Clinical factors associated with a *Candida albicans* Germ Tube Antibody positive test in Intensive Care Unit patients

Pemán et al.
Clinical factors associated with a Candida albicans Germ Tube Antibody positive test in Intensive Care Unit patients

Javier Pemán1*, Rafael Zaragoza2, Guillermo Quindós3, Miriam Alkorta4, María S Cuétara5, Juan J Camarena6, Paula Ramírez2, María J Giménez1, Estrella Martín-Mazuelos8, María J Linares-Sicilia9, José Pontón3, the study group Candida albicans Germ Tube Antibody Detection in Critically Ill Patients (CAGTAUCI)

Abstract

Background: Poor outcomes of invasive candidiasis (IC) are associated with the difficulty in establishing the microbiological diagnosis at an early stage. New scores and laboratory tests have been developed in order to make an early therapeutic intervention in an attempt to reduce the high mortality associated with invasive fungal infections. Candida albicans IFA IgG has been recently commercialized for germ tube antibody detection (CAGTA). This test provides a rapid and simple diagnosis of IC (84.4% sensitivity and 94.7% specificity). The aim of this study is to identify the patients who could be benefited by the use of CAGTA test in critical care setting.

Methods: A prospective, cohort, observational multicentre study was carried out in six medical/surgical Intensive care units (ICU) of tertiary-care Spanish hospitals. Candida albicans Germ Tube Antibody test was performed twice a week if predetermined risk factors were present, and serologically demonstrated candidiasis was considered if the testing serum dilution was $\geq 1:160$ in at least one sample and no other microbiological evidence of invasive candidiasis was found.

Results: Fifty-three critically ill non-neutropenic patients (37.7% post surgery) were included. Twenty-two patients (41.5%) had CAGTA-positive results, none of them with positive blood culture for Candida. Neither corrected colonization index nor antifungal treatment had influence on CAGTA results. This finding could corroborate that the CAGTA may be an important biomarker to distinguish between colonization and infection in these patients. The presence of acute renal failure at the beginning of the study was more frequent in CAGTA-negative patients. Previous surgery was statistically more frequent in CAGTA-positive patients.

Conclusions: This study identified previous surgery as the principal clinical factor associated with CAGTA-positive results and emphasises the utility of this promising technique, which was not influenced by high Candida colonization or antifungal treatment. Our results suggest that detection of CAGTA may be important for the diagnosis of invasive candidiasis in surgical patients admitted in ICU.

Background

Invasive candidiasis (IC), especially in the critical care setting, has become an excellent target for prophylactic, empiric, and pre-emptive therapy interventions due to its increased incidence, high morbidity, mortality rate, and associated healthcare costs [1,2]. Although the past few years have brought exciting developments in antifungal pharmacotherapy, the mortality rate remains excessively high [3-7]. Poor outcomes are associated with the difficulty in establishing the microbiological diagnosis at an early stage of IC (blood culture results are positive in only 50% and the antigen or antibody detection also show low sensitivity rates at the present time) [2] and the delay in receiving antifungal treatment [8,9]. Moreover, it is well known that inadequate empirical antibiotic treatment is an independent determinant.

*Correspondence: pemán_jav@gva.es
1Department of Microbiology, Hospital Universitario La Fe, (Av Campanar, 21), Valencia, (46009), Spain
Full list of author information is available at the end of the article.

© 2011 Pemán et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
of hospital mortality and mycoses are among the types of infection with the highest rates of inappropriate initial treatment [10] and consequently worse prognosis [11].

New scores and laboratory tests have been developed in order to make an early therapeutic intervention in an attempt to reduce the high mortality associated with invasive fungal infections [12,13]. Substantial progress has been made in diagnosis of IC with the development of a variety of methods for the detection of antibodies and antigens. However, no single test has found widespread clinical acceptance and there is a consensus that diagnosis based on a single specimen lacks sensitivity [14,15].

