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EVALUATION OF PANFUNGAL MARKER (1,3)-β-D-GLUCANIN DIAGNOSIS OF INVASIVE INFECTIONS WITH *CANDIDA* SPECIES

ЕВАЛУАЦИЈА НА ПАНФУНГАЛНИОТ МАРКЕР (1,3)-B-D-ГЛИКАН ЗА ДИЈАГНОЗА НА ИНВАЗИВНИ ИНФЕКЦИИ СО *CANDIDA* SPECIES

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Apstract

Introduction. Although blood culture is considered a gold standard in diagnosis of invasive infections, it is still not reliable and fast enough for diagnosis of candidemia. Determination of serum (1,3)-β-D-glucan is a highly sensitive and specific test for invasive mycosis, and could probably be of benefit to patients with high risk for invasive infections with *Candida* species.
Aim. The aim of this study was to prospectively evaluate the diagnostic performance of serum (1.3)-β-D-glucan BDG (Fungitell) assay, in comparison with blood culture, for diagnosis of invasive infections with *Candida* species.
Methods. Blood and sera from 120 patients divided in 4 groups, hospitalized at the University clinics in Skopje, during a 2-year period, were investigated for invasive *Candida* infections. Blood was examined with conventional methods (automated BacT/Alert system, Gram stain and culture on fungal media). Identification of *Candida* species was performed with VITEK-2 system. Serum (1,3)-β-D-glucan was determined by means of Fungitell assay.
Results. Positive blood culture was registered in 23.33%, 43.33%, 23.8% and in 3.33% of samples only in groups I, II, III and IV, respectively. Positive findings with (1,3)-β-D-glucan Fungitell assay at the same time with blood culture were detected in 83.33%, 76.67%, 30% and 26.67% in groups I, II, III and IV, respectively. The average concentration of BDG was highest in group I, followed by group II, group IV and group III.
Conclusion. Our results suggest that a positive (1,3)-β-D-glucan assay could be a superior test in addition to the blood culture for diagnosis of candidemia and highlights the value of this test as a diagnostic adjunct in the serodiagnosis of an invasive candidiasis.

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Keywords: *Candida*, candidemia, blood culture, (1,3)-β-D-glucan, serodiagnosis

Апстракт

Вовед. Хемокултурата, иако се смета за златен стандард во етиолошката дијагноза на инвазивните инфекции, сè уште не е сигурен и брз метод за дијагноза на кандидемија. Одредувањето на серумскиот (1,3)-β-Д-гликан е високо сензитивен и специфичен тест за инвазивни микози, кој може да биде од голема корист за лицата со висок ризик за инвазивни инфекции со *Candida* species.
Цел. Целта на оваа студија епроспективно евалуирање на дијагностичкиот потенцијал на серумскиот (1,3)-β-Д-гликан, во споредба со хемокултурата, за дијагноза на инвазивни инфекции со *Candida* species.
Методи. Крв и серум од 120 пациенти класифицирани во четиригрупи, хоспитализирани на универзитетските клиники во Скопје, во период од две години, беа испитувани за инвазивни инфекции со *Candida* species. Крвта беше обработувана со конвенционални методи (автоматизиран БацТ/Алерт систем за хемокултури, боење по Грам и култура на фунгални подлоги). Идентификацијата на *Candida* species се изведуваше со ВИТЕК-2 системот. Серумскиот (1,3)-β-Д-гликан се одредуваше со Фунгителл тестот.
Резултати. Позитивна хемокултура беше консеквентно регистрирана во 23.33%, 43.33%, 23.8% и 3.33% примероци во групите I, II, III и IV. Позитивен наод на (1,3)-β-Д-гликан истовремено со хемокултурата, Фунгителл тестот детектираше кај 83.33%, 76.67%, 30% и 26.67% во групите I, II, III и IV, консеквентно. Просечната концентрација на БДГ беше највисока во групата I, следена од групите II, IV и III.

