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Candida albicans Germ-Tube Antibody: Evaluation of a New Automatic Assay for Diagnosing Invasive Candidiasis in ICU Patients

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Abstract Testing for *Candida albicans* germ-tube antibody IFA IgG assay (CAGTA) is used to detect invasive candidiasis infection. However, most suitable assays lack automation and rapid single-sample testing. The CAGTA assay was adapted in an automatic monotest system (invasive candidiasis [CAGTA] VirClia® IgG monotest (VirClia®), a chemiluminescence assay with ready-to-use reagents that provides a rapid objective result. CAGTA assay was compared with the monotest automatic VirClia® assay in order to establish the diagnostic reliability, accuracy, and usefulness of this method. A prospective study with 361 samples from 179 non-neutropenic critically ill adults patients was conducted, including 21 patients with candidemia, 18 with intra-abdominal candidiasis, 84 with *Candida* spp. colonization, and 56 with culture-negative samples, as well as samples from ten healthy subjects. Overall agreement between the two assays (CAGTA and VirCLIA) was 85.3%. These assays were compared with the gold-standard method to determine the sensitivity, specificity as well as positive and negative predictive values. In patients with candidemia, values for CAGTA and VirCLIA assays were 76.2 versus 85.7%, 80.3 versus 75.8%, 55.2 versus 52.9%, and 91.4 versus 94.3%, respectively. The corresponding values in patients with intra-abdominal candidiasis were 61.1 versus 66.7%, 80.3 versus 75.8%, 45.8 versus 42.9%, and 88.3 versus

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89.3%, respectively. No differences were found according to the species of *Candida* isolated in culture, except for *Candida albicans* and *C. parapsilosis*, for which VirClia® was better than CAGTA. According to these results, the automated VirClia® assay was a reliable, rapid, and very easy to perform technique as tool for the diagnosis invasive candidiasis.

Keywords *Candida albicans* germ-tube antibody (CAGTA) · Critically ill patients · ICU · Invasive candidiasis · Serum diagnostic test

Introduction

Invasive candidiasis (IC) has become an emerging and difficult-to-treat fungal infection in clinical practice [1]. A rise in the incidence and mortality from candidemia during the last decade has been reported [2, 3]. *Candida* spp. are among the most common etiologic agents of hospital-acquired systemic infections [4]. There are at least 15 distinct *Candida* spp. that attack humans, but >90% of invasive disease is caused by the five most common pathogens, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* [5].

The conventional culture method (gold standard) has a low sensitivity for blood samples (approximately 50%) and is time-consuming. This has resulted in an increasing use of empirical or prophylactic antifungal agents in patients without documented IC, favoring resistance [6–8]. Non-culture diagnostic techniques based on serological biomarkers have been introduced to improve the diagnosis of IC [9–13]. Invasive candidiasis CAGTA (*Candida albicans* germ-tube antibody) IFA IgG (Vircell SL, Granada, Spain) is a commercially available testing method that enables the detection of specific IgG antibodies against antigens located on the cell wall surface of the mycelium of *Candida* spp. in human serum/plasma by indirect immunofluorescence. Recently, studies have shown the usefulness of CAGTA especially in combination with other biomarkers for the diagnosis of invasive candidiasis in patients admitted to the intensive care unit (ICU) [13–16]. However, this method is laborious and requires a long hands-on time when many samples have to be processed.

The present study was conducted to assess the performance of a pre-market assay for anti-mycelium antibody detection (Invasive candidiasis CAGTA VirClia® IgG Monotest, Vircell SL, Granada, Spain) (VirClia®) adapted for automation on the VirClia® instrument (Vircell SL, Granada, Spain). This assay was evaluated and compared with conventional manual CAGTA by using clinical samples taken from patients with culture-proven invasive candidiasis and from culture-negative healthy controls.

Patients and Methods

Design and Study Population

Adult non-neutropenic critically ill patients admitted for at least 7 days in medical-surgical ICUs of tertiary care hospitals throughout Spain were selected from two prospective, cohort and observational studies conducted by our group [13, 14].

Exclusion criteria were as follows: neutropenia (total leukocyte count <1000/mm^3), life expectancy of less than one week, and an Acute Physiology and Chronic Health Evaluation (APACHE II) score ≥35 on ICU admission; chemotherapy prior to ICU admission; documented *Candida* infection during the week prior to ICU admission; non-invasive candidiasis targeted treatment with antifungal drugs before ICU admission or before inclusion in the study; refusal to sign the informed consent; limitation of the therapeutic effort; and failure to complete protocol specifications (inadequate data collection).

