# *β-d-Glucan and* Candida albicans *germ tube antibody in ICU patients with invasive candidiasis*

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# β-D-Glucan and *Candida albicans* germ tube antibody in ICU patients with invasive candidiasis

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**Take-home message:** In unselected, non-neutropenic, critically ill patients, two consecutive measurements of (1→3)-β-D-glucan (BDG) greater than 80 pg/mL allowed discrimination between invasive candidiasis (IC) and high-grade *Candida* colonization, with a higher sensitivity (80 % vs. 65 %) and similar specificity compared with data previously reported in selected patients. Significant changes of BDG and *Candida albicans* germ tube antibody (CAGTA) kinetics in IC patients treated with antifungals were not observed (in most cases BDG levels remained persistently elevated); also, in patients neither colonized nor infected or with low-grade *Candida* spp. colonization, none of the confounding factors analyzed (treatment with amoxicillin–clavulanate, piperacillin–tazobactam, recent surgery, Gram-positive bloodstream infection, renal replacement therapy, enteral nutrition) was associated with a significant increase in BDG positivity.

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**Abstract** *Purpose:* To assess the performance of (1→3)-β-D-glucan (BDG) and *Candida albicans* germ tube antibody (CAGTA) for the diagnosis of invasive candidiasis (IC) in a prospective cohort of 107 unselected, non-neutropenic ICU patients. *Methods:* BDG (cutoff positivity ≥80 pg/mL) and CAGTA (cutoff positivity ≥1/160) assays were performed twice a week. Confounding factors included amoxicillin–clavulanate and piperacillin–tazobactam treatments, recent surgery, Gram-positive bloodstream infection, renal replacement therapy, and enteral nutrition. Patients were classified as neither colonized nor infected ($n = 29$), *Candida* spp. colonization ($n = 63$) (low grade, $n = 32$; high grade, $n = 31$), and invasive candidiasis (IC) ($n = 15$). *Results:* BDG levels were higher in patients with IC and high-grade colonization than in the remaining groups ($p = 0.012$), and two consecutive measurements ≥80 pg/mL discriminated IC from the remaining groups (sensitivity 80 %, specificity 75.7 %). For the discrimination between IC and *Candida* spp. colonization, the AUC for the maximum value of BDG was 0.667 (95 % CI 0.544–0.790) and for the maximum value of CAGTA 0.545 (95 % CI 0.395–0.694). Significant changes of BDG and CAGTA kinetics in IC patients treated with antifungals were not observed. In patients neither colonized nor infected or with low-grade *Candida* spp. colonization, none of the confounding factors was associated with a significant increase in BDG positivity. *Conclusions:* Two consecutive BDG levels ≥80 pg/mL allowed discrimination among IC and high-grade colonization. Systemic antifungal therapy could not be monitored with biomarker kinetics, and BDG levels were not subject to interference by confounding factors in either colonized or infected patients or with low-grade colonization.

**Keywords** Invasive candidiasis · *Candida* spp. colonization · (1→3)-ß-D-glucan · *Candida albicans* germ tube antibody · Enteral nutrition · Antifungal treatment · Biomarker kinetics

## Introduction

Invasive candidiasis (IC) not only remains a severe complication in ICU patients [1] but also an increase in the incidence and mortality of candidemia during the last decade has been reported [2, 3]. Timely administration of antifungals is crucial to improve prognosis but the diagnostic sensitivity of blood cultures is only about 50 %, which results in an increasing use of empirical or prophylactic antifungal treatment in patients without documented IC, favoring the development of resistance [4]. Non-culture diagnostic techniques based on serological biomarkers, such as (1→3)-β-D-glucan (BDG) [5–13] and anti-mycelium antibodies (*Candida albicans* germ tube antibody [CAGTA]) [14–18], have been introduced to improve the initial diagnosis of IC.

We investigated the following primary objectives: the performance of BDG and CAGTA for the diagnosis of IC, the kinetics of these biomarkers to monitor systemic antifungal treatment, and the influence of confounding factors on BDG levels in unselected, non-neutropenic ICU patients.