Candida albicans IFA IgG has been recently commercialized for Candida albicans germ tube antibody (CAGTA) detection. This test is based on the detection, by an indirect immunofluorescence assay, of antibodies against the surface of C. albicans germ tubes. The test provides a rapid and simple diagnosis of IC in the clinical microbiology laboratory (84.4% sensitivity and 94.7% specificity) [16,17]. Although the performance of the test has been studied in haematological patients (87.5% sensitivity and 95.2% specificity) [18], no large clinical study has been performed in the intensive care unit (ICU) setting with this new tool. Two recently published mortality analyses of our group showed a significant decrease of mortality in those patients with a CAGTA-positive result in ICU patients [19,20], especially in those with patterns of increasing CAGTA titres who had been treated with antifungal agents. Thus, antifungal treatment should be considered when CAGTA titres increase in critically ill patients [20]. However, the limitation of these data, the complexity and the cost of this technique make it mandatory to find which patients could benefit by the determination of CAGTA in order not to apply this technique universally. For these reasons, the aims of the present study were to determine the clinical features of the patients with a CAGTA-positive result in the ICU setting, in an attempt to define the population of critically ill patients who could be benefit by this technique analyzing the factors associated to a positive result in order to make rentable the use of this test.

**Methods**

**Patients and methods**

During a two-year period (January 2005-December 2006) a prospective observational multicenter study was conducted at six Spanish University hospitals: Hospital Doctor Peset -HDP- (563 hospital beds/16 ICU beds, Valencia), Hospital La Fe -HLF- (1,292/21, Valencia), Hospital de Cruces -HDC- (931/18, Barakaldo), Hospital Severo Ochoa -HOSO- (365/12, Leganés), Hospital Reina Sofía -HRS- (1,301/34, Córdoba), and Hospital Nuestra Señora de Valme -HNSV- (639/14, Seville). The cohort described is the same as has been previously published by our group [19,20], but the data showed here are novel and have not been included in our cited articles. The institutional review boards at each participating institution approved the study and informed consent was obtained in each patient included in the study.

**Study design and data collection**

The study was developed following a pre-designed protocol with several predetermined analyses. A critically ill patient was eligible for investigation and was enrolled in the study if any of the following five inclusion criteria were present: i) acute pancreatitis of more than seven days of evolution; ii) prolonged ICU stay (>14 days) and three or more risk factors (diabetes mellitus, extra-renal depuration, parenteral nutrition, more than seven days of broad spectrum antibiotic therapy, and major abdominal surgery); iii) liver transplant; iv) neutropenia or bone marrow transplant (BMT); and v) high Candida colonization. Exclusion criteria were as follow: i) pregnancy; ii) younger than 18 years old, iii) previous IC; or iv) life expectancy lower than seven days. Prior antifungal therapy was not an exclusion criterion. All patients were consecutively included in the study.

Severity of illness at inclusion of study was calculated with the APACHE II score (Acute Physiology and Chronic Health Evaluation). For all eligible patients, screening cultures for Candida colonization were performed weekly until seven days after ICU discharge. These samples were obtained from bronchial aspirates, oropharynx, urine, rectum, and peri-catheter skin as part of the surveillance study. A corrected colonization index (CCI) was used to assess the intensity of Candida colonization [21-23], and patients with CCI ≥0.4 were considered as highly colonized. J. Pemán and R. Zaroza reviewed all patients’ forms before including them in the database.

**Microbiological studies**

CAGTA detection (Candida albicans IFA IgG, Vircell, Spain) was performed twice a week. A positive result, defined as serologically proven candidiasis, was considered when a serum titre ≥1:160 was detected in at least one sample and no other microbiological evidence of IC was found. Blood and sterile sites cultures were drawn following physicians’ criteria and processed with automated systems (BACTEC, Becton Dickinson Diagnostic Instrument Systems, Madrid, Spain or BacTAlert, bioMérieux España, Madrid, Spain). Identification of yeasts at species level was made with the API 20C, API 32C, or the YST card of the Vitek system (bioMérieux España, Madrid, Spain) at each institution. The decision to add antifungal therapy for patients with suspected IC was at the discretion of the prescribing physician based...
on clinical criteria, but it was not influenced by CAGTA results (physicians had no access to the results). Clinical, microbiological and outcome variables were recorded.

Data analysis
All quantitative data are presented as mean ± SD unless otherwise stated. The chi-square or Fisher’s exact test was used to compare categorical variables and student’s t-test was applied for quantitative homocedastic variables with normal distribution. Two tailed p value of 0.05 or less was considered for statistical significance. Data analysis was performed with the SPSS software for Windows version 11.5 (SPSS, Chicago, IL). An external statistical company assessed this analysis independently.