Screening and Microbiological Cultures

Screening for *Candida* spp. was made by means of surveillance cultures twice weekly from the 7th day of ICU admission. Samples were taken from feces or rectal swabs, urine, tracheal aspirates (or protected specimen brush or bronchoalveolar lavage), oropharyngeal swabs (in patients without mechanical ventilation), peripheral blood, vascular lines, wound/drainage exudates, or infected foci at the discretion of the attending physician. Samples were seeded directly into *Candida* CHROMagar® Chromogen culture medium (Hardy Diagnostics, Santa Maria, CA, USA). Blood cultures were processed using the automated BACTEC® system (Becton–Dickinson

Diagnostic Instrument System, Paramus, NJ, USA) or other standardized methods. A positive result was considered with the presence of *Candida* growth in the culture medium, and the identification at the species level was required. *Candida* score [15] was calculated on patients when culture data were available.

Serological Biomarkers

Blood samples (5 mL) were collected in tubes without anticoagulant, centrifuged at 1800 rpm for 10 min, and stored at −70 °C until analysis. None underwent more than two freeze–thaw cycles, and serum and reagents were tempered and homogenized before processing.

Anti-mycelium antibodies (Ab) were detected by an immunofluorescence assay (Invasive candidiasis CAGTA IFA IgG Vircell SL, Granada, Spain) (CAGTA) according to the manufacturer's instructions. The cutoff value for positive CAGTA was ≥1/160. Afterward, a new test for anti-mycelium Ab detection with the Invasive Candidiasis CAGTA VirClia® IgG Monotest (VirClia®) was made on the VirClia® instrument according to the manufacturer's instructions. The cutoff value for positive results was ≥1.1. Indeterminate results (cutoff values between 0.9 and 1.09) were retested according to the manufacturer's instructions. Briefly, VirClia® is an easy-to-perform indirect chemiluminescent immunoassay (automated protocol) in a monotest format with ready-to-use reagents. It allows pipetting from primary sample tube with no further manipulation needed and provides an objective and rapid result.

For each patient, CAGTA measurement was made by both assays during or before the IC episode. All samples were tested in parallel using the standard assay protocols. None of the tests were performed in real time and, therefore, were not available for the clinician's decision making.

Definitions

Invasive candidiasis or proven *Candida* infection was defined as (a) primary candidemia (C) (presence of *Candida* spp. in one or more blood cultures taken from peripheral veins) and (b) intra-abdominal candidiasis (IAC) on the basis of macroscopic findings and direct examination or positive culture for *Candida* spp. from the peritoneal fluid collected during an operation or within 24 h from external drainage. Patients were also classified into the groups of neither colonized nor infected (NC), and *Candida* spp. colonization (CC) [13]. In addition, serum from healthy subjects who were included as negative controls showed neither clinical signs nor symptoms, nor laboratory data related to the presence of an infection process.

Statistical Analysis

For the comparison with the gold standard, a positive result was considered when at least one serum sample from a patient had a positive result. Results were considered negative when all samples from the same patient gave a negative result. Categorical variables are expressed as frequencies and percentages, and continuous variables as a mean, or as a median and interquartile range (25th–75th percentile) (IQR). For the gold standard, blood culture or peritoneal fluid culture results (candidemic patients or patients with intra-abdominal candidiasis, considered as true positive results) were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The 95% confidence intervals (CIs) were calculated to test the significance of the estimates. Data from NC and healthy subjects were considered as true negative results. Agreement between assays was determined by unweighted kappa (κ) statistics and 95% CIs. For statistical analysis, IBM SPSS Statistics for Windows, version 23.0 (Armonk, NY, USA) was used.

Results

Study Population

A total of 361 serum samples from 179 ICU patients were classified according mycological cultures into the following groups: 21 patients with candidemia and 47 (13%) serum samples, 18 patients with IAC and 54 (15.0%) samples, 84 patients with CC and 168 (46.5%) samples, and 46 culture-negative patients with 82 (22.7%) samples. Also, we studied ten (2.8%) serum samples from ten healthy subjects included as negative controls.

The following strains were isolated in positive blood cultures from patients with candidemia: *Candida albicans* (7 isolates), *C. parapsilosis* (7), *C. glabrata* (6), and *C. krusei* (1). From patients with IAC, *Candida*

albicans (11 isolates), *C. glabrata* (4), *C. dubliniensis* (1), *C. tropicalis* (1), and *C. famata* (1) were recovered.

