher in Group B as compared to Groups A and C. Gram-negative bacteremia ( $n = 8$ ) led to more false-positive BDG as compared to Gram-positives ( $n = 4$ ). *Escherichia coli* ( $n = 4$ ) and *Klebsiella* ( $n = 2$ ) were the most common causes followed by bacteremia caused by *Acinetobacter* ( $n = 2$ ) and *Staphylococcus aureus* ( $n = 2$ ). Mean BDG level in these patients with bacteremia was 117.4 pg/mL [Table 2].

Most of the patients (148) were started on antifungals on the day IC or candidemia was suspected and BDG was ordered. Echinocandins were used in 125 (81.1%) patients. Caspofungin was the most common antifungal agent used in 82 (53.2%) patients followed by anidulafungin in 32 (20.7%) and fluconazole in 29 (18.8%) patients. Major changes in prescription of antifungals occurred in Group B after BDG result. Antifungals were stopped in 15 (25%) patients in Group B as an alternative diagnosis was made even before BDG was available. Of the remaining 45, for 10 patients in whom BDG was  $<80$  pg/mL, antifungals were stopped. Antifungals were continued in 35 patients in Group B after observing BDG results as they were  $>80$  pg/mL. The mean antifungal duration was  $16.12 \pm 2.4$ ,  $5.1 \pm 1.0$ , and  $15.9 \pm 3.9$  days in Groups A, B, and C, respectively. It was significantly less in Group B as compared to that of Groups A and C ( $P < 0.001$ ).

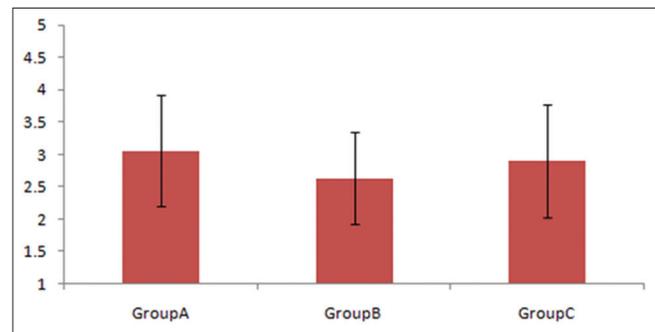
**Table 2: Mean  $\beta$ -d-glucan values among patients with bacteremia**

Bacteremia (pathogen)	Mean BDG (pg/mL)
<i>Klebsiella pneumoniae</i> (n=4)	112.2
<i>Escherichia coli</i> (n=2)	120.5
<i>Acinetobacter baumannii</i> (n=2)	121.6
<i>Staphylococcus aureus</i> (n=2)	154.0
<i>Streptococcus pyogenes</i> (n=1)	96.7
<i>Staphylococcus hominis</i> (n=1)	99.4
Overall mean	117.4

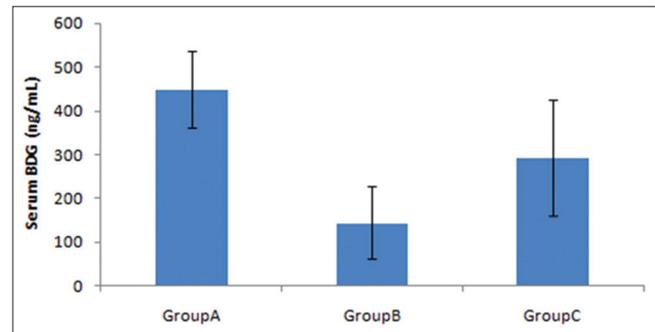
BDG:  $\beta$ -D-glucan

Out of 154 patients, 34 died within the same admission leading to crude mortality of 22.07%. Distribution of mortality was comparable across the groups. Among patients who had a BDG of  $\geq 400$  pg/mL, 26 (out of 48) patients died, a significantly higher mortality rate than those with BDG of  $<400$  pg/mL (54.6% vs. 7.5%;  $P < 0.001$ ).

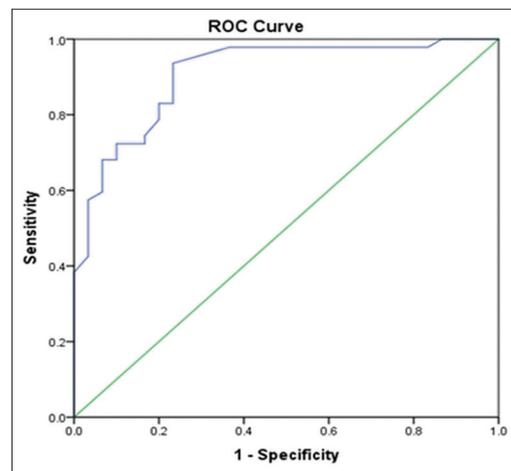
Sensitivity, specificity, NPV, and PPV were 97.8%, 16.6%, 83.3%, and 66.7%, respectively, at a cutoff of 80 pg/mL. The receiver operating characteristic curve showed sensitivity, specificity, NPV, and PPV of 97.8%, 63.6%, 95.0%, and 80.7%, respectively (area under the curve = 0.908 and  $P < 0.001$ ), at a cutoff of 143.5 pg/mL [Figure 3].