Results
Fifty-three miscellaneous critically ill patients were included in the study, 37.7% of whom needed surgical procedure during their stay in the ICU. The hospital distribution of patients was as follows: 17 (HDC), 12 (HLF), 11 (HDP), 9 (HSO), 3 (HNSV) and 1 (HRS). The most frequent patient inclusion criteria were prolonged ICU stay and presence of ≥3 IC risk factors (60%) followed by CCI ≥0.4 (23%), neutropenia or BMT (7.5%), acute pancreatitis (6%) and liver transplant (4%). Five patients (9%) presented extended ICU stay and ≥3 IC risk factors plus high CCI. The age and APACHE II score averages were 71.5 ± 18.4 years, and 14.9 ± 5.4 points, respectively. The principal causes of ICU admission were septic shock (28.3%) and respiratory failure (28.3%), followed by post-surgery standard care (24.5%) and coma (9.4%). The patient groups did not differ significantly in demographic characteristics or inclusion criteria among participating centres.

Culture-based methods yielded negative for blood and sterile sites cultures for Candida spp. when drawn in all study patients. However, 43.3% of the patients were highly colonized during the study.

Twenty-two patients (41.5%) had CAGTA-positive results (10 patients had one positive sample, 8 had two, and 4 had ≥3 positive determinations), none of them with positive blood culture for Candida. The CAGTA titres observed in these patients ranged from 1/160 to 1/2560. There were no differences between CAGTA-positive and -negative patients in age, sex, inclusion criteria, cause of admission, and presence of hepatic failure. The presence of acute renal failure at the beginning of the study was more frequent in CAGTA-negative patients, but no extra-renal depuration was needed. APACHE II score was also statistically higher in this group. Previous surgery was statistically more frequent in CAGTA-positive patients in the univariate analysis (Table 1). No statistical differences were found, in the rate of highly colonized patients, between positive and negative CAGTA results at the end of the study (77.2% vs. 89.6%, respectively; p = 0.26).

Empiric anti fungal treatment was applied in 50.9% of cases during the study (after the inclusion); the regimens most frequently prescribed were fluconazole (48.1%) and voriconazole (14.8%). There was no difference in the global administration of antifungal treatment between CAGTA-positive and -negative patients (45.4% vs. 54.8%; p = 0.45); however, the use of voriconazole showed a tendency to be more frequent in CAGTA-positive patients (5.9% vs. 30%; p = 0.08) (Table 2).

Discussion
Mortality rates of IC episodes in the critical care setting remain excessively elevated due to diagnosis difficulties and, subsequently, inappropriate treatment. IC diagnosis usually requires a high index of suspicion and is difficult because the infection lacks pathognomonic signs, blood cultures are often negative and, in many instances, it is not possible to obtain specimens for histology; therefore, serology could be an aid in its diagnosis, as it provides specific biomarkers [2,24,25]. Demonstration of IC may not be apparent because the infectious burden is beneath the analytical sensitivity of the chosen diagnostic modality, or infection remains limited in the context of neutrophil recovery or follows administration of prophylactic or empirical antifungal therapy. Serological tests for the diagnosis of IC by detecting antibodies against different antigens of Candida must differentiate Candida colonization or superficial infection from tissue invasion and candidemia requiring antifungal therapy. Sera from patients with IC recognize a manno protein of 230-250 kDa located on the germ tube cell wall surface. Pontón et al. [26,27] have developed an indirect immunofluorescence assay to detect antibodies (CAGTA) against this antigen present in C. albicans germ tubes, which has been a useful biomarker for the diagnosis of IC in different groups of patients, including intravenous heroin users, BMT recipients, patients with haematological disorders and ICU patients [16,18,28,29]. The test has shown an overall sensitivity of 77-89% and a specificity of 91-100%. Although the titres found in immunocompromised patients are lower than those found in immunocompetent patients, the overall performance of the test is similar. Detection of CAGTA in patients with invasive infections caused by Candida species other than C. albicans (Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida dubliniensis, Candida guilliermondii and Candida krusei) may also be positive, although titers are lower than in candidiasis by C. albicans [16,27,29-31].