Clinical Performance of the VirClia® Assay

Compared with the Manual Protocol (CAGTA)

A total of 361 clinical serum samples provided the following results: 165 serum samples with positive results from both test systems and 143 were found to be negative by both systems. Fifty-three samples (14.7%) gave discordant results; 21 sera were positive by CAGTA but negative by VirClia®, and 32 of them were positive by VirClia® but negative by CAGTA. Overall agreement for the two assays was 85.3% (308/361). The kappa value was 0.71 (95% CI 0.63–0.78).

Compared with Blood or Peritoneal Fluid Culture Results

Of a total of 39 patients (21 candidemia and 18 IAC) 27 (69.2%) having proven invasive candidiasis provided a positive result by the CAGTA assay, and 12 patients had a negative-CAGTA result. Meanwhile, 30/39 (76.9%) gave a positive result with the VirClia® assay.

Considering this analysis by the group of patients, we noted that 16/21 (76.2%) candidemia patients were positive by CAGTA and 18/21 (85.7%) provided a positive result by VirClia®. For patients with IAC, 11/18 (61.1%) patients were positive by CAGTA and 12/18 (66.7%) by VirClia®. Patients with CC, 58/84 (69.0%) were positive by CAGTA, and 59/84 (70.2%) were positive by VirClia®.

Results of sensitivity, specificity, and positive and negative predictive values of CAGTA and VirClia® are shown in Table 1.

According to the isolated *Candida* spp. in patients with candidemia or IAC, we found better results using the VirClia® assay for all *Candida* spp. (85.7 vs. 76.2%, $p = 0.162$) in candidemia, and 66.7 versus 61.1% ($p = 0.579$) in IAC. Overall, in patients with invasive candidiasis, the results for the VirClia® assay were better than for the CAGTA assay (76.9 vs. 69.2%, $p = 0.083$). In the analysis by species, VirClia® showed better results than CAGTA for *C. albicans* (88.9 vs. 77.8%, $p = 0.163$). For *C. parapsilosis*, both assays showed a lower detection capacity, although VirClia® gave better results than did CAGTA (57.1 vs. 42.9%, $p = 0.356$). The two assays showed similar results for most of the other *Candida* spp. (Table 2).

When discordant results were assessed (Table 3), negative results from both assays were found in three strains of *C. parapsilosis* in patients with candidemia. Two patients with candidemia gave results only with the VirClia® assay when *C. glabrata* and *C. parapsilosis* were isolated. For patients with IAC, five were negative in both assays when *C. glabrata* (2), *C. albicans* (2), and *C. famata* were isolated. In one patient with a *C. glabrata* isolate, this strain was detected only by the CAGTA assay, whereas in two patients with *C. albicans*, these strains were detected only by the VirClia® assay (Table 2).

Costs

Reagent demand and costs rise up to 25% by adopting the semi-automated assay. However, it should be noted that hands-on time and time to result are considerably reduced so a large-scale testing of serum could be possible.

Time to Result

The CAGTA assay has a variable time to result due to the numbers of samples tested in one run, but the minimum time was about 3 h, including the hands-on time and determination. With the automatic VirClia® protocol, the time to result varied from 50 to 130 min (1–24 samples), including instrument setup and the hands-on time was only 5 min maximum depending on the number of samples per run. Finally, interpretation of the results is summarized in a report generated by the instrument.

Discussion

This study shows that specific detection IgG antibodies against antigens of the mycelium of *Candida* spp. by means of an automated method are similar to that of the conventional test, with reliable results in a suitable time to result and hands-on time.

Culture patient blood samples remain the gold-standard technique, although the low sensitivity of this technique is well known since in most patients with suspected candidemia, blood cultures may remain

Table 1 Sensitivities, specificities, positive (PPV) and negative (NPV) predictive values of CAGTA and VirCLIA assays as compared to the clinical gold standard in patients with invasive candidiasis, candidemia, and intra-abdominal candidiasis

