

Evaluation of a Novel Plasma (1,3)- β -D-Glucan Detection Assay for Diagnosis of Candidemia in Pediatric Patients

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We evaluated a novel plasma (1,3)- β -D-glucan (BDG) detection assay for the diagnosis of candidemia in children. The median BDG levels were 73.4 pg/ml in patients with candidemia and <10 pg/ml in patients without candidemia ($P < 0.001$). Receiver operating characteristic analysis revealed a cutoff point of 14 pg/ml and an area under the curve of 0.802. At these values, the assay demonstrated 68% sensitivity, 91% specificity, 66% positive predictive value, and 91% negative predictive value. Plasma BDG levels were undetectable in 18 candidemia cases.

As the number of pediatric patients with invasive candidiasis has increased in recent years (1), rapid diagnosis of the disease has become urgently needed so that appropriate treatment can be initiated without delay. (1,3)- β -D-glucan (BDG) is a fungal cell wall polysaccharide present in a variety of fungi, including *Candida* species. Testing BDG in serum by Fungitell assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA) has been approved by the U.S. Food and Drug Administration to aid the diagnosis of invasive fungal infections. Clinical studies have demonstrated that the Fungitell assay has a sensitivity of 75% and a specificity of 80% in the diagnosis of candidiasis in intensive care unit patients in the United States (2, 3).

The GKT assay (Gold Mountain River Tech Development Co., Ltd., Beijing, China) is a novel assay to detect BDG in plasma. The assay is manufactured by developing blood extractions from the horseshoe crab *Tachypleus tridentatus* into limulus amoebocyte lysate (LAL). BDG can be detected in plasma via the activation of factor G in LAL, leading to a cascade of proteolytic coagulation that can be measured by a reader. The assay is also modified by removing bacterial endotoxin-sensitive factor C from the LAL, making it specific for BDG detection. It is important to recognize that the GKT assay is designed to detect BDG in the plasma; this differs from the Fungitell assay used in the United States, which detects BDG in the serum, and from the Fungitec-G assay (Seikagaku Corp., Tokyo, Japan) used in Japan that detects BDG in the serum or plasma. In addition, sample pretreatment process and test result readouts vary among these kits. Furthermore, each kit is developed from different horseshoe crab species, and the amoebocytes from different horseshoe crab species may possess different affinities toward the BDG molecules. Therefore, each kit has its own cutoff, and one cannot be applied to the other. Although the GKT assay has been approved by the China State Food and Drug Administration as an adjunct test to aid in the diagnosis of deep fungal infections, little is found in the English literature about its performance characteristics for the diagnosis of candidemia, particularly in pediatric patients. In this study, we evaluated the GKT assay for the diagnosis of candidemia in pediatric patients.

Pediatric patients (0 to 14 years old) admitted to Xinhua Hospital, a 2,000-bed tertiary teaching hospital affiliated with Shanghai Jiaotong University School of Medicine, Shanghai, China, from April 2009 to February 2012 were included in the study. The hospital serves a population of 1.2 million people. This study was

approved by the Xinhua Hospital institutional review board. Patient demographic information and pertinent clinical data, including history of long-term use of antibiotics, presence of indwelling central venous catheters, fever, and low birth weight, were reviewed. None of these patients included in the study received antifungal prophylaxis prior to their blood culture collection. For each patient, 4 ml of blood was drawn for blood culture. Blood culture samples were processed in pediatric bottles using the BacT/Alert system (bioMérieux, Marcy l'Etoile, France). Positive samples were subcultured onto Sabouraud dextrose agar (Oxoid, United Kingdom), and subsequent culture isolates were identified to the species level using the YST panel with the Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France). Patients with candidemia were defined as isolating *Candida* species in blood obtained from at least two separate peripheral blood samples collected at different sites. Patients without candidemia were defined as having negative blood culture results for fungi and no clinical evidence of fungal infections (excluding neonatal intensive care unit patients).

BDG detection was performed in plasma that was collected at the same time that blood was drawn for culture. Detection of BDG in plasma was performed according to the manufacturer's instructions. Briefly, 100 μ l of plasma was mixed with 900 μ l of pretreatment reagents and then heated at 70°C for 10 min. After cooling down, the solution was mixed with the treatment reagents for 1 h and then transferred to a reading tube. Detection of the presence of BDG was based on the absorbance change, and the result was

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TABLE 1 Plasma BDG levels in pediatric patients with or without candidemia

Patient	No. of patients	BDG level (pg/ml) (median [range])	P	Sensitivity (%)	Specificity (%)
Candidemia ^a					
Age, ≤28 days	8		0.024 ^b		
<i>C. albicans</i>	3	3,378.0 (1,797.0–3,895.0)		100	91
<i>C. parapsilosis</i>	5	41.4 (<10.0–74.3)		60	91
Age, >28 days to 12 years	48		0.029 ^b		
<i>C. albicans</i>	7	453.04 (14.8–3,434.0)		100	91
<i>C. guilliermondii</i>	12	139.1 (<10.0–636.0)		83	91
<i>C. parapsilosis</i>	17	70.8 (<10.0–983.3)		65	91
<i>C. famata</i>	10	<10.0 (<10.0–498.1)		30	91
<i>C. albicans</i> and <i>C. parapsilosis</i>	1	<10.0			
<i>C. lusitanae</i>	1	18.9			
Subtotal	56	73.5 (<10.0–3,895.0)	0.001 ^c	68	91
Noncandidemia	210	<10.0 (<10.0–1,000.0)			

^a Candidemia cases were divided with each age subgroup based on the different causal *Candida* species.