Заклучок. Нашите резултати укажуваат дека позитивен (1,3)-β-Д-гликан може да биде супериорен тест, покрај хемокултурата за дијагноза на кандидемија, и ја истакнува користа од овој тест како дијагностичка поддршка во серодијагнозата на инвазивната кандидијаза.

Клучни зборови: *Candida*, кандидемија, хемокултура, (1,3)-β-Д-гликан, серодијагноза

Introduction

The incidence of invasive fungal infections (IFI) caused by *Candida* species has dramatically increased over the last decades, despite the availability of effective treatments, and is directly associated with increased morbidity and mortality [1], especially among the growing population of immunocompromised patients or patients receiving critical care in intensive care units (ICUs) [2]. The most frequent predisposing factors for development of invasive fungal infections are prolonged stay in ICUs, broad spectrum antimicrobial agents, prolonged use of corticosteroids, chemotherapy and radiotherapy, prematurity, intravascular catheters and parenteral nutrition, immunosuppression and disruption of mucous membranes, and HIV/AIDS are among [1]. *Candida* species are ranked on the 4th position as agents of nosocomial septicemia in many studies across USA, and cause approximately 9-12% of all septicemias [3] and on the 6th or 7th position as causes of nosocomial septicemia in many European studies [4]. To achieve a favorable prognosis of these deadly infections, an early initiation of an antifungal treatment is necessary. It relies on a timely and accurate diagnosis, which in turn is still challenging. In the absence of specific signs and symptoms, there is a need to evolve an appropriate diagnostic approach. Histopathologic demonstration of organisms in tissue specimens or growth of fungal agents in culture media is still the "gold standard" method, but obtaining such specimens may be difficult, and conventional microbiological methods' results (blood culture) are relatively insensitive, since they are positive in less than 50% of all invasive *Candida* infections, are time-consuming and not generally accessible in all laboratories [5]. Therefore, diagnosis of invasive fungal infections remains challenging, due to a limited choice of sensitive early diagnostic markers. As a consequence of the difficulties with diagnosis, a significant effort has gone into developing non-culture-based diagnostic techniques for detecting invasive candidiasis [6]. Recently, particular emphasis has been placed on the detection of fungal markers within biological samples. Among possible culture-independent serum markers, *Candida* mannoproteins (13), and (1,3)-β-D-glucan (BDG) offered some promise [7,8].

(1,3)-β-D-glucan (BDG) is a panfungal marker that is a component of the cell wall (cell wall polysaccharide) found in many pathogenic fungi, including *Candida* species, which can be present early in the blood and fluids from patients suffering from IFI. Serum β-D-glucan concentrations show a constant rise before clinical and microbiological evidence of infections, then decrease, and eventually become negative if patients respond to antifungal therapy. Conversely, patients not responding do not show a decrease or show a continuous rise. The Fungitell test (Associates of Cape Cod) is a chromogenic kinetic test that was approved in 2003 by the U.S. Food and Drug Administration for the presumptive diagnosis of IFI [9]. It may allow earlier diagnosis of IFI, which is otherwise feasible with traditional methods.
The aim of this study was to prospectively evaluate the diagnostic performance of serum (1,3)-β-D-glucan BDG (Fungitell), in comparison with blood culture, for diagnosis of invasive infections with *Candida* species.

Study design

A prospective diagnostic study was performed at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje, during a 2-year period, from March 2012 until May 2014.

