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Combination of *Candida* biomarkers in patients receiving empirical antifungal therapy in a Spanish tertiary hospital: a potential role in reducing the duration of treatment

M. Carmen Martínez-Jiménez[1,2], Patricia Muñoz[1–4]*, Maricela Valerio[1,2], Antonio Vena[1,2], Jesús Guinea[1–3] and Emilio Bouza[1–4]

[1]*Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain;* [2]*Instituto de Investigación Sanitaria del Hospital Gregorio Marañón, Madrid, Spain;* [3]*CIBER Enfermedades Respiratorias-CIBERES (CB06/06/0058), Madrid, Spain;* [4]*Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain*

*Corresponding author. Servicio de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, C/ Dr Esquerdo 46, 28007 Madrid, Spain. Tel: +34-91-586-6725; Fax: +34-91-504-4906; E-mail: pmunoz@hggm.es

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Objectives: Initiation of empirical antifungal therapy for invasive candidiasis (IC) is usually based on clinical suspicion. Serological biomarkers have not yet been studied as a means of ruling out IC. We evaluated the potential role of two combined biomarkers in stopping unnecessary antifungals in patients at risk of IC in the ICU and in other wards.

Methods: This was a prospective observational study including adults starting empirical antifungal treatment for suspected IC, at Gregorio Marañón Hospital, Madrid (Spain). Patients were stratified according to admission department (ICU or other wards) and final diagnosis (no IC or proven or probable IC). Type of candidiasis (candidaemia or deep-seated candidiasis) was also considered. The *Candida albicans* germ tube antibody (CAGTA) test and the β-D-glucan (BDG) test were performed on serum samples collected by venepuncture on days 0, 3 and 5 after starting empirical antifungal therapy.

Results: Sixty-three ICU patients and 37 non-ICU patients were included. High-risk gastrointestinal surgery and sepsis in non-surgical patients were the main indications for empirical treatment (30% each). Patients had no IC (58%), proven IC (30%) or probable IC (12%). Overall, sensitivity and negative predictive value of the combination of both the CAGTA test and the BDG test were 97% for the entire population. The best performance was observed in ICU patients (sensitivity and negative predictive value of 100%). Among patients without IC, all biomarkers were negative in 31 patients.

Conclusions: Serial determination of CAGTA/BDG during empirical antifungal therapy has a high sensitivity and negative predictive value. If properly confirmed, this strategy could be used to discontinue antifungal treatment in at least 31% of patients as a complementary tool in antifungal stewardship programmes.

Introduction

Overuse of antifungal drugs is explained partially by the high morbidity and mortality associated with candidaemia and invasive candidiasis (IC). In addition, conventional microbiological techniques are slow and lack sensitivity.[1,2] In most patients with suspected IC, blood cultures remain negative 5 days after initiation of antifungal agents.[3,4] However, clinicians still find it difficult to decide when it is safe to stop empirical antifungal therapy even in patients with negative cultures, since a potential role of the antifungal drug is difficult to exclude in patients with either good or poor evolution.

Non-culture diagnostic techniques based on serological biomarkers, such as anti-mycelium antibodies [*Candida albicans* germ tube antibody (CAGTA) test], (1→3)-β-D-glucan (BDG), mannan antigen and anti-mannan antibodies, have been tested to improve the initial diagnosis of IC, but not to exclude it.[5–7] Besides, there is little information on the behaviour of biomarkers in patients with deep-seated candidiasis and negative blood cultures.[7] The limitations of individual tests include false positives and negatives and difficult interpretation of indeterminate results.[8] The value of combining tests to evaluate continuation of empirical antifungal treatment after 5 days of therapy has not been assessed.

Our aim was to evaluate the diagnostic value of the combination of two *Candida* biomarkers, CAGTA and BDG,[9] in ruling out disease in patients receiving empirical therapy for suspected IC.

Methods

We performed an observational, prospective study from June 2012 to January 2014 in Gregorio Marañón General Hospital (Madrid, Spain), serving a population of ∼715000 inhabitants. Inclusion criteria were the following: non-pregnant adult patients with non-haematological conditions receiving systemic empirical antifungal treatment for suspected IC according to local and international guidelines,[10–13] who agreed to participate after giving their written informed consent. Exclusion criteria were the following: known proven IC at inclusion; previous antifungal therapy for >5 days; or therapy to treat invasive fungal infections other than IC.

Serum samples were collected by venepuncture on days 0, 3 and 5 after starting antifungal treatment to determine CAGTA and BDG. The biomarker results were not reported to the attending clinicians. Routine microbiological tests (blood culture, catheter culture and culture of other clinical samples) and radiological tests were also performed according to conventional clinical practice and local guidelines. At the end of the study, each patient's history was independently reviewed by three investigators blind to the biomarker results. If the classification of the cases was discordant or doubtful, then episodes were reconsidered and classified in a clinical session (unanimous agreement was required). The results were analysed for the whole cohort and also after stratification according to whether the patient had been admitted to a critical care unit or to a medical or surgical ward.