**Figure 1: Mean candidal score across the three groups**



**Figure 2: Mean (1,3)- $\beta$ -d-glucan values across the three groups**



**Figure 3: Receiver operating curve showing trend of sensitivity and specificity in this study**

## DISCUSSION

The glucan component is composed mainly of glucose polymers linked via  $\beta$ -1,3-glycosidic bonds, forming the backbone of the fungal cell wall.<sup>[7]</sup> The basis of the Fungitell<sup>®</sup> assay relies on beta glucan's ability to activate the limulus amoebocyte lysate clotting cascade present in the blood of *Limulus polyphemus*, the North American horseshoe crab.<sup>[8]</sup> The resulting clotting can be measured via kinetic spectrophotometry and thus gives an indirect measure of BDG level in serum. BDG levels rise with many pathogenic fungi with exception of *Cryptococcus* species and the yeast phase of *Blastomyces dermatitidis*, as well as the zygomycetes.<sup>[5]</sup> Sensitivity and specificity values of BDG can range from 38% to 100% and 45% to 99%, respectively, with similar ranges observed for the PPV (30% to 89%) and NPV (73% to 97%) for IC/candidemia.<sup>[9-11]</sup>

Apart from its diagnostic utility in IC/candidemia, BDG can also be used in the prognosis of patients with candidemia. Sims *et al.* showed that severe sepsis (91% vs. 28%) and mortality (36% vs. 6%) were significantly higher in patients with BDG result of 400 pg/mL or more compared to those with a BDG <400 pg/mL.<sup>[12]</sup> In our study, mortality rates were higher in a subset of patients with >400 pg/mL.

A number of confounding factors affect BDG values which include hemodialysis with cellulose membranes, serosal exposure to gauze, administration of blood products, albumin, immunoglobulin, coagulation factors, or plasma protein fraction filtered through BG-containing filters.<sup>[5]</sup>  $\beta$ -lactam antibiotics, especially amoxicillin-clavulanate and piperacillin-tazobactam and, concurrent bacteremia (both Gram positive and Gram negative) can also falsely elevate BDG.<sup>[13,14]</sup>

In our study, we found that both Gram-negative (*E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter*) and Gram-positive (*Staphylococcus aureus*) bacteria cause high BDG with a mean of 117.4 pg/mL. We found that  $\beta$ -lactam antibiotics, IV albumin, and bacteremia were significantly higher in Group B and other factors such as IVIG and mesh had a trend to be higher in Group B. Since it is practically impossible to avoid these factors in ICU settings, we tried to validate the cutoff for BDG, taking these factors into consideration. With standard cutoff of 80 pg/mL, sensitivity was 97.8% but specificity was meager (16%), likely because of these factors which falsely elevated BDG in Group B. With increase in BDG cutoff of 143.5 pg/mL, there was no loss of sensitivity, but specificity increased to 63.6%. We could also demonstrate an increase in NPV from 83.3% to 95%.

With poor availability of diagnostic methods for IC/candidemia, overuse of antifungals is rampant.<sup>[4]</sup> Owing to its good NPV, BDG has been shown to be an effective tool to aid stopping antifungal therapy.<sup>[15]</sup> In our study, antifungal agents were discontinued in all patients with BDG <80 pg/mL in Group B. In our study, all patients had a high risk for *Candida* (all the three groups had mean candidal score of >2.5) and would have got prolonged antifungal therapy based on conventional

methods of diagnosing IC/candidemia in ICU (clinical suspicion, risk factors, and candidal score). Using BDG for its NPV helped stopping "unnecessary" antifungal agents in ten of our patients preventing them from getting exposed to longer empirical therapy. As each dose of caspofungin (which was the most common antifungal used) costs 14,000 INR (215 USD), up to 10 days of antifungal therapy was saved per patient, amounting to 1.4 lac INR (2150 USD) per patient. Similar cost-effectiveness of using BDG in ICU settings has been proven in another study.<sup>[15]</sup>

Our study had several limitations; first, it was a retrospective data analysis in a single center. Second, BDG is known to rise serially and most of our patients had only a single BDG estimation. Third, BDG is not specific for *Candida*, so exclusion of non-*Candida* fungal infection is not possible with 100% accuracy.

## CONCLUSION

Our study supports the use of BDG in the exclusion of IC in ICU patients, with a value <80 pg/ml (the lower limit cutoff), facilitating the early discontinuation of empirically started antifungal therapy and resulting in considerable cost savings. A value >523 pg/ml (the highest possible reading) was also strongly correlated with IC and facilitated continuation of antifungals even though IC was not confirmed. A value of >400 may also carry a poorer prognosis. This study also suggests a higher cutoff of at least 143.5 pg/mL which will be more specific for IC, although this may need further validation with larger trials.

We recommend that clinicians routinely utilize the BDG assay along with blood cultures for both the diagnosis and exclusion of IC in high-risk critically ill patients.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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