^b Plasma BDG levels were statistically different among candidemia cases caused by different *Candida* species.

^c The median (range) value of plasma BDG in the 56 patients with candidemia was significantly higher than that in 210 patients without candidemia.

determined by a microbiology kinetic reader provided by the manufacturer. The limit of detection of the GKT plasma BDG detection assay was <10 pg/ml.

Patient demographic and clinical characteristics were summarized using descriptive statistics, i.e., counts and median (range) numbers. Nonparametric analysis methods were chosen due to the non-Gaussian distribution of BDG values. The Mann-Whitney U test was used to determine the statistical difference between levels of BDG in patients with candidemia and in those without the disease. The Kruskal-Wallis test was used to test the statistical difference of BDG levels in cases of candidemia caused by different *Candida* species. Receiver operating characteristic (ROC) curves were plotted to determine the best cutoff value for the plasma BDG assay to diagnose candidemia. Sensitivity, specificity, and exact binomial 95% confidence intervals (CIs) for each were calculated. Analyses were performed using IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) and MedCalc software (version 11.6.1.0; Mariakerke, Belgium). All tests were two sided and were considered significant at a *P* value of <0.05.

Fifty-six patients with culture-confirmed candidemia were identified over the study period from a total of 2,256 patients tested (2.5% prevalence of the infection). The median (range) age was 2 months (20 days to 11 years); 28 patients (50%) were male. Thirty-eight patients had premature birth, 10 had gastrointestinal abnormalities, 6 had congenital heart diseases, and 3 had neurological abnormalities. To identify patients without candidemia, a total of 210 patients with negative fungal cultures and without evidence of fungal infections were randomly selected from 13 different wards (8 to 22 patients per ward) over the same study period. The median (range) age was 2 months (10 days to 11 years); 103 patients (49%) were male.

The median (range) value of plasma BDG in the 56 patients with candidemia was 73.5 pg/ml (<10 to 3,895 pg/ml), which was higher than that in 210 patients without candidemia (median, <10 pg/ml; range, <10 to 1,000 pg/ml) (*P* = 0.001) (Table 1). *Candida parapsilosis* (39%) was the most common cause of candidemia in our pediatric patients, followed by *Candida guilliermondii* (21%), *Candida albicans* (18%), and *Candida famata* (18%) (Table 1). We have further broken down our pediatric patients into neonate (≤28 days old) and nonneonate (>28 days to

12 years) groups. We found that plasma BDG levels varied among candidemia patients caused by different *Candida* species: the highest BDG levels were seen in candidemia cases caused by *C. albicans*, followed by *C. guilliermondii*, *C. parapsilosis*, and *C. famata* (Table 1). These findings were not affected by age groups (*P* = 0.024 versus 0.029). Our findings were different from previous reports using the Fungitell assay to assess BDG levels in serum from adult candidemia patients. Del Bono et al. has reported that no differences were found when comparing median BDG values in patients with *C. albicans* candidemia versus non-*C. albicans* candidemia (4). A similar observation was also reported by Odabasi et al. (2).

ROC analysis for plasma BDG values demonstrated a cutoff point of 14 pg/ml, with an area under the curve of 0.802 (Fig. 1). At this value, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the diagnosis of candidemia in our pediatric patients were 68% (95% CI, 50% to 80%),

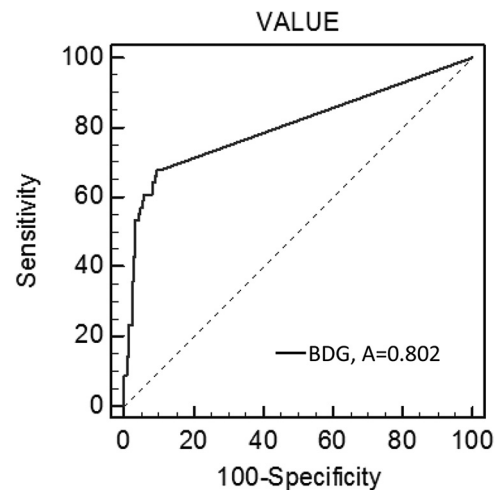


FIG 1 Receiver operating characteristic (ROC) curve of BDG cutoff values (pg/ml) for determining positive and negative plasma (*n* = 266 specimens from 56 patients with culture-confirmed candidemia and from 210 patients without candidemia). Area under the ROC curve, 0.802.