Clinical and microbiological data were prospectively collected using a pre-established protocol that included: patients' demographics; risk factors for IC; severity of illness at the time of study inclusion; sepsis criteria; type and length of antifungal therapy; results of radiological and microbiological tests; overall mortality during hospitalization; and length of hospital stay. As for microbiological variables, we collected the type of IC, origin of infection and species isolated.

Possible reasons for false-positive BDG results (albumin administration,[14–16] intravenous immunoglobulin,[15–18] β-lactam antibiotics,[19] transfusion of blood products,[15,16,18,20–22] renal replacement therapy,[15,16] abdominal surgery,[15,16] bacteraemia[15,16,23] and bilirubin >10×1.1 mg/dL[15]) were also collected. Patients from whom fewer than three serum samples were obtained were excluded from the analysis.

In order to explore the role of biomarkers in clinical settings with different pre-test probabilities of having fungal infection, we further classified our patients according to their main pathophysiological pattern into the four groups suggested by Eggimann and Pittet[24] and Leon *et al*.:[25] immunosuppressed patients (onco-haematological patients and solid organ transplant recipients); high-risk digestive surgery patients (including tertiary peritonitis, anastomotic leakage, recurrent digestive perforation and necrotizing pancreatitis); other abdominal surgery patients; and other patients (non-abdominal surgery and medical patients).

Definitions

Proven IC was defined as identification of *Candida* species in blood cultures or positivity in histopathological, cytopathological or microscopic examination of normally sterile clinical samples obtained by biopsy or needle aspiration and/or recovery of *Candida* species by culture of a sample obtained by means of a sterile procedure from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease.[13]

Intra-abdominal candidiasis was defined as the recovery of *Candida* spp. from a deep intra-abdominal sample obtained by a sterile procedure (including surgery, puncture and drains placed for <24 h) in patients showing clinical or radiological abnormalities consistent with an infectious disease process.[26]

Probable IC was defined as fever (≥38.5°C) or hypothermia (<35°C) with leucocytosis (white blood cell count ≥12000 cells/mm^3) and/or haemodynamic instability (mean arterial pressure <65 mmHg) otherwise unexplained despite at least 3 days of broad-spectrum antibiotics and both of the following: (i) *Candida* spp. isolated from at least two non-sterile sites (±3 days); and (ii) no alternative microbiological diagnosis. Symptomatic urinary tract infection due to *Candida* spp. with no other microbiological diagnosis and endoscopically visualized oesophagitis with histological and microbiological biopsy exclusion of other causes than *Candida* on microscopy or culture were also considered probable IC.[16]

IC was excluded when the criteria for proven and probable IC were not fulfilled and/or another diagnosis was established.

Patients with IC were further classified into candidaemia without deep-seated *Candida* infection (mainly catheter-related candidaemias), IC without candidaemia (mainly intra-abdominal infections) and IC with candidaemia.[27] Deep-seated candidaemia was defined as the presence of at least one positive blood culture with another positive specimen from a normally sterile site related to a specific focus of infection.

Microbiological methods

Identification of *Candida* spp

Candida species were identified using ID 32C (bioMérieux, Marcy-l'Étoile, France).

Serological detection of CAGTA

Serum samples were processed according to the manufacturer's recommendations (Vircell Microbiologist S.L., Granada, Spain). Samples were considered positive above a cut-off ≥1/160.

Serological detection of BDG

The Fungitell® assay was performed according to the manufacturer's instructions and BDG concentrations were read and analysed with a BioTek ELX808™ Microplate Reader and GEN5 Software (BioTek U.S., VT, USA). The cut-offs for BDG proposed by the manufacturer were as follows: positive, ≥80 pg/mL; indeterminate, ≥60 to <79 pg/mL; and negative, <60 pg/mL. We considered BDG to be positive if the value was ≥80 pg/mL and negative if the value was <80 pg/mL, so that indeterminate results were classified as negative results.[9]

Data analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated on days 0, +3 and +5 for each biomarker independently and in combination.

Two possible models were used to ensure optimal sampling. In model 1 (non-restrictive), the biomarkers were considered positive if any of the six determinations (CAGTA and BDG on days 0, +3 and +5) was positive. In model 2 (partially restrictive model), at least two consecutive positive CAGTA and/or BDG results were required.[7,16,25] Both models were assessed for each biomarker independently and for the combination of CAGTA and BDG.

The performance of the biomarkers was analysed in patients with proven IC, proven plus probable IC and no IC. When we analysed the 'only proven IC model', probable cases were included in the no IC group.