Material and methods

This study analyzed primarily sterile specimens (blood and serum) from 120 patients classified in 4 different groups according to the clinical diagnosis and risk factors for development of invasive candidiasis (group I (n=30)-patients with primary immune deficiency, group II (n=30)-patients with prolonged stay in ICU receiving broad spectrum antibacterial treatment, group III (n= 30)-patients with mucosal candidiasis and group IV (n=30)-cystic fibrosis patients). Invasive fungal infection was defined according to the revised definitions by the EORTC/MSG (European organization for research and treatment of cancer/mycoses study group) consensus group, with the necessary modification that BDG was not included in the microbiological criteria [10]. Blood culture was performed with automated BacT/Alert system (*bioMerieux*, France), Gram stain and culture on selective Sabouraud and CALBmedium (Oxoid). Identification of *Candida* species was performed with automated VITEK-2 system (*bioMérieux,* France) [11]. Serological (1,3)-β-D-glucan detection was performed with Fungitell assay (Associates of Cape Cod) (9). Briefly, total of 5 µl of serum were briefly pretreated with 20 µl alkaline reagent solution (0.125 M KOH/0.6 M KCl) for 10 min at 37°C and then 100 µl reconstituted Fungitell reagent was added to the sample placed into triplicate wells of a 96-well microtiter plate.The reaction was incubated for 40 min at 37°C and the optical density was measured at 405/490 nm with a spectro-

photometer. The mean rate of optical density change was determined for each well, and the BG concentration was determined by comparison to a standard curve. Interpretation of BG values was as follows: <60 pg/ml, negative; 60 to 79 pg/ml, indeterminate; ≥80 pg/ml, positive. The test results of the BDG assay were not available for the clinician for decision making (BG results were not used for the management or classification of IFI). Proven and probable IFI were considered to be true-positive cases for analysis. Patients with possible IFI were considered to have true-negative cases.

Results

According to the gender structure of the participants in our study, men were more frequently identified in groups I, III and IV (60%, 56.67%, 53.33%, respectively), and women in the group II (56.67%). The average age of patients with primary immune deficiency was 39.13± 21.8 years, 65.63±29.8 of patients in the second group, 51.91±18.4 of the third group of patients and 16.23± 6.4 years of the fourth group of patients (Table 1).

Table 1. Characteristics of patients according to age and gender

Variable	Groups of patients				
	group I n=30 (%)	**group II n=30 (%)**	**group III n=30 (%)**	**group IV n=30 (%)**	**Total n=120 (%)**
Gender					
women	12(40%)	17(56.67%)	13(43.33%)	14(46.67%)	56(46.7%)
men	18(60%)	13(43.33%)	17(56.67%)	16(53.33%)	64(53.33%)
Age					
years, mean±SD	39.13±21.8	65.63±29.8	51.91±18.4	16.23±6.4	43.0±24.1
min-max	3-68	21-83	18-93	7-30	3-82
[a]*p = 0.6*					

p (Chi-square test)

Distribution of the examined participants in all groups, according to clinical diagnosis with EORTC/MSG criteria, showed that a proven fungal infection was most frequently registered in patients with prolonged ICU stay receiving a broad spectrum antibiotic treatment (56.67%), followed by patients with primary immune deficiency (33.33%) (Table 2). Differences in distribution of proven, probable and possible fungal infection were statistically significant between group I versus groups III and IV, and between group II versus groups III and IV.

Table 2. Characteristics of patients according to EORTC/MSG criteria classification

Variable	Groups of patients				
	group I n=30 (%)	**group II n=30 (%)**	**group III n=30 (%)**	**group IV n=30 (%)**	**Total n=120 (%)**
Classification					
proven	10(33.33%)	17(56.67%)	0	1(3.33%)	28(23.33%)
probable	12(40%)	11(36.67%)	6(20%)	8(26.67%)	37(30.83%)
possible	8(26.67%)	2(6.67%)	24(80%)	21(70%)	55(45.83%)
	Chi-square: 54.08[a]*p*< 0.001				
	I vs II ns		II vs III *p*< 0.001		III vs IV ns
	I vs III *p*< 0.001		II vs IV *p*< 0.001		
	I vs IV *p*< 0.0009*				