## Patients and methods

### Design and study population

Between 1 January 2011 and 30 December 2012, unselected, non-neutropenic critically ill patients admitted for at least 7 days to a medical-surgical ICU in Sevilla, Spain, were included in a prospective, cohort, and observational study. The study protocol was approved by the institutional review board, and informed consent was obtained from the patients or their legal representatives. At ICU entry, patients with neutropenia (total leukocyte count $<1000/mm^3$) were excluded as were those with other conditions (see Supplementary Material).

### Screening, microbiological cultures, and *Candida* score

Surveillance cultures for the screening *Candida* spp. were performed twice a week from the seventh day of ICU admission (see Supplementary Material for details of sites where samples were obtained and microbiological cultures). Results were considered positive in the presence of *Candida* growth in the culture medium. Identification at the species level was required. *Candida* score [19] was calculated on collection of samples and once culture data were available.

### Serological biomarkers and BDG confounding variables

Blood samples (15 mL) were collected in three tubes without anticoagulant, centrifuged at 1800 rpm for 10 min, separated into aliquots, and stored at −80 °C until analysis. The BDG assay (Fungitell®, Associates of Cap Cod Inc., Easy Falmouth, MA, USA) was performed according to the manufacturer's recommendations. The cutoff value is 80 pg/mL. CAGTA detection was performed by an immunofluorescence test (Vircell® kit assay, Granada, Spain) according to the manufacturer's instructions. The cutoff value for positive CAGTA is ≥1/160. For each patient, maximum values recorded for *Candida* score and serologic biomarkers at or before the episode of IC were used in the analysis. When an episode of IC did not develop, the highest value of all recorded values was used. None of the tests were performed in real time and, therefore, were not available for clinician's decision-making.

Variables reported that may affect serum levels of BDG at the time of blood sampling and within 3 days pre- and post-sampling were collected, including antimicrobials (amoxicillin–clavulanate, piperacillin–tazobactam), recent surgery (within 3 days after a major procedure), Gram-positive bloodstream infection, renal replacement therapy, and enteral nutrition. BDG levels in the different products for enteral nutrition were determined. A total of nine separate batches of commonly used enteral diets in our ICU were tested. For each batch, an aliquot was processed using the same procedure as that applied to serum. For evaluating the effects of enteral nutrition, patients who received the selected diet for at least 5 days were included in the analysis.

### Study protocol and collection of data

Once the patient was included in the study, the following data were recorded twice a week and for four consecutive weeks until ICU discharge or death: surveillance culture, Acute Physiology and Chronic Health Evaluation (APACHE II) score, Sepsis-related Organ Failure Assessment (SOFA) score, *Candida* score, presence or absence of sepsis, severe sepsis, or septic shock, and BDG-related confounding variables, and results of BDG and CAGTA. Blood samples for the measurement of BDG and CAGTA were drawn at the same time periods. Patients were followed until ICU and/or hospital discharge, or death. Other variables recorded are detailed in the Supplementary Material.

### Definitions

*Candida* colonization was considered unifocal when *Candida* spp. were isolated from one site and multifocal when *Candida* spp. were simultaneously isolated from various noncontiguous sites, even if two different *Candida* spp. were isolated. Low-grade *Candida* colonization was defined when *Candida* spp. were isolated in one or more foci, in one or two consecutive surveillance controls. High-grade *Candida* spp. colonization was defined as colonization of at least three body sites on two or more consecutive screening days [20]. Invasive candidiasis was defined as (1) primary candidemia (presence of *Candida* spp. in one or more blood cultures obtained from peripheral veins), (2) intra-abdominal candidiasis, and (3) catheter-related bacteremia. The definitions of intra-abdominal candidiasis [21] and catheter-related candidemia are described in the Supplementary Material.

Patients were classified into the groups of neither colonized nor infected, low-grade *Candida* spp. colonization, high-grade *Candida* spp. colonization, and IC. In the presence of candidemia, catheters were removed and fundoscopy was performed.