We used a two-tailed Fisher exact test to study the correlation between CAGTA and/or BDG positivity in the total population and by risk groups. Normally distributed values are expressed as mean ± SD, whereas non-normally distributed values are expressed as median (IQR). Receiver operating characteristic (ROC) curves were plotted to compare model 1, model 2 and CAGTA and BDG combinations for each timepoint (days 0, 3 and 5 from starting empirical antifungal therapy). A *P* value <0.05 was considered statistically significant.

Data were entered in Access 2007, and IBM SPSS Statistics for Windows, version 20.0 (IBM, Corp., Armonk, NY, USA) was used to perform the statistical analysis.

Ethics approval and patient consent/privacy

The study was approved by the local ethics committee (CEIC-A1, ref. 247/13) and the Spanish Agency for Medicines and Health Care Products (Agencia Española del Medicamento y Productos Sanitarios, AEMPS).

Results

A total of 260 patients received empirical antifungal treatment during the study period. Of these, 160 were not included in the final analysis for the following reasons: 27 (10.4%) did not sign the informed consent; 56 (21.5%) had fewer than three serum samples (due to death, life expectancy <48 h and transfer to another hospital or hospital discharge); 47 (18.1%) had demonstrated IC at inclusion; and 30 (11.5%) had invasive fungal infections other than candidiasis.

The main demographic and clinical characteristics of the 100 patients included are shown in Table 1. Most patients were male (67%) and the mean age was 60.8 ± 15.7 years. Overall, 63 patients were admitted to the ICU (44% surgical and 19% medical) and 37 to other wards (13% surgical and 24% medical). The main indications for empirical antifungal prescription were high-risk gastrointestinal surgery and sepsis in non-surgical patients (30% each one). The main underlying conditions and the reasons for potential false-positive results with the biomarkers are shown in Table 1.

The 100 patients were finally classified as no IC (58), proven IC (30) and probable IC (12). *C. albicans* accounted for 60% of the isolates in proven IC, mainly in the ICU (88.9% of cases). The diagnosis of proven IC was more frequent in non-ICU patients (56.7%) than in ICU patients (14.3%) ($P<0.001$). IC without candidaemia (26/30), mostly due to intra-abdominal infections (66.7%), was the principal type of IC found in our study (Table 1). Echinocandins accounted for 73% of empirical therapy (84.1% in the ICU and 54.0% in other wards), and median (IQR) duration of antifungal therapy was 10.0 (6.0–14.0) days. The duration of antifungal therapy in patients without candidiasis was 8.5 (4.0–12.0) days. Overall mortality was 31% (42.9% in ICU patients versus 10.8% in other wards).

Finally, Table 1 shows the efficacy of the combination of biomarkers in ICU patients and non-ICU patients following models 1 and 2. The combination of CAGTA and BDG yielded a sensitivity of 100% in ICU patients and 95.2% in non-ICU patients with model 1. The sensitivity in model 2, however, was 88.9% in the ICU and 80.9% in non-ICU wards.

Table 2 and Table S1 (available as Supplementary data at *JAC* Online) show the behaviour of biomarkers alone and in combination and considering IC as either proven or both proven and probable. As for CAGTA, sensitivities ranged from 53.3% to 60.0% and NPVs from 72.7% to 83.6% when results were evaluated on the three different days and when only proven or proven plus probable cases were considered. No clear improvement in sensitivity and NPV was demonstrated when the three days were analysed together with model 1 or with model 2 (sensitivity 60.0% and NPV 83%).

On the other hand, BDG yielded better results when the three days were analysed together with model 1 (sensitivity 86.7% and NPV 90.2%) than with model 2 (sensitivity 73.3% and NPV 86.0%) or for each timepoint independently (Table S1).

However, the results were better when both tests were used in combination (sensitivity 80.0%–93.3% and NPV 87.5%–95.6%). When the three samples were analysed together, model 1 sensitivity reached 96.7% with an NPV of 97.1%. Nevertheless, with model 2, neither the sensitivity nor the NPV improved (sensitivity 83.3% and NPV 90%).

As expected, the inclusion of probable IC as cases lowered the sensitivity and NPV but increased the specificity and PPV. In this scenario, only model 1 showed sensitivity and NPV >90% (Table S1).

The ROC curves (Figure 1) of the combined biomarkers (model 1 or 2 and for each timepoint—days 0, +3 and +5) showed that the best sensitivities were achieved with the combination of CAGTA and BDG using model 1 (sensitivity 96.7%, specificity 47.1%, AUC 0.719) and with the combination of CAGTA and BDG on day +5 (sensitivity 93.3%, specificity 61.4%, AUC 0.774).