Regarding the distribution of host factors, 53.33% of patients in group I had hematological malignancy, 30% had AIDS; 10% presented with hypogamaglobulinemia and 3.33% each had chronic granulomatous disease or CARD9 deficit. In group II, host factors distribution was: neonatal sepsis (16.66%), abdominal surgery (13.33%), solid organ cancer (13.33%), diabetes mellitus (13.33%), neonatal meningitis (10%), sepsis in pediatric ICU (6.66%), urosepsis (6.66%), renal failure with hemodialysis (6.66%), endocarditis (6.66%), pancreatitis with diabetes mellitus (3.33%), and burns (3.33%). In group III, 56.66% of patients had esophageal candidiasis with comorbidities like COPD, asthma, obesity, diabetes mellitus, alcoholism, ulcus disease and cancer, and 43.33% ICU patients with diabetes mellitus as comorbidity, presented with signs and symptoms of candiduria. In group IV, CF patients with acute exacerbations were analyzed.

The blood culture was positive in 23.33% patients in group I, 43.33% in group II, 23.08% in group III and one patient in group IV. The statistical analysis confirmed that positive blood culture was a significantly less frequent finding in patients with cystic fibrosis, compared to both groups of patients with primary immune deficiency (p=0.023), and patients with prolonged ICU stay and antibiotic treatment (p=0.00025). The most frequent isolates from blood culture in group I were *C. tropicalis* and *C. krusei*, followed by *C. albicans* and *C. parapsilosis.* In group II, *C. albicans* and *C. pelliculosa* were equally presented, followed by *C. parapsilosis* and *C. Glabrata* (Table 3). Positive findings with (1-3)-β-D-glucan Fungitell BDG assay at the same time with

Table 3. Yeast species in blood culture

Variable	Groups of patients				
	group I n=30 (%)	group II n=30 (%)	group III n=30 (%)	group IV n=30 (%)	Total n=120 (%)
Blood culture					
negative	23(76.67%)	17(56.67%)	10(76.92%)	29(96.67%)	79(76.7%)
positive	7(23.33%)	13(43.33%)	3(23.08%)	1(3.33%)	24(23.3%)
	Chi-square: 13.4 p = 0.0038**				
	I vs IV p= 0.023*				
	II vs IV p= 0.00025**				
C.parapsilosis	1	2	0	0	3
C.tropicalis	3	1	0	0	4
C.albicans	1	4	1	1	7
C.krusei	2	0	0	0	2
C.pelliculosa	0	4	0	0	4
C.glabrata	0	2	2	0	4

p (Chi-square test) *p < 0.05 **p< 0.01

Table 4. Characteristics of patients according to positivity of (1-3)-β-D-glucan test

Variable	Groups of patients				
	group I n=30 (%)	group II n=30 (%)	group III n=30 (%)	group IV n=30 (%)	Total n=120 (%)
(1-3)-β-D-glucan test					
negative	5(16.67%)	5(16.67%)	21 (70%)	19(63.33%)	50 (41.66%)
positive	25(83.33%)	23 (76.67%)	9 (30%)	8 (26.67%)	65 (54.16%)
intermediate	0	2 (6.67%)	0	3 (10%)	5 (4.16%)
	[a]p<0.001				
	I vs III [a]p=0.00003**			II вс III [a]p=0.000066**	
	I vs IV [a]p=0.00004**		II вс IV [a]p=0.00009**		

[a]p (Chi-square test) **p<0.01

blood culture were detected in 25/30 (83.33%) patients of group I, 23/30 (76.67%) of group II, 9/30 (30%) of group III, and 8/30 (26.67%) patients of group IV. Described differences were confirmed as statistically significant between group I and group III (p=0.00003), and between group I and group IV (p=0.00004), and also between group II and group III (p=0.000066), and between group II and group IV (p=0.00009) (Table 4).

In the statistical analysis, intermediate results were not included.