### Statistical analysis

The percentages were compared using the Chi square ($\chi^2$) test, the means by the $F$ test, and the medians by the Kruskal–Wallis test. In order to evaluate the diagnostic performance for IC of the maximum values of BDG and CAGTS receiver operating characteristics (ROC) curves were calculated and the areas under the curves (AUCs) were estimated by means of the 95 % confidence intervals (CI). For the BDG thresholds of 80 pg/mL, the sensitivity, specificity, and predictive values were obtained. Statistical significance was set at $p < 0.05$.

## Results

### Study population and salient findings

Of 148 eligible patients, 41 (27.7 %) were excluded. The flowchart of the study population and reasons for exclusion are shown in Fig. 1. The study population included 107 patients (66.4 % men, mean age 62.7 years). There were 29 patients in the neither colonized nor infected

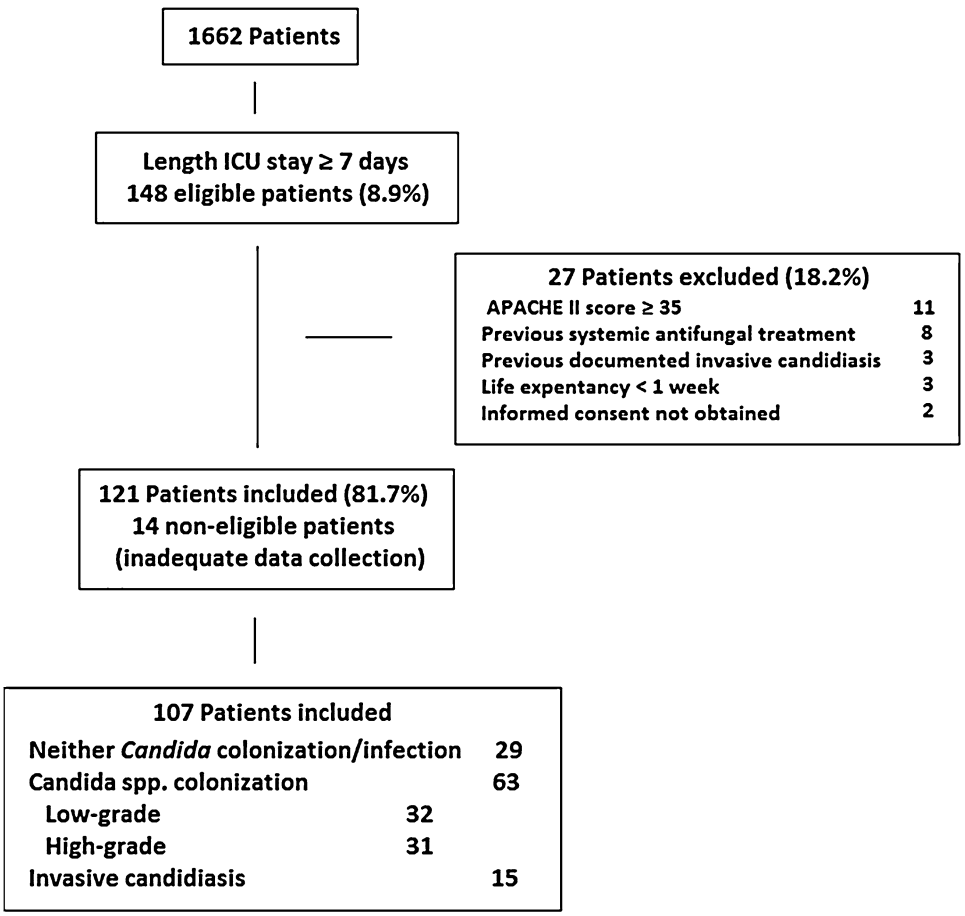


**Fig. 1** Flowchart of the study patients (*ICU* intensive care unit)

**Table 1** Results of *Candida* score, BDG levels, and CAGTA positivity in the four study groups

| Data | Neither colonized nor infected ($n = 29$) | *Candida* spp. colonization | | Invasive candidiasis ($n = 15$) | *P* value |
|---|---|---|---|---|---|
| | | Low grade ($n = 32$) | High grade ($n = 31$) | | |
| Age, years, mean (±SD) | 62.6 ± 16.9 | 64.4 ± 14.9 | 59.0 ± 14.7 | 66.7 ± 10.4 | 0.339 |
| Male/female (%) | 72.4/27.6 | 71.9/28.1 | 51.6/48.4 | 73.3/26.7 | 0.235 |
| ICU admission, mean (±SD) | | | | | |
| APACHE II | 17.4 ± 6.7 | 18.4 ± 5.9 | 17.4 ± 5.4 | 17.8 ± 7.7 | 0.721 |
| SOFA | 9.2 ± 3.3 | 9.3 ± 4.4 | 8.9 ± 3.3 | 8.0 ± 4.5 | 0.559 |
| *Candida* score, median (IQR) | 3 (2–3)[a] | 3 (2–4)[a,b] | 4 (3–4)[c] | 4 (3–4)[b,c] | <0.001 |
| CAGTA positivity, no. (%) | 6 (20.7)[a] | 13 (40.6)[a] | 22 (71.0)[b] | 5 (33.3)[a] | <0.001 |
| BDG, pg/mL, median (IQR) | 85 (31–237)[a] | 47 (31–240)[a] | 250 (83–3450)[b] | 242 (90–525)[a,b] | 0.006 |
| BDG ≥ 80 pg/mL, no. (%) | 17 (58.6)[a,b] | 13 (40.6)[a] | 24 (77.4)[b] | 12 (80.0)[a,b] | 0.009 |
| BDG in two consecutive measurements ≥ cutoff pg/mL, no. (%) | ($n = 22$) | ($n = 21$) | ($n = 31$) | ($n = 15$) | |
| 80 | 1 (3.4) | 4 (12.5) | 13 (41.9) | 12 (80.0)[†] | <0.001 |