Table 3 shows the behaviour of the biomarkers in the whole population and in ICU versus non-ICU patients. Overall, 96.7% of proven IC (29/30 cases) met the premise of model 1, while only 83.3% (25/30 cases) met the conditions of model 2. When patients with probable IC were also considered, the combination of biomarkers was positive in 10/12 cases (83.3%) in both models. The three results classified as potential false negatives (all negative biomarkers) occurred in patients fulfilling the definitions (one proven and two probable IC), although the significance of the *Candida* isolate was doubtful. The first one corresponded to *C. glabrata* recovered from a mixed culture of a percutaneous cholecystostomy sample in a patient with pancreatitis (classified as proven candidiasis according to definitions). The other two corresponded to *Candida* spp. (from two non-sterile sites) recovered from respiratory cultures in an HIV-infected patient with severe oral candidiasis and possible oesophagitis and from a patient with severe pancreatitis. Given their poor clinical situation, both patients were given systemic antifungals, and the cases were classified as probable candidiasis.

Among patients without IC, all biomarkers were negative in 31/58 patients (53.4%) and there were 27 false-positive results. False positives were more common in the ICU (51.2%) than in non-ICU wards (33.3%). Potential causes for these false positive results, such as abdominal surgery within 48 h (17), administration of albumin (12) and renal replacement therapy (11), were significantly more common in the ICU. Multifocal *Candida* colonization was significantly more common in patients with proven (66.7%) or probable (100%) IC than in patients without infection (19%) ($P<0.001$).

Discussion

Our study suggests that the combination of CAGTA and BDG could be used to safely stop antifungals on day 5 in a substantial proportion (31%) of patients receiving empirical antifungal therapy, both in the ICU and in non-ICU wards.

Experts accept that reducing unnecessary use of antifungals should be one of the major goals of stewardship programmes. Empirical antifungal therapy accounts for around 42% of antifungal use in tertiary hospitals[4,25,28–30] and is prescribed mainly to critically ill patients admitted to the ICU.[3,28,31] Although clinical guidelines support and recommend this therapy in high-risk

Table 1. Demographic characteristics of patients receiving empirical antifungal therapy

	Total ($n=100$)	ICU ($n=63$)	Non-ICU ($n=37$)	*P*
Male, *n* (%)	67 (67.0)	41 (65.1)	26 (70.3)	0.594
Age (years), mean ± SD	60.8 ± 15.7	60.8 ± 15.0	60.8 ± 17.1	0.881
Length of stay in hospital (days), median (IQR)	56.5 (29.2–91.7)	61.0 (30.0–91.0)	50.0 (21.5–101.5)	0.620
Length of stay in hospital (days) until empirical antifungal treatment, median (IQR)				
proven IC	21.5 (12.7–35.2)	32.0 (11.0–66.0)	21.0 (10.5–30.0)	0.263
probable IC	13.0 (6.2–28.0)	13.0 (6.0–22.0)	30.0[c]	0.500
no IC	9.5 (4.7–20.0)	11.0 (5.0–20.0)	9.0 (4.0–27.0)	0.790
Underlying disease on admission, *n* (%)				
solid tumours	22 (22.0)	16 (25.4)	6 (16.2)	0.285
intestinal perforation	20 (20.0)	15 (23.8)	5 (13.5)	0.214
severe cardiovascular disorders	19 (19.0)	12 (19.0)	7 (18.9)	0.987
acute pancreatitis	11 (11.0)	3 (4.8)	8 (21.6)	0.009
cirrhosis	7 (7.0)	4 (6.3)	3 (8.1)	0.739
solid organ transplantation (previous)	7 (7.0)	3 (4.8)	4 (10.8)	0.252
HIV infection	2 (2.0)	1 (1.6)	1 (2.7)	0.700
genitourinary disorders	2 (2.0)	—	2 (5.4)	0.062
autoimmune disease	2 (2.0)	2 (3.2)	—	0.274
others	8 (8.0)	7 (11.1)	1 (2.7)	0.135
Stratification of patients with suspected IC, *n* (%)				
high-risk digestive tract surgery[a]	30 (30.0)	22 (34.9)	8 (21.6)	0.161
other abdominal surgery	18 (18.0)	10 (15.9)	8 (21.6)	0.470
non-abdominal surgery	13 (13.0)	9 (14.3)	4 (10.8)	0.618
non-surgical	30 (30.0)	18 (28.6)	12 (32.4)	0.684
immunosuppressed[b]	9 (9.0)	4 (6.3)	5 (13.5)	0.227
Classification of the episode, *n* (%)				
proven IC	30 (30.0)	9 (14.3)	21 (56.7)	<0.001
IC without candidaemia[c]	26 (26.0)	8 (12.7)	18 (48.6)	<0.001
candidaemia without deep-seated infection[d]	3 (3.0)	1 (1.6)	2 (5.4)	0.280
IC with candidaemia[e]	1 (1.0)	0 (0.0)	1 (2.7)	0.190
probable IC	12 (12.0)	11 (17.5)	1 (2.7)	0.028
no IC	58 (58.0)	43 (68.2)	15 (40.5)	0.007
Origin of proven IC, *n* (%)				
intra-abdominal infection[f]	20 (66.7)	6 (66.7)	14 (66.7)	0.002
prosthetic (non-catheter) infection[g]	3 (10.0)	—	3 (14.3)	0.022
empyema	2 (6.7)	1 (11.1)	1 (4.8)	0.700
others[h]	5 (16.7)	2 (22.2)	3 (14.3)	0.441
Candida spp. in proven IC, *n* (%)				
albicans	21 (70.0)	8 (88.9)	13 (61.9)	0.183
glabrata	4 (13.3)	1 (11.1)	3 (14.3)	0.815
parapsilosis	2 (6.7)	—	2 (9.5)	0.326
other	3 (9.9)	—	3 (9.9)	0.495
Potential causes of false-positive results, *n* (%)				
blood product transfusion	50 (50.0)	34 (54.0)	16 (43.2)	0.300
abdominal surgery within 48 h	49 (49.0)	37 (58.7)	2 (5.4)	0.011
piperacillin/tazobactam	38 (38.0)	26 (41.3)	12 (32.4)	0.379
albumin	30 (30.0)	23 (36.5)	7 (18.9)	0.05
renal replacement therapy	25 (25.0)	22 (34.9)	3 (8.1)	0.003
bacteraemia[i]	22 (22.0)	17 (27.0)	5 (13.5)	0.116
bilirubin ≥10×1.1 mg/dL	5 (5.0)	3 (4.8)	2 (5.4)	0.887
intravenous immunoglobulin	6 (6.0)	4 (6.3)	2 (5.4)	0.848