In Table 5 the concentrations of the (1-3)-β-D-glucan marker (BDG) are presented. At the same time with blood culture, statistically significantly higher concentration of BDG was obtained in group I compared to group III (p=0.000008) and group IV (p=0.000008), and, statistically significantly higher concentration of

Table 5. Descriptive statistics BDG concentration (pg/ml)

	Descriptive statistics BDG concentration (pg/ml)			
	mean ± SD	min-max	median (IQR)	*p*-value
group I	184.77 ± 106.9	33 - 447	177 (117 – 268)	H=44.4 [d]p<0.001
group II	164.43 ± 98.9	38 - 378	148 (88 – 218)	I vs III[c]p=0.000008**
group III	59.87 ± 27.5	34 - 124	46 (41 – 88)	I vs IV[c]p=0.000008**
group IV	61.17 ± 27.3	25 - 133	47 (41 – 85)	II vs III[c]p=0.0000029** II vs IV[c]p=0.0000028**

[c]p (Mann0Whitney U test) [d]p (Kruskal-Wallis test)

BDG was obtained in group II compared to group III (p=0.0000029) and group IV (p=0.0000028). The average concentration of BDG was highest in group I (184.77±106.9 pg/ml), followed by group II (164.43± 98.9 pg/ml), group IV (61.17±27.3 pg/ml), and group III (59.87±27.5 pg/ml). The median concentration of BDG was 177 pg/ml (range117-268), 148 pg/ml (range 88-218), 46 pg/ml (range 41-88), and 47 pg/ml (range 41-85) in all four groups, respectively.

Positive BDG test in group I was registered in 25 (83.33%) patients' sera. The concentration of BDG in the positive sera was in the range 82-447 pg/ml. The average concentration of BDG was 214±91.9 pg/ml. Sensitivity, specificity, PPV, NPVof BDG were100%, 62.5%, 88%, 100%, respectively (Table 6).

Positive BDG test in group II was registered in 23 (76.67%) patients' sera. The concentration of BDG in the positive sera was in the range 88-378 pg/ml, with an

Table 6. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group I

Method	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	31.82	100	100	34.78
β-D-glucan test	100	62.5	88	100

Table 7. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group II

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	46.43	100	100	11.76
β-D-glucan test	88.46	100	100	40

average concentration of 197.74±88.6 pg/ml. Sensitivity, specificity, PPV, NPV of BDG were 88.46%, 100%, 100%, 40%, respectively (Table 7).

In group III positive BDG test was registered in 9 (30%) patients' sera. The concentration of BDG in the positive sera was in the range 88-124 pg/ml, with an average concentration of 99.22±12.8 pg/ml. Sensitivity, specificity, PPV, NPV of BDG were 100%, 87.5%, 66.67%, 100%, respectively (Table 8).

In group IV positive BDG test was registered in 8 (26.67%) patients' sera. The concentration of BDG in the positive sera was in the range85-133 pg/ml, with an average concentration of 99.25±16.5 pg/ml (Table 9).

Table 8. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group III

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture	0	72.73	0	80
β-D-glucan test	100	87.5	66.67	100

Table 9. Comparative diagnostic performances of blood culture and (1-3)-β-D-glucan test in group IV

Test	Se(%)	Sp(%)	PPV(%)	NPV(%)
Blood culture				
β-D-glucan test	100	100	100	100

This test did not identify false negative or false positive results in group IV; all 8 cases of CF with positive BDG assay were in the group of probable/proven IFIs according to EORTC/MSG criteria, and all negative BDG cases were classified as possible infections.