| 100 | 1 (3.4) | 3 (9.4) | 10 (32.3) | 10 (66.7) | <0.001 |
| 200 | 1 (3.4) | 2 (6.2) | 7 (22.6) | 8 (53.3) | <0.001 |
| 300 | 0 | 0 | 7 (22.6) | 7 (46.7) | <0.001 |

Superscripts indicate statistically significant differences ($p < 0.05$) (a vs. b; a vs. c; b vs. c)
*CAGTA Candida albicans* germ tube antibodies, *BDG* (1→3)-β-D-glucan
[†] In five patients, the biomarker anticipated the event

**Table 2** Sensitivity, specificity, and positive and negative predicted values of BDG positivity for the threshold of 80 pg/mL in 89 patients who had at least two determinations

| | BDG ≥80 pg/mL at least in one measurement | BDG ≥80 pg/mL in two consecutive measurements |
|---|---|---|
| Sensitivity (%) | 80.0 (51.9–95.7) | 80.0 (51.9–95.7) |
| Specificity (%) | 44.6 (34.2–55.3) | 75.7 (64.3–84.9) |
| Negative predictive value (%) | 93.2 (82.9–94.3) | 95.9 (89.3–98.5) |
| Positive predictive value (%) | 19.0 (14.7–24.3) | 34.9 (25.0–46.3) |

95 % confidence intervals in parentheses

group, 63 in the *Candida* spp. colonization group (low grade 32, high grade 31), and 15 in the IC group. Characteristics of the study population according to colonization and infection status as well as risk factors and details of antifungal treatment are shown in Tables 1 and 2 of the Supplementary Material.

## Serological biomarkers and *Candida* score

A total of 465 measurements of biomarkers were performed (4.3 per patient). Fifty-six measurements of BDG were >523 pg/mL and were retested for quantification as were 91 samples to confirm the initial value, with a reliability of 87.9 %.

Maximum values of *Candida* score and BDG as well as the number of patients with positive CAGTA and BDG levels ≥80 pg/mL are shown in Table 1. There were statistically significant differences in the distribution of *Candida* score, CAGTA positivity, median BDG levels, and number of patients with positive BDG (≥80 pg/mL) among the groups of neither colonized nor infected, low-grade and high-grade *Candida* spp. colonization, and IC. However, significant differences in the *Candida* score, BDG level, and number of patients with BDG levels ≥80 pg/mL between the groups of IC and high-grade *Candida* spp. colonization were not found.