TABLE 2 Plasma BDG levels in candidemia patients following antifungal drug treatment

Patient	Week	Culture result	BDG level (pg/ml)	Antifungal drug used	Clinical outcome	
1	0 ^a	<i>C. albicans</i>	3,434	Fluconazole	Survived	
	1		954.3			
	2		174.9			
	3	Negative	102.3			
	4		13.87			
2	0 ^a	<i>C. albicans</i>	759.7	Fluconazole	Survived	
	1					
	2		781.3			Added caspofungin
	3	Negative	671.2			
	4		186.8			
3	0 ^a	<i>C. famata</i>	498.1	Fluconazole Added flucytosine	Survived	
	1		1,817			
	2	Negative	384.2			
	3		<10			
	4		<10			
4	0 ^a	<i>C. parapsilosis</i>	207.2	Fluconazole	Survived	
	1		295.7			
	2	Negative	<10			
	3		<10			
	4					
5	0 ^a	<i>C. parapsilosis</i>	139.9	Fluconazole	Survived	
	1		1,000			
	2		867.5			
	3	Negative	728			
	4		<10			
6	0 ^a	<i>C. parapsilosis</i>	<10	Fluconazole	Survived	
	1		<10			
	2	Negative	259.9			
	3		<10			
	4		<10			
7	0 ^a	<i>C. parapsilosis</i>	72.6	Fluconazole	Survived	
	1	Negative	<10			
	2		<10			
	3		<10			
	4		<10			

^a Time when the blood culture was positive.

91% (95% CI, 86% to 94%), 66% (95% CI, 52% to 78%), and 91% (95% CI, 87% to 95%), respectively. Our cutoff value is different from the one established with the Fungitell assay (60 pg/ml) and the one used with the Fungitec-G assay (20 pg/ml).

Seven candidemia patients had multiple plasma specimens collected at different time points, which, thus, allowed us to measure serial plasma BDG levels in each of them after antifungal drug treatment (Table 2). In all cases, the plasma BDG levels declined by 4 weeks. In 5 patients, BDG levels were still detectable at the time that cultures were negative.

Plasma BDG levels were undetectable in 18 candidemia cases, of which 9 were caused by *C. parapsilosis*, 7 by *C. famata*, and 2 by *C. guilliermondii*. It is possible that *C. parapsilosis* and *C. famata* have smaller amounts of BDG in their cell walls, as suggested by their lower susceptibility to echinocandins (antifungals targeting

BDG synthesis) (4, 5). As a result, 70% of our patients with *C. famata* candidemia had undetectable BDG values. Likewise, low BDG levels were observed in our patients with *C. parapsilosis* candidemia within both neonatal and nonneonatal pediatric populations (Table 1), which is similar to the findings reported by other investigators (3, 4, 6). These data suggest that the BDG detection may be suboptimal in the diagnosis of candidemia cases caused by these two *Candida* species.

BDG was detectable in plasma from 19 (9%) patients with negative blood cultures, with concentrations ranging from 11 to 1,000 pg/ml. It is true that the sensitivity of a blood culture in detecting candidemia is not high (7), but none of these patients presented with evidence of fungal infections. False-positive BDG detection may be attributable to multiple factors, such as blood dialysis, surgical gauze usage, bacterial infections (*Pseudomonas aeruginosa*), or receipt of albumin or immunoglobulin products (8–10). One study also showed that the baseline level of BDG in pediatric patients without candidemia was slightly higher than that of adult patients (68 pg/ml versus 48 pg/ml) using the serum BDG Fungitell assay (11). We selected patients for chart review whose plasma BDG values were >100 pg/ml. A total of five patients were identified, and none of them had evidence of fungal infections. Although we could not identify any factor directly contributing to false positives in these five cases, we found that one patient had a plasma transfusion done 1 day prior to the BDG testing, and another patient had a positive blood culture growing *Klebsiella pneumoniae*.

In summary, we demonstrated that the GKT plasma BDG assay has a moderate efficacy (68% sensitivity and 66% PPV) to aid the diagnosis of candidemia in pediatric patients. The assay is useful to rule out the candidemia due to its high NPV (91%). The assay also showed prognostic value in monitoring antifungal treatment response (though this result is based on the study of a small number of available cases). Since poor sensitivity of blood culture may be exacerbated in children due to the low volume of blood collection, the plasma BDG assay that only requires 100 μ l of plasma can provide early and rapid diagnostic value to aid the diagnosis of candidemia in children, thus allowing initiation of appropriate antifungal therapy without delay. However, low sensitivity of the plasma BDG assay was noticed in detecting candidemia caused by *C. famata* and *C. parapsilosis*.

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