Continued

Table 1. *Continued*

	Total ($n=100$)	ICU ($n=63$)	Non-ICU ($n=37$)	*P*
amoxicillin/clavulanic acid	4 (4.0)	2 (3.2)	2 (5.4)	0.583
multifocal *Candida* colonization	43 (43.0)	27 (42.9)	16 (43.2)	0.970
Empirical antifungal treatment				
empirical drug, *n* (%)				
echinocandins	73 (73.0)	53 (84.1)	20 (54.0)	0.001
fluconazole	20 (20.0)	8 (12.7)	12 (32.4)	0.017
amphotericin B	5 (5.0)	1 (1.6)	4 (10.8)	0.041
other azoles	2 (2.0)	1 (1.6)	1 (2.7)	0.700
overall (treatment days), median (IQR)	10.0 (4.2–15.0)	9.0 (4.0–12.0)	12.0 (5.5–21.5)	0.014
proven IC	12 (4.0–21.0)	10.0 (2.0–18.0)	13.0 (6.5–21.5)	0.209
probable IC	10.5 (7.0–14.7)	10.0 (7.0–14.0)	26.0[j]	0.167
no IC	8.5 (4.0–12.0)	8.0 (4.0–11.0)	10.0 (4.0–16.0)	0.230
echinocandins	10.0 (6.0–14.0)	9.0 (5.0–12.0)	12.0 (7.2–19.7)	0.027
fluconazole	10.5 (3.2–25.0)	7.0 (3.2–13.2)	14.5 (3.2–26.0)	0.427
amphotericin B	4.0 (3.5–21.0)	4.0[j]	11.0 (3.2–22.5)	0.800
Sepsis criteria, *n* (%)				
sepsis	35 (35.0)	15 (23.8)	20 (54.1)	0.020
severe sepsis	24 (24.0)	12 (19.0)	12 (32.4)	0.130
septic shock	30 (30.0)	27 (42.9)	3 (8.1)	<0.001
multiorgan failure	11 (11.0)	9 (14.3)	2 (5.4)	0.171
Overall mortality, *n* (%)	31 (31.0)	27 (42.9)	4 (10.8)	0.001
no IC	16 (27.6)	15 (20.8)	1 (2.7)	0.035
proven IC	7 (23.3)	5 (55.5)	2 (9.5)	0.006
probable IC	8 (66.7)	7 (63.6)	1 (100.0)	0.460
Models of CAGTA and BDG combinations in proven IC				
model 1				
sensitivity, % (95% CI)	96.7 (80.9–99.8)	100.0 (62.9–99.0)	95.2 (74.1–99.7)	—
specificity, % (95% CI)	47.1 (35.4–59.2)	42.6 (29.5–56.7)	62.5 (35.9–83.7)	—
PPV, % (95% CI)	43.9 (31.9–56.6)	22.5 (11.4–38.9)	76.9 (55.9–90.2)	—
NPV, % (95% CI)	97.1 (82.9–99.8)	100.0 (82.2–99.6)	90.9 (57.1–99.5)	—
accuracy, % (95% CI)	62.0 (51.7–71.4)	50.8 (38.0–63.5)	81.1 (64.3–91.4)	—
model 2				
sensitivity, % (95% CI)	83.3 (64.593.7)	88.9 (50.7–99.4)	80.9 (57.4–93.7)	—
specificity, % (95% CI)	64.3 (51.9–75.1)	59.3 (45.1–72.1)	81.2 (53.7–95.0)	—
PPV, % (95% CI)	50.0 (35.7–64.3)	26.7 (13.0–46.2)	85.0 (61.1–96.0)	—
NPV, % (95% CI)	90.0 (77.4–96.3)	97.0 (82.5–99.8)	76.5 (49.8–92.2)	—
accuracy, % (95% CI)	70.0 (59.9–78.5)	63.5 (50.3–75.0)	81.1 (64.3–91.4)	—