The number of patients with positive CAGTA was significantly higher among patients with high-grade *Candida* spp. colonization than in the remaining groups (Table 1). However, in the subgroup of surgical patients, differences between CAGTA-positive and CAGTA-negative patients were not found (30 vs. 40.4 %, $p = 0.351$). Also, ICU mortality rate was similar in patients with positive and negative CAGTA values (26 vs. 40.4 %, $p = 0.177$).

For the discrimination between IC and *Candida* spp. colonization, the AUC for the maximum value of BDG was 0.667 (95 % CI 0.544–0.790) and for the maximum value of CAGTA was 0.545 (95 % CI 0.395–0.694) (see

ROC curves in the Supplementary Material). For a threshold of BDG ≥ 80 pg/mL at least on one measurement, the sensitivity was 80 % (95 % CI 51.9–95.7) and the specificity 44.6 % (95 % CI 34.2–55.3). However, when BDG levels ≥80 pg/mL were found in two consecutive measurements, the sensitivity was maintained at 80 % but the specificity increased to 75 % (95 % CI 64.3–84.9) (Table 2).

## BDG and CAGTA kinetics

Kinetics of biomarkers in the 13 patients with IC treated with systemic antifungal therapy (azoles in 7 patients, echinocandins in 4, and liposomal amphotericin B in 2) are shown in Table 3. There were six patients with negative CAGTA in all determinations, whereas CAGTA values were positive before diagnosis and before starting antifungal therapy in two patients each (case nos. 103 and 111). In relation to BDG measurements, all values were negative in only one patient (case no. 83). Five patients had two consecutive BDG increases >80 pg/mL before culture-based diagnosis of IC (case nos. 38, 60, 90, 98, and 111) and six patients had positive results before starting antifungal therapy (case nos. 38, 60, 69, 90, 98, and 111). Significant changes in the time-course levels of both biomarkers in patients with IC treated with antifungals as a whole or separately by type of antifungal agent were not observed.

## BDG confounding factors

Levels of BDG in the nine enteral feeding diets analyzed are shown in Table 4 (top panel). In three products, the BDG content was higher than 500,000 pg/mL, where as in the remaining six products, ranged between 17,300 and 331,000 pg/mL.

In relation to the number of confounding factors, one factor was present in 43 patients, two factors in 26, three factors in 7, four factors in 5, and five factors in 2. Table 4 (bottom panel) shows data of the 61 patients, neither colonized nor infected ($n = 29$) or with low-grade *Candida* spp. colonization ($n = 32$) in which the increase in BDG could not be attributed to the presence of IC or high-grade *Candida* spp. colonization. Of these 61 patients, the cutoff level of ≥80 pg/mL was not surpassed in 56, whereas in the remaining 5 patients (case nos. 37, 42, 48, 77, and 112), two consecutive BDG values ≥80 pg/mL were registered. In two patients (case nos. 37 and 42), enteral nutrition was the only confounding variable, and in one patient (case no. 112), two confounding factors