Discussion

Early diagnosis of invasive *Candida* infections is crucial in order to initiate antifungal agents early. Delay in the administration of appropriate antifungal treatment increases mortality from invasive candidiasis. Unfortunately, clinical and radiological signs are often unspecific and conventional culture methods are not sensitive enough [6]. To overcome many obstacles during laboratory work, different tests have been developed, which have been evaluated for diagnosis of invasive candidiasis. In this study we have evaluated the performance of a new (1,3)-β-D-glucan (BDG) detection system (Fungitell) as a diagnostic adjunct for invasive fungal infections. Different studies have evaluated clinical performance of the (1,3)-β-D-glucan assay with focus on specific target populations, like hematological [8], pediatric patients [12], or ICU [13]. It has also been proposed as an early biomarker of invasive fungal infections and is included in diagnostic criteria of invasive fungal infections in the 2008 revised IFI diagnosis criteria of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [10]. In order to investigate the diagnostic potential of the BDG test to enhance diagnosis of invasive *Candida* infections, we have examined sera from 120 patients that were divided into 4 groups according to the clinical diagnosis and EORTC/MSG criteria, for presence of elevated concentrations of (1,3)-β-D-glucan and compared them with blood cultures. According to EORTC/MSG criteria, a proven fungal infection was most frequently registered in patients with prolonged ICU stay receiving antibiotic treatment with broad spectrum (group II) (56.67%), followed by patients with primary immune deficiency (group I) (33.33%). Differences in distribution of proven, probable and possible fungal infection were statistically significant between group I versus groups III and IV, and especially between group II versus groups III and IV. This is in consistency with the work of Montagna *et al*. [14] where most of the cases with invasive fungal infection tend to occur in older patients (more than 60 years), as was the average age in our group of patients with prolonged stay in ICU setting (65.63±29.8) that presented the highest rate of proven infections with *Candida* species. This can be attributed to the increased incidence of invasive mycoses with advanced age which most often is associated with impaired immunity. In our study 33.32 % of invasive fun-

gal infections in group II were in the NICU (neonatal intensive care unit) and PICU setting (pediatric intensive care unit). Of all the patients in this group, 56.67% had proven and 36.67% had probable fungal infections. Positive blood cultures were identified in 24 patients in all groups. Most episodes of candidemia developed in group I, and were caused by *C. tropicalis* and *C. krusei*, followed by *C. albicans*, *C. parapsilosis* and *C. tropicalis*. In group II, *C. albicans* and *C. pelliculosa* were equally presented, followed by *C. parapsilosis* and *C. glabrata.* In the third group, 3 cases of candidemia were confirmed with positive blood culture (one patient with *C. albicans*, *C. glabrata* in 2 patients). In our CF patients, only one patient had positive blood culture with *C. albicans*. Although *C. albicans* is still considered the most frequent etiological agent of candidemia, in the recent few decades, a significant increase of candidemia caused by non-*albicans Candida* species [15] has been registered, which are usually less susceptible to antifungal drugs. Our study confirms high prevalence of non-*albicans Candida* species candidemia, as has been demonstrated in many reports from around the world [16,17].

The Fungitell assay for detection of (1,3)-β-D-glucan confirmed elevated concentrations of BDG in: group I-25/30 (83.33%), group II-23/30 (76.67%), group III-9/30 (30%), and group IV-8/30 (26.67%). In group I, this test detected 10/10 (100%) of proven and 12/12 (100 %) of probable cases of IFIs and 3/6 (37.5%) in possible infections. In group II, the test detected 13/17 (76.47%) of proven and 10/11 (90.91%) of probable cases of IFIs. In group III, 6/6 (100%) of probable and 3/24 (100%) of possible infections were detected by the assay. In group IV, the Fungitell assay was positive in 8/30 (26.67%) of proven/probable infections. BDG-positive results have been obtained by the Fungitell assay for most of the cases with candidemia caused by different *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*). Interestingly, the BDG values in two cases of proven infection (candidaemia caused by *C. Pelliculosa* in a neonate and one adult case of candidemia with *C. albicans*) did not reach any of these cut-offs and the test remained negative. However, the BDG detection was not performed at the same time with the blood sample as the one that was *Candida* culture positive, although the sample used was collected at the same time.