	IC (overall)	C	IAC
CAGTA			
Sensitivity % (95% CI)	69.2 (53.6–81.4)	76.2 (54.9–89.4)	61.1 (38.6–79.7)
Specificity % (95% CI)	80.3 (69.2–88.1)	80.3 (69.2–88.1)	80.3 (69.2–88.1)
PPV % (95% CI)	67.5 (52.0–79.9)	55.2 (37.5–71.6)	45.8 (27.9–64.9)
NPV % (95% CI)	81.5 (70.4–89.1)	91.4 (81.4–96.3)	88.3 (77.8–94.2)
VirCLIA			
Sensitivity % (95% CI)	76.9 (61.7–87.4)	85.7 (65.4–95.0)	66.7 (43.7–83.7)
Specificity % (95% CI)	75.8 (64.2–84.5)	75.8 (64.2–84.5)	75.8 (64.2–84.5)
PPV % (95% CI)	65.2 (50.8–77.3)	52.9 (36.7–68.5)	42.9 (26.5–60.9)
NPV % (95% CI)	84.7 (73.5–91.8)	94.3 (84.6–98.1)	89.3 (78.5–95.0)

CI confidence interval. Gold-standard method: positive blood or peritoneal fluid culture. *IC* invasive candidiasis, *C* candidemia, *IAC* intra-abdominal candidiasis

Table 2 Results of CAGTA and VirCLIA assay in serum samples from patients according *Candida* spp. isolated in blood or peritoneal fluid culture

Candidemia origin (*n*)	CAGTA+ (%)	VirCLIA+ (%)	*p* value
All species			
Overall (39)	27 (69.2)	30 (76.9)	0.083
C patients (21)	16 (76.2)	18 (85.7)	0.162
IAC patients (18)	11 (61.1)	12 (66.7)	0.579
C. albicans			
Overall (18)	14 (77.8)	16 (88.9)	0.163
C patients (7)	7 (100)	7 (100)	n.s
IAC patients (11)	7 (63.3)	9 (81.8)	0.167
C. parapsilosis			
Overall (7)	3 (42.9)	4 (57.1)	0.356
C patients (7)	3 (42.9)	4 (57.1)	0.356
IAC patients (0)	0 (0.0)	0 (0.0)	n.a
C. glabrata			
Overall (10)	7 (70.0)	7 (70.0)	n.s
C patients (6)	5 (83.3)	6 (100)	0.363
IAC patients (4)	2 (50)	1 (25)	0.391

n.a non-applicable, *n.s* non-significant. Global data include C and IAC patients. *C* candidemia, *IAC* intra-abdominal candidiasis. Patients with *C. krusei* (1), *C. dubliniensis* (1), *C. tropicalis* (1) and *C. famata* (1) were not included in this analysis

negative up to 5 days after starting treatment with the antifungal agents [7, 16–18]. In addition, false-negative results may be found in patients with disseminated candidiasis [19].

Non-culture diagnostic techniques based on serological biomarkers, such as the anti-mycelium antibody assay, have been introduced to improve early diagnosis of invasive candidiasis [11–14, 20]. However, there is limited data on the usefulness of CAGTA in critically ill patients. Two previous reports assessed the predictive value of CAGTA for invasive candidiasis in a cohort of 53 critically ill non-neutropenic patients, of whom 43.3% were highly colonized, and 41.5% patients had CAGTA-positive results [9, 10]. Sensitivity and specificity values 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

Table 3 Discordant results in patients with proven candidemia (C) and intra-abdominal candidiasis (IAC) between both assays and the gold-standard method

No. of patient	Diagnosis	CAGTA result	VirCLIA result	Strain isolated
1	C	N	N	*C. parapsilosis*
2	C	N	N	*C. parapsilosis*
3	C	N	N	*C. parapsilosis*
4	C	N	P	*C. glabrata*
5	C	N	P	*C. parapsilosis*
6	IAC	N	N	*C. glabrata*
7	IAC	N	N	*C. albicans*
8	IAC	N	N	*C. albicans*
9	IAC	N	N	*C. glabrata*
10	IAC	N	N	*C. famata*
11	IAC	P	N	*C. glabrata*
12	IAC	N	P	*C. albicans*
13	IAC	N	P	*C. albicans*

N negative, *P* positive

of positive CAGTA results in patients with previous surgery and a significantly lower ICU-related mortality among CAGTA-positive patients. However, Martinez-Jiménez et al. [11] showed that the presence of a positive CAGTA test in a sample from a patient with candidemia suggests deep-seated candidiasis. Also, several studies have assessed the clinical value of CAGTA particularly in combination with other serum biomarkers [11, 12, 14, 20].

This biomarker has some limitations, i.e., false-positive and false-negative results of unknown cause. In fact, in the present study, several patients from the non-colonized neither infected group were found to have a positive CAGTA (13/46, 28.3%) or VirClia® (16/46, 34.8%) result and were classified as false positives. This rate of false-positive results was slightly higher than in studies previously published by our research team in the same group of patients [13, 14, 20].