[a]High-risk digestive tract surgery included tertiary peritonitis, anastomotic leakage, recurrent digestive perforation and necrotizing pancreatitis.
[b]Immunosuppressed patients included onco-haematological patients, solid organ transplant recipients and others.
[c]Nineteen (73.1%) intra-abdominal infections; 3 (11.5%) prosthetic (non-catheter) infections; 2 (7.7%) empyema; 1 (3.8%) urinary tract infection; and 1 (3.8%) meningitis.
[d]Two (6.7%) catheter-related candidaemias [1 (11.1%) in ICU patients and 1 (4.8%) in non-ICU patients] and 1 (3.3%) primary candidaemia [1 (4.8%) in non-ICU patients].
[e]One (3.3%) candidaemia related to intra-abdominal infection [1 (4.8%) in non-ICU patients].
[f]Nineteen (63.3%) intra-abdominal infections included peritonitis, intra-abdominal abscess and intra-abdominal drain placed for <24 h [6 (66.7%) in ICU patients and 13 (61.9%) in non-ICU patients].
[g]Three (10.0%) prosthetic (non-catheter) infections [3 (14.3%) in non-ICU patients] included 1 (4.8%) pacemaker, 1 (4.8%) aorta-abdominal bypass and 1 (4.8%) peripheral vascular bypass.
[h]Others: 1 (3.3%) meningitis [1 (11.1%) in ICU patients]; 1 (3.3%) urinary infection [1 (4.8%) in non-ICU patients], 2 (6.7%) catheter-related candidaemias [1 (11.1%) in ICU patients and 1 (4.8%) in non-ICU patients] and 1 (3.3%) primary candidaemia [1 (4.8%) in non-ICU patients].
[i]Bacteraemia [$n=22$ (22%)]: 13 (59.1%) due to Gram-positive bacteria included 4 (18.2%) *Enterococcus* spp., 4 (18.2%) CoNS, 3 (13.6%) *Staphylococcus aureus* and 2 (9.1%) mixed cultures of Gram-negative bacteria + *Enterococcus* spp.
[j]Only one case.

Table 2. Analysis of biomarker results alone or in combination and requesting only one positive result (model 1) or at least two consecutive positive results (model 2) for the total days (days 0, 3 and 5 from starting empirical antifungal treatment) in patients with proven IC

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Model 1 CAGTA	60.0 (40.7–76.8)	85.7 (74.8–92.6)	64.3 (44.1–80.7)	83.3 (72.3–90.7)
Model 2 CAGTA	60.0 (40.7–76.8)	87.1 (76.5–93.6)	66.7 (46.0–82.8)	83.6 (72.6–90.9)
Model 1 BDG	86.7 (68.4–95.6)	52.9 (40.6–64.8)	44.1 (31.4–57.5)	90.2 (75.9–96.8)
Model 2 BDG	73.3 (53.8–87.0)	70.0 (57.1–80.1)	51.2 (35.7–66.4)	86.0 (73.6–93.3)
Model 1 CAGTA/BDG	96.7 (80.9–99.8)	47.1 (35.4–59.4)	43.9 (31.9–56.6)	97.1 (82.9–99.8)
Model 2 CAGTA/BDG	83.3 (64.5–93.7)	64.3 (51.9–75.1)	50.0 (35.7–64.3)	90.0 (77.4–96.3)

Combination CAGTA/BDG was considered positive if at least one of CAGTA or BDG was positive.
Data for each timepoint (days 0, 3 and 5 from starting empirical antifungal therapy) for CAGTA, BDG and for the combination CAGTA and BDG are available in Table S1. We also provide the same results analysing proven plus probable cases together.