**Table 3** CAGTA and BDG kinetics in 13 patients with invasive candidiasis (IC) treated with systemic antifungals

| Case no. | *Candida albicans* germ tube antibody (CAGTA) levels | | | | | | | | Type of *Candida* isolated |
|---|---|---|---|---|---|---|---|---|---|
| 26 | NEG | NEG | *NEG* | *1/320* | *NEG* | | | | *C. parapsilosis* |
| 38 | NEG | *1/320* | *1/1280* | *1/320* | | | | | *C. parapsilosis* |
| 44 | | *1/320* | *1/320* | *1/320* | | | | | *C. albicans* |
| 57 | *NEG* | *NEG* | *NEG* | | | | | | *C. glabrata* |
| 60 | NEG | NEG | NEG | NEG | NEG | NEG | *NEG* | *NEG* | *C. parapsilosis* |
| 69 | NEG | *NEG* | *NEG* | *NEG* | *NEG* | | | | *C. tropicalis* |
| 81 | *NEG* | *NEG* | *1/320* | *1/640* | *1/1280* | *1/1280* | *1/1280* | *1/640* | *C. glabrata* |
| 83 | NEG | *NEG* | *NEG* | NEG | NEG | NEG | NEG | | *C. parapsilosis* |
| 90 | NEG | NEG | NEG | *NEG* | *NEG* | NEG | NEG | | *C. albicans* |
| 91 | *NEG* | *NEG* | NEG | NEG | NEG | | | | *C. parapsilosis* |
| 98 | NEG | NEG | NEG | *NEG* | *NEG* | *NEG* | *1/160* | *NEG* | *C. glabrata* |
| 103 | 1/1280 | *1/1280* | *1/1280* | *1/1280* | *1/1280* | *1/1280* | | | *C. albicans* |
| 111 | 1/640 | NEG | 1/160 | 1/160 | *1/640* | *1/640* | *1/640* | | *C. glabrata* |
| Case no. | (1→3)-β-D-Glucan (BDG) levels | | | | | | | | Type of *Candida* isolated |
| 26 | 31 | 31 | *100* | *120* | *85* | | | | *C. parapsilosis* |
| 38 | 31 | 31 | 80 | *188* | | | | | *C. parapsilosis* |
| 44 | *150* | *180* | *90* | *200* | | | | | *C. albicans* |
| 57 | *450* | *300* | *300* | | | | | | *C. glabrata* |
| 60 | 31 | 80 | 80 | 31 | 40 | 31 | 31 | *31* | *C. parapsilosis* |
| 69 | 550 | *560* | *31* | *31* | *31* | | | | *C. tropicalis* |
| 81 | *450* | *300* | *150* | *400* | *300* | *500* | *500* | *500* | *C. glabrata* |
| 83 | 31 | *31* | *70* | 31 | 31 | 55 | 31 | | *C. parapsilosis* |
| 90 | 31 | 31 | 1260 | *1400* | *31* | 31 | 31 | | *C. albicans* |
| 91 | *31* | *31* | 82 | 31 | | | | | *C. parapsilosis* |
| 98 | 500 | 500 | 270 | *153* | *250* | *1120* | *3340* | *7700* | *C. glabrata* |
| 103 | *242* | *1390* | *4880* | *427* | *305* | *4000* | | | *C. albicans* |
| 111 | 31 | 31 | 570 | 250 | *500* | *2390* | *383* | | *C. glabrata* |

Biomarker levels during systemic antifungal therapy are marked in italics

**Table 4** Content of BDG of various enteral feeding products (top panel) and influence of potential confounding factors on BDG levels in neither colonized nor infected patients ($n$ = 29) or with low-grade *Candida* spp. colonization ($n$ = 32) (bottom panel)

| Product | Batch number | Fluid volume (mL) | BDG level (pg/mL) |
|---|---|---|---|
| Nutrison Multi-fibre (Nutricia Medical Nutrition) | 101136166 | 500 | >500,000 |
| Nutrison Advanced Diason (Nutricia Medical Nutrition) | 29HDO637 | 500 | >500,000 |
| Novasource Diabet plus (Nestlé Healthcare Nutrition) | 22298428200 | 500 | >500,000 |
| Osmolite HN (Abbott Laboratories) | 100-907-411 | 500 | 331,000 |
| Nutrison Advanced Peptisorb (Nutricia Medical Nutrition) | 04121114 | 500 | 136,000 |
| Atempero Enteral (Vegenat, S.A.) | F332.2 | 500 | 68,000 |
| Ensure HN (Abbott Laboratories) | 217T5NR | 500 | 48,200 |
| Insosurce Protein (Nestlé Healthcare Nutrition) | 2275428200 | 500 | 31,400 |
| Promote (Abbott Laboratories) | 24438NR | 500 | 17,300 |

| Factor | BDG levels exceeded the cutoff of 80 pg/mL twice consecutively | | |
|---|---|---|---|
| | No ($n$ = 56) | Yes ($n$ = 5) | $P$ value* |
| Amoxicillin–clavulanate | 4 (7.1) | 0 | 1 |
| Piperacillin–tazobactam | 3 (5.4) | 0 | 1 |
| Recent surgery | 8 (14.3) | 1 (20) | 0.563 |
| Renal replacement therapy | 5 (8.9) | 1 (20) | 0.415 |
| Gram-positive bloodstream infection | 3 (5.4) | 0 | 1 |
| Enteral nutrition | 26 (46.4) | 2 (40) | 1 |