This study includes a cohort of different groups of patients for analyzing VirClia® assay (automated version of the CAGTA assay), thus giving a total agreement of 85.3% between the two assays.

Sensitivity, specificity, and negative predictive values for CAGTA assay were slightly lower than those for VirClia® in patients with invasive candidiasis. These results of the diagnostic performance for invasive candidiasis in ICU patients with CAGTA are within the ranges reported by other authors, such as 53.3–96% for sensitivity and 64.3–100% for specificity in similar cohorts of patients [11, 13, 14, 20–23].

In patients with invasive candidiasis (candidemia and IAC), our data showed a higher sensitivity for candidemia than for IAC but similar results for specificity and predictive values. This finding indicates that this new assay could be used with reliable results for patients with candidemia and intra-abdominal infection.

Although the CAGTA assay was originally designed for detecting *C. albicans*, it has been evaluated for other *Candida* species, including *C. parapsilosis*, *C. glabrata, C. tropicalis*, *C. krusei*, *C. dubliniensis*, and *C. guilliermondii* [10, 13, 21]. In our experience, the accuracy of both tests was slightly higher for VirClia® than for CAGTA (76.9 vs. 69.2%, $p = 0.083$) for all *Candida* spp. For *C. albicans* and *C. parapsilosis*, the VirClia® assay showed better results as compared with CAGTA (88.9 vs. 77.8% and 57.1 vs. 42.9%, respectively). For other *Candida* spp., similar results with both assays were obtained. Our results with CAGTA for *C. tropicalis* were better than those reported in another study (11) (100 vs. 33.3%), although only one strain of *C. tropicalis* was isolated in our cohort. Results for *C. albicans* and *C. parapsilosis* were less favorable as compared with other studies (77.8 vs. 85.7% and 42.9 vs. 100%, respectively), although results for *C. glabrata* were similar (70.0 vs. 75.0%) (11).

However, this is the first study available in which serum VirClia® determinations were used as a biomarker to use for the diagnosis of invasive candidiasis in ICU patients.

This study has the main limitation that the gold-standard method has a low sensitivity, so that patients diagnosed with CC with a positive CAGTA result could be classified as confirmed candidemia (58/84, 69.0% in this analysis). Martín-Mazuelos et al. (2015) speculated that patients colonized before the development of invasive candidiasis may present an immunological response with an increase in specific antibodies, so that patients colonized by *Candida* could have "occult" candidemia, undiagnosed by standard methods. Because of this, the statistical data recorded in this comparison would improve significantly. Secondly, we were able to study only a relatively small number of cases. Thirdly, this biomarker should be used in combination with other diagnostic tests to improve the diagnostic performance in patients with invasive candidiasis [11–14].

The use of CAGTA as a test for diagnosing invasive candidiasis has some difficulties due to the complexity and its long waiting time to result. For these reasons, a faster and automatic test, such as the VirClia® assay, is clinically relevant.

In summary, sensitivity and NPV values confirmed this method as tool for the diagnosis of invasive candidiasis, so the automated VirClia® assay was a reliable, rapid and very easy to perform technique in both single-sample and large-scale sampling testing. Also, additional staff training is not necessary for test performance in comparison with the CAGTA assay. In addition, the results for *C. albicans* and *C. parapsilosis* which are the most frequent *Candida* spp. in patients with candidemia [24, 25] showed that this new assay may be superior to the CAGTA technique. The short time to result is a further advantage of this novel technique. However, implementation of the VirClia® assay should be investigated in prospective studies, including the combination of other *Candida* biomarkers for rapid discrimination of ICU patients with and without invasive candidiasis. This would contribute to a more rational use of empirical or preemptive antifungal therapy.

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Compliance with Ethical standards

Conflict of interest None of the authors has any conflict of interest to be disclosed.

Ethical Approval The study was conducted with the approval of the Ethics Committee of Hospital Universitario de Valme, Seville, Spain (CEIC-A1, ref. 350/12).