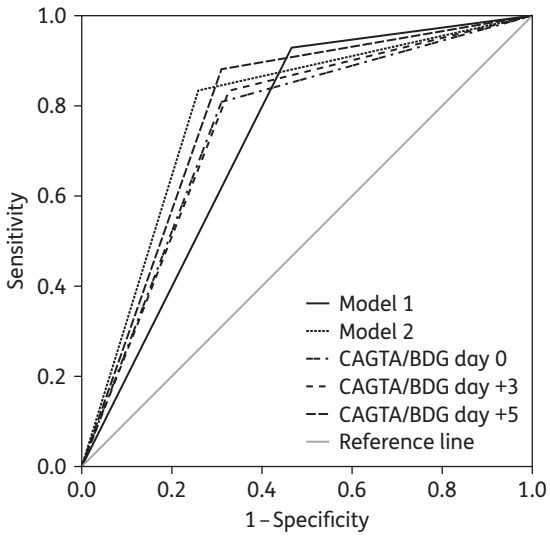

Figure 1. ROC curves showing the efficacy of model 1, model 2 and CAGTA and BDG combinations for each timepoint (days 0, 3 and 5 from starting empirical antifungal therapy). Model 1 (non-restrictive model): if at least one test result (CAGTA or BDG) was positive on any of the three days. Model 2 (partially restrictive model): if at least two consecutive CAGTA and/or BDG test results were positive. Model 1: sensitivity, 96.7%; specificity, 47.1%; AUC, 0.732 (0.633–0.830). Model 2: sensitivity, 83.3%; specificity, 64.3%; AUC, 0.787 (0.694–0.880). CAGTA/BDG day 0: sensitivity, 80.0%; specificity, 60.0%; AUC, 0.750 (0.651–0.848). CAGTA/BDG day +3: sensitivity, 83.3%; specificity, 58.6%; AUC, 0.753 (0.655–0.851). CAGTA/BDG day +5: sensitivity, 93.3%; specificity, 61.4%; AUC, 0.785 (0.693–0.877).

patients,[12] prescriptions in this group were more often inappropriate than in others (57% in our experience), mainly because of excessive duration of therapy[29] and incorrect indication, as recently demonstrated elsewhere.[3,4,29,30]

Our results show that the problem in the ICU (high suspicion and relatively low prevalence of infection among treated patients; 14.3% in our series) differs from other wards (low suspicion and high prevalence of infection in empirically treated patients; 56.7% in our series). In fact, higher mortality rates have been recorded for candidaemia occurring in the internal medicine department than in the ICU in some series.[32] Patients in medical and surgical wards may have very significant risk factors for development of invasive mycoses.[33–36] Although we only included patients receiving antifungal therapy, ours is one of the few studies that have analysed both types of population, reflecting real-life use of empirical antifungals in a tertiary institution.

Our study also shows that IC was finally proven in 30% of empirically treated patients (42% if we include probable cases), thus demonstrating that the population included in our series was at high risk. Accordingly, the indication to start antifungals early would be acted on in most cases.[1,37] The main problem is that antifungals are maintained for approximately 10 days in patients without IC (11.7 days in non-ICU wards), with the associated unnecessary risk of toxicity and expense. The low sensitivity of blood cultures complicates the decision to stop antifungal treatment safely. Thus, alternative approaches that do not rely on waiting for positive culture results are urgently needed.[4] The most interesting result of our study was that the combination of negative CAGTA and BDG tests would have enabled antifungal therapy to be stopped early in 53% of the patients with no IC (48.8% in ICUs and 66.7% in non-ICU wards) on day 5, with a sensitivity of at least 97%.

The few studies that have analysed CAGTA have done so mainly in onco-haematological patients and in patients admitted to the ICU. The diagnostic performance of the test has shown sensitivities of around 84%–87% and specificities of 95%.[25,38] Other potential advantages are the low cost and potential usefulness when discriminating between colonization and infection, and between deep-seated candidaemia and catheter-related candidaemia.[33,38] When CAGTA was used alone, we found that it had a sensitivity of 60%, a specificity of 85% and an NPV of 83%. The slightly lower specificity obtained in the present work with CAGTA in comparison with previous studies is probably due to the difference in study populations (empirical antifungal therapy versus patients with candidaemia).[9] In addition, there was little variation between the three sequential days tested. Interestingly, 13% of the patients (four) with IC had an initial negative CAGTA that became positive on day +5. Other authors have also described this pattern in 31.8% of ICU patients.[39]

Different studies have examined the role of BDG in the diagnosis of IC and report a mean sensitivity of 75% and specificity of 80%.[2,7] In our study, BDG had a sensitivity of 96.7% with a specificity of 47.1%. In general, specificity and PPV are lower in ICU patients (specificity 42.6% and PPV 22.5% in the ICU versus

Table 3. Results for patients with at least one positive IC biomarker or with all of them negative

	Proven IC ($n=30$)[a]			Probable IC ($n=12$)			Proven+probable IC ($n=42$)[a]			No IC ($n=58$)[a]		
	overall ($n=30$)	ICU ($n=9$)	non-ICU ($n=21$)	overall ($n=12$)	ICU ($n=11$)	non-ICU ($n=1$)	overall ($n=42$)	ICU ($n=20$)	non-ICU ($n=22$)	overall ($n=58$)	ICU ($n=43$)	non-ICU ($n=15$)
Model 1[b]	29 (96.7%)	9 (100.0%)	20 (95.2%)	10 (83.3%)	9 (81.8%)	1 (100.0%)	39 (92.9%)	18 (90.0%)	21 (95.5%)	27 (46.6%)	22 (51.2%)	5 (33.3%)
Model 2[c]	25 (83.3%)	8 (88.9%)	17 (81.0%)	10 (83.3%)	9 (81.8%)	1 (100.0%)	35 (83.3%)	17 (85.0%)	18 (81.8%)	15 (25.9%)	13 (30.2%)	2 (13.3%)
All IC biomarkers were negative	1 (3.3%)	0 (0.0%)	1 (4.8%)	2 (16.7%)	2 (18.2%)	0 (0.0%)	3 (7.1%)	2 (10.0%)	1 (4.5%)	31 (53.4%)	21 (48.8%)	10 (66.7%)