Data are frequencies (%)
*Fisher test

were present: recent surgery and renal replacement therapy. Statistical analysis did not show the influence of these variables on BDG levels.

## Discussion

In this study, two consecutive measurements of BDG ≥80 pg/mL, 24–48 h apart, allowed one to diagnose IC. This observation is very useful to discriminate between patients with IC and patients with *Candida* spp. colonization, particularly high-grade colonization, in which a single measurement of BDG ≥80 pg/mL may be incorrectly interpreted as *Candida* infection. Also, a single measurement of BDG cannot differentiate IC, particularly candidemia, from patients with a high-grade colonization in which "occult" candidemia undiagnosed by standard methods may be present [22]. In this scenario, two consecutive BDG positive assays may help to better discriminate between IC and high-grade colonization. Additionally, it has been suggested that translocation of BDG into the bloodstream may explain BDG antigenemia in colonized patients without culture-based evidence of IC [9]. As *Candida* infection is a dynamic process, repeated testing of biomarkers during ICU admission is required. The occurrence of two or more sequential concentrations of ≥80 pg/mL (positive test) has been found to give the best overall option for diagnosis [6, 23, 24]. Tissot et al. [9] in a study of selected patients (89 patients at high risk of intra-abdominal candidiasis) showed that sensitivity and specificity of two consecutive BDG measurements ≥80 pg/mL were 65 and 78 %, respectively, with an area under the ROC curve of 0.74. In the present study of unselected ICU patients, we obtained a higher sensitivity (80 %) and similar specificity (75.7 %). Other studies also showed that two consecutive positive samples increases specificity and positive predictive values without affecting sensitivity [9, 11, 23, 24].

Another interesting aspect relates to whether detectable BDG antigenemia will develop prior to the onset of clinical symptoms or after positive blood cultures. In our study, of the 12 patients with IC and two consecutive BDG-positive samples, the first increases before culture-based diagnosis of IC (5 patients) and the start of antifungal therapy (6 patients) occurred at a median of 8 (3–17) and 7 (2–22) days, respectively, as compared to 5 and 6 days in the study by Tissot et al. [9]. These differences may be explained by the type of patients and IC: surgical patients and intra-abdominal candidiasis (29 episodes, two of them with associated candidemia) in the study by Tissot et al. [9] as compared to medical-surgical patients and a predominance of candidemias ($n$ = 10) over intra-abdominal candidiasis ($n$ = 5) in our study.

Assessment of BDG kinetics following the start of antifungal treatment in patients with proven or probable IC may be useful as a prognostic marker for patient response. Some authors have reported that a decrease of BDG values in IC patients is associated with treatment success [9, 25, 26]. In contrast, increasing BDG levels have been associated with treatment failure or fatal outcome [9]. However, recent investigations on BDG for

monitoring response of IC to antifungal therapy have shown that BDG clearance is slow [27, 28]. In our study, significant changes of BDG kinetics in IC patients treated with antifungals were not found, and in most cases BDG levels remained persistently elevated. The presence of a central venous catheter that was involved in candidemia or the presence of renal failure might have caused a slow clearance of BDG [29]. In our study, among 13 patients with IC treated with antifungal agents, only two had catheter-related bacteremia and five had renal failure and required renal replacement therapy. In both cases, differences in BDG kinetics as compared with IC patients without catheter-related bacteremia or renal replacement therapy were not observed. These inconsistent findings are not surprising as the precise pharmacokinetics of release and the route of BDG elimination remain unclear.