References

1. Eggimann P, Bille J, Marchetti O. Diagnosis of invasive candidiasis in the ICU. Ann Intensive Care. 2011;. doi:10.1186/2110-5820-1-37.
2. Lortholary O, Renaudat C, Sitbon K, et al. Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002–2010). Intensive Care Med. 2014;40(9):1303–12.
3. Colombo AL, Guimaraes T, Sukienik T, et al. Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. Intensive Care Med. 2014;40(10):1489–98.
4. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39(3):309–17.
5. Pappas PG, Kauffman CA, Andes DR, et al. Executive summary: clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America. Clin Infect Dis. 2016;62(4):409–17.
6. Fernandez J, Erstad BL, Petty W, Nix DE. Time to positive culture and identification for *Candida* blood stream infections. Diagn Microbiol Infect Dis. 2009;64(4):402–7.
7. Clancy CJ, Nguyen MH. Finding the ''missing 50%'' of invasive candidiasis: how non-culture diagnostics will

improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56(9):1284–92.
8. Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M. Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? Intensive Care Med. 2014;40(9):1241–55.
9. Zaragoza R, Peman J, Quindos G, et al. Clinical significance of the detection of *Candida albicans* germ tube-specific antibodies in critically ill patients. Clin Microbiol Infect. 2009;15(6):592–5.
10. Peman J, Zaragoza R, Quindos R, et al. Clinical factors associated with a *Candida albicans* germ tube antibody positive test in intensive care unit patients. BMC Infect Dis. 2011;11:60.
11. Martinez-Jimenez MC, Muñoz P, Guinea J, et al. Potential role of *Candida albicans* germ tube antibody (CAGTA) in the diagnosis of deep-seated candidemia. Med Mycol. 2014;52(3):270–5.
12. Martínez-Jiménez MC, Muñoz P, Valerio M, et al. Candida biomarkers in patients with candidaemia and bacteraemia. J Antimicrob Chemother. 2015;70(8):2354–61.
13. Martín-Mazuelos E, Loza A, Castro C, et al. β-D-Glucan and *Candida albicans* germ tube antibody in ICU patients with invasive candidiasis. Intensive Care Med. 2015;41(8):1424–32.
14. León C, Ruiz-Santana S, Saavedra P, et al. Contribution of *Candida* biomarkers and DNA detection for the diagnosis of invasive candidiasis in ICU patients with severe abdominal conditions. Crit Care. 2016;20(1):149. doi:10.1186/s13054-016-1324-3.
15. León C, Ruiz-Santana S, Saavedra P, et al. Usefulness of the *Candida* score for discriminating between *Candida* colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. Crit Care Med. 2009;37(5):1624–33.
16. Alexander BD, Pfaller MA. Contemporary tools for the diagnosis and management of invasive mycoses. Clin Infect Dis. 2006;43(Suppl 1):15–27.
17. Lam SW, Eschenauer GA, Carver PL. Evolving role of early antifungals in the adult intensive care unit. Crit Care Med. 2009;37(5):1580–93.
18. Shorr AF, Gupta V, Sun X, Johannes RS, Spalding J, Tabak YP. Burden of early-onset candidemia: analysis of culture-positive bloodstream infections from a large U.S. database. Crit Care Med. 2009;37(9):2519–26.
19. Azoulay E, Dupont H, Tabah A, et al. Systemic antifungal therapy in critically ill patients without invasive fungal infection. Crit Care Med. 2012;40(3):813–22.
20. Leon C, Ruiz-Santana S, Saavedra P, et al. Value of b-D-glucan and *Candida albicans* germ tube antibody for discriminating between *Candida* colonization and invasive candidiasis in patients with severe abdominal conditions. Intensive Care Med. 2012;38(8):1315–25.
21. Moragues MD, Ortiz N, Iruretagoyena JR, et al. Evaluation of a new commercial test (*Candida albicans* IFA IgG) for the serodiagnosis of invasive candidiasis. Enferm Infecc Microbiol Clin. 2004;22(2):83–8.
22. Linares MJ, Javier MR, Villanueva JL, et al. Detection of antibodies to *Candida albicans* germ tubes in heroin addicts with systemic candidiasis. Clin Microbiol Infect. 2001;7(4):218–26.
23. García-Ruiz JC, del Carmen Arilla M, Regúlez P, Quindós G, Alvarez A, Pontón J. Detection of antibodies to *Candida albicans* germ tubes for diagnosis and therapeutic monitoring of invasive candidiasis in patients with hematologic malignancies. J Clin Microbiol. 1997;35(12):3284–7.
24. Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R, et al. Epidemiology and predictive factors for early and late mortality in Candida bloodstream infections: a population-based surveillance in Spain. Clin Microbiol Infect. 2014;20(4):O245–54.
25. Caggiano G, Coretti C, Bartolomeo N, Lovero G, De Giglio O, Montagna MT. Candida bloodstream infections in Italy: changing epidemiology during 16 years of surveillance. Biomed Res Int. 2015;. doi:10.1155/2015/256580.