[a]$P<0.05$.
[b]Model 1 (non-restrictive model): if at least one test result (CAGTA or BDG) was positive on any of the three days.
[c]Model 2 (partially restrictive model): if at least two consecutive CAGTA and/or BDG test results were positive.

62.5% and 76.9% in non-ICU wards). This finding, which has been reported elsewhere, may be due to the higher exposure to causes of false positivity in the ICU (51.2% in the ICU versus 33.3% in non-ICU wards).[5,16] BDG is a promising tool in intra-abdominal candidiasis, as demonstrated by Tissot *et al.*,[7] who showed that two consecutive positive BDG results have a sensitivity and specificity of 65% and 78%, respectively.

Our study demonstrates that the option with the highest NPV is the combination CAGTA/BDG. Accordingly, the combination of CAGTA and BDG may be a very promising tool, not for establishing the diagnosis of IC but for ruling out the infection in patients who receive empirical antifungal therapy. The best results (sensitivity 96.7% and NPV 97.1%) were achieved when the combination was considered positive if any of the six tests performed were positive (CAGTA and BDG on days 0, +3 and +5). With this non-restrictive model (model 1), only one patient with potential proven candidiasis (*Candida* recovered from a cholecystostomy with multiple bacterial isolates) had a negative result. This patient was in a medical ward; thus, the sensitivity and NPV of the model was 100% for ICU patients and 95.2% and 90.9% for patients in non-ICU wards. Other previous studies emphasized the superiority of the diagnostic value of combining CAGTA and BDG.[6,9,25,40]

Our results suggest that the most effective strategy would probably be to provide the clinician with the results of IC biomarkers on day +5 of empirical therapy (sensitivity 93.3% and NPV 95.6%), when the definite results of the blood cultures are also available. Our approach would have made it possible to stop antifungals in the 31 patients whose biomarker results were negative and who did not have IC. Considering that the mean duration of therapy was 10 days, we calculated that avoiding 5 days of therapy in 31 patients would account for 15.6% of the antifungals used in this study (~€78 000 in cost). Furthermore, since our hospital consumes ~€3 million in antifungals per year, a saving of 15% would enable us to reduce costs by €468 000 per year. The relatively low cost of these six diagnostic tests for 100 patients (nearly €12 000; €10 for each CAGTA and €30 for each BDG test) highlights the cost-effectiveness of our approach. More effective designs, such as performing the biomarker tests earlier or only performing the tests on day 5, could also be studied. In our experience, performing the tests only on day 5 yielded a sensitivity of 93.3% and an NPV of 95.6%. No differences were observed when we analysed the use of the tests on days 3 and 5 (sensitivity 93.3% and NPV 94.9%).

Our study is subject to a series of limitations. The first is the high rate of false-positive results using the non-restrictive model. Overall, false positives accounted for 55% of the positive results in ICU patients and 19% in non-ICU patients. This rate could be reduced; however, as our objective was to include the combination strategy in our antifungal stewardship model, patient safety was our main goal. While it is useful to know that administration of albumin or intravenous immunoglobulins, renal replacement therapy and multifocal colonization could yield false-positive BDG results, patients requiring these therapies are sometimes also at high risk of candidiasis.[7,14] However, the risk of inducing unnecessary treatment exists and we cannot either exclude that discontinuation of the antifungals in patients with no IC does not cause harm to them, so, in our opinion, a randomized clinical trial is needed. Till then, the implications of IC biomarker results for therapeutic decisions should be determined by

experts in the field.[30,38,41] Besides, we have not analysed the potential impact of different degrees of *Candida* colonization on the levels of biomarkers.

The second limitation is that our study was performed in a single centre, although it reflects real use of antifungals in a general hospital. Nevertheless, our results should be replicated elsewhere before our conclusions can be incorporated into antifungal strategies.

Finally, a third possible limitation is the difficulty in making a diagnosis of intra-abdominal candidiasis. Although we have been very strict with the criteria and infection was considered as proven only when *Candida* was recovered from normally sterile samples, its role in polymicrobial cultures may be sometimes difficult to establish.

In summary, serial determination of CAGTA and BDG during the first days of empirical antifungal therapy has a very high sensitivity and NPV. If confirmed in a randomized clinical trial, this strategy could be used to discontinue antifungal treatment in at least 31% of patients. It could be also be used as a complementary tool in antifungal stewardship programmes.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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