The greatest limitation of BDG testing is its poor specificity [13, 30]. Several factors that may increase BDG levels for reasons other than invasive fungal infection have been identified [12], including hemodialysis with cellulose membranes, thrombocyte infusion with leukocyte-removing filters, the administration of human blood products (immunoglobulins or albumins), the use of antibiotics such as amoxicillin–clavulanate or piperacillin–tazobactam [31, 32], the presence of serious bacterial infections [12, 33], the use of surgical gauzes containing glucan, and severe mucositis [11] and possibly enteral nutrition for their BDG content. We assessed the influence of treatment with amoxicillin–clavulanate, piperacillin–tazobactam, recent surgery, Gram-positive bloodstream infection, renal replacement therapy, and enteral nutrition in the group of neither colonized nor infected and low-grade *Candida* spp. colonization. None of these variables was associated with a significant increase in BDG positivity. Recent studies have also shown that BDG levels are not affected by the use of antibiotics, such as ampicillin–sulbactam and piperacillin–tazobactam [32, 34] or the presence of Gram-positive bacteremia (in hematology patients) [35, 36]. In six healthy volunteers given different dose regimens of the nutritional supplement ImunixX (iXX Pharma), in which the active substance is 1,3-β-D-glucan, and using the Fungitell® assay, all BDG levels were negative [37].

In the present study, CAGTA-positive results were significantly more frequent in the group of high-grade *Candida* spp. colonization than in the remaining groups, but this biomarker was not useful for differentiating high-grade colonization from IC. We can speculate that heavily colonized patients before development of IC may present an immunological response with an increase in specific antibodies. In a previous study of our group in patients with severe abdominal conditions, CAGTA showed a sensitivity of 71 %, specificity of 57.3 %, positive predictive value of 38.6 %, and negative predictive value of 83.9 %, with an area under the ROC curve of 0.670 for the diagnosis of IC. These values improved with the combined use of CAGTA and BDG. The characteristics of patients in both studies, patients with severe abdominal conditions vs. unselected patients, may account for the differences between the studies. There is limited information on the usefulness of CAGTA in critically ill patients. There are only two previous reports by the same group [15, 16] assessing the predictive value of CAGTA for IC in a cohort of 53 critically ill non-neutropenic patients in whom 23 (43.3 %) were highly colonized; 22 patients (41.5 %) had CAGTA-positive results. The sensitivity and specificity of CAGTA detection were not established in these studies because none of the patients had a positive blood culture for *Candida*. Other findings of these studies included a higher rate of CAGTA-positive results in patients with previous surgery and a significantly lower ICU-related mortality among CAGTA-positive patients. These results were not confirmed in our study.

In relation to the kinetics of this biomarker, relevant changes in the titers of CAGTA in IC patients treated with antifungal agents were not observed. Zaragoza et al. [38] reported three patterns in 53 critically ill non-neutropenic patients: increasing titers (31.8 %), decreasing titers (36.4 %), and no change in titer kinetics (22.8 %), and found that statistically significant lower mortality rates were found in patients with patterns of increasing CAGTA titers who had been treated with antifungal agents.

Potential limitations of the study include the small sample size, which in turn is not unusual in diagnostic studies exploring the value of serum biomarkers for the detection of fungal infections in the ICU setting, and the fact that BDG testing was performed in batches or frozen samples and we cannot exclude that some negative results may have stemmed from sample instability.

In summary, two consecutive measurements of BDG ≥80 pg/mL obtained at 48-h intervals allow clinicians to discriminate high-grade *Candida* spp. colonization from IC and, consequently, to ensure more appropriate recommendations of early antifungal treatment. Clinicians can also rely on positivity of BDG values because potential confounding factors, frequently present in ICU patients, did not appear to affect BDG-positive tests. It is important to note that the very high content of BDG identified in some products commonly used for enteral nutrition did not influence the plasma levels of this biomarker. The use of CAGTA as the only biomarker is not useful to differentiate *Candida* spp. colonization from IC. Antifungal use did not impact on kinetics of BDG and CAGTA. These are important clues that are ready to be used for the diagnosis of IC in non-neutropenic critically ill patients in daily practice.

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