In this study the sensitivity of the BDG test was 100%, 88.46%, 100%, 100%, in groups I, II, III and IV respectively, compared to sensitivities of 77.6% (18), 86.7% [19], and 100% [20] in other reports for diagnosis of candidemia. Many studies have reported wide discrepancies on sensitivity (40%-100%) and specificity (45%-99%) of the assay [21-23], probably due to the heterogeneity of their designs, and probably because they have used different cut-off values (10 to 120 pg/ml) for a positive result. In a recent study, Pickering *et al.* evaluated 39 sera samples from 15 patients with blood culture positive yeast infections using a cut-off value of 80 pg/ml (19). Thirty (77%) samples were positive for BDG (range 84 to 1359 pg/ml), and 13 of the 15 patients had at least one specimen positive. In a recent multi-center study of 107 patients with proven candidiasis, 81% had a positive result for BDG at a cut-off of 60 pg/ml and 78% had positive results at a cut-off of 80 pg/ml (18). Other studies have also reported low BDG sensitivity in different IFI and various patient populations. Koo *et al*. (24) and Ostrosky-Zeichner *et al*. (18), who both analyzed the performance of the BDG assay, regardless of the category of patients or type of IFI, observed an average sensitivity of 64%. Koo *et al*. (24) reported even lower BDG sensitivity in patients receiving a hematopoietic stem cell transplant or patients with febrile neutropenia (43% and 38%, respectively).

Specificity of the BDG test in our study was 62.5%, 100%, 87.5%, 100%; PPV was 88%, 100%, 66.67%, 100%, and NPV was 100%, 40%, 100% and 100% in groups I, II, III and IV, respectively. Specificity in group I was considerably lower than that reported by Pazos *et al*. [20] for adult patients with hematological cancer (89.6%). On the other hand, Digby *et al*. have reported positive BDG results in patients hospitalized in ICU with proven bacterial infections [26]. Since these investigators used very low (>20 pg/ml) cut-off values for a positive BDG marker, rather than the recommended cut-off (≥80 pg/ml), it is impossible to accurately interpret their results. In this study population, BDG demonstrated high sensitivity, but poor specificity. But, the high negative predictive value in our study (100%) and other studies as well (>95%) [26] show that their primary value, based on a negative result, is to exclude the presence of IFI instead.

False-positive BDG results have been previously described in many studies. Prattes *et al*. found that unmodified cellulose containing membranes increased the serum BDG levels. False-positive BDG results have also been confirmed after mucosal damage from chemotherapy or radiotherapy, which could allow BDG from dietary sources or from *Candida* colonizing the gastrointestinal tract to enter the bloodstream; as well after receipt of antibiotics from fungal sources [22]. False-positive BDG results are possible after treatment with immunoglobulin or antitumoral polysaccharides such as lentinan and schizophyllan [27], which can elevate BDG levels in the absence of IFI. In our study we also had patients on hemodialysis, but we had no relevant details on the background of the treatment, so we could not be certain if some of the elevated BDG levels actually demonstrated false positive results (data not shown). False-positive BDG results have also been described during some bacterial infections [19]. In our study we had few patients who presented with clinical signs of sepsis, with negative blood culture for fungi, but positive blood culture for bacteria (data not shown). There is a high probability that these were polymicrobial

infections that were also described in many studies and could be an additional reason for false positive results of the BDG test [28]. For all these reasons, BDG assay must be used and interpreted with a great caution. This phenolmenon should be carefully evaluated in further studies, where probably a serial monitoring of sera could clarify more precisely these dilemmas about false-positive results.

To summarize, our findings suggest that a positive (1,3)-β-D-glucan (BDG) assay could be a superior test in addition to the blood culture for diagnosis of candidemia and highlights the value of the BDG assay as a diagnostic adjunct in the serodiagnosis of an invasive candidiasis. This test may also be useful in the evaluation of patients at high risk of IFI. In clinical practice, proper use of this test would require knowledge of its performances and features, particularly for the factors associated with a false-positive test result.

Conflict of interest statement. None declared.

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