Candida albicans IFA IgG has been recently commercialized for CAGTA detection. This test has been compared in a retrospective study to the standard test using
172 sera from 51 haematological and intensive care patients [16]. The commercially available test was similar to the standard test and provided faster and easier diagnosis of IC in the clinical microbiology laboratory. To our knowledge, this is the first time that the new commercial kit for CAGTA detection has been studied in the ICU setting. It is well known that the global sensitivity rate of blood culture is not too high (50%) in the hospital population [32]. Moreover, this ratio is considerably lower in the ICU setting as León et al. [12] observed in the Candida score study, where the ratio of proven IC was only 5.7%. However, until now this procedure is still the gold standard for candidemia diagnosis. The reasons for the low number of positive blood cultures in critical care setting are unknown but are likely to be related to the prophylactic and empiric treatments administered to these patients [33,34]. In our study, no blood cultures were positive. The low ratio of positive blood cultures in critically ill patients obtained in all studies in this setting, including this one, confirms the compelling necessity of finding another sound gold standard for validating new diagnostic tools [12,35,36]. Then, serological data may be used as evidence to implicate Candida spp. when mycological and/or histological data are negative or cannot be acquired. The high prevalence of CAGTA-positive results in the population studied (41.5%) corroborates the adequacy of the inclusion criteria used in this study as a predictive biomarker of Candida infection and the need of consolidating the CAGTA determination in a delimited group of ICU population. For these reasons and for the excellent sensitivity and specificity previously reported [16], all the CAGTA-positive patients could be considered as “probable” IC. The preliminary results presented in this study

Table 1 Main clinical characteristics of patients according to CAGTA results

| No. of patients (%) | Total | CAGTA + | CAGTA - | p value |
|---------------------|-------|---------|---------|---------|
| Patients            | 53    | 22 (41.5) | 31 (58.5) | -       |
| Age (years)         | 71.5 ± 18.4 | 69.2 ± 22.2 | 73.1 ± 15.3 | 0.46    |
| Male:Female (ratio) | 1.65  | 1.2      | 2.3     | 0.25    |
| APACHE II (score)   | 14.9 ± 5.4 | 12.2 ± 4.4 | 16.9 ± 5.2 | 0.001   |
| Septic shock        | 15 (28.3) | 4 (18.2) | 11 (35.4) | 0.16    |
| Respiratory failure | 15 (28.3) | 7 (31.8) | 8 (25.8) | 0.63    |
| Coma                | 5 (9.4) | 2 (9.1) | 3 (9.7) | 0.92    |
| Renal failure       | 24 (45.3) | 6 (27.3) | 18 (58.1) | 0.02    |
| Extra-renal depuration | 16 (30.2) | 4 (18.2) | 12 (38.7) | 0.1     |
| Hepatic failure     | 19 (35.8) | 9 (40.9) | 8 (25.8) | 0.24    |
| Neutopenia          | 4 (7.5) | 0     | 4 (12.9) | 0.1     |
| Broad spectrum antibiotics | 36 (67.9) | 17 (77.3) | 19 (61.3) | 0.21    |
| Parenteral nutrition | 33 (62.3) | 14 (63.7) | 19 (61.3) | 0.86    |
| Hepatic transplant  | 2 (3.8) | 1 (4.5) | 1 (3.2) | 0.80    |
| Acute pancreatitis  | 3 (5.7) | 2 (9.1) | 1 (3.2) | 0.36    |
| Diabetes mellitus   | 12 (22.6) | 4 (18.2) | 8 (25.8) | 0.51    |
| Previous surgery    | 20 (37.7) | 12 (54.5) | 8 (25.8) | 0.03    |

CAGTA: Candida albicans germ tube antibody.

Table 2 Distribution of antifungal treatment administered according to CAGTA results

| No. of patients (%) | Total | CAGTA + | CAGTA - | p value |
|---------------------|-------|---------|---------|---------|
| Fluconazole         | 13 (48.1) | 6 (60) | 7 (41.2) | 0.34    |
| Voriconazole        | 4 (14.8) | 3 (30) | 1 (5.9) | 0.08    |
| Fluconazole + caspofungin* | 4 (14.8) | 0 | 4 (23.5) | 0.09    |
| Liposomal amphotericin B | 2 (7.4) | 0 | 2 (11.8) | 0.25    |
| Liposomal amphotericin B + fluconazole* | 2 (7.4) | 0 | 2 (11.8) | 0.25    |
| Caspofungin         | 1 (3.7) | 0 | 1 (5.8) | 0.43    |
| Fluconazole + liposomal amphotericin B +caspofungin* | 1 (3.7) | 1 (10) | 0 | 0.18    |

CAGTA: Candida albicans germ tube antibody.

*Sequential treatment.
evidence the need for a large multicentre study to validate the CAGTA detection as an alternative diagnosis tool in the ICU setting [16,30].

This study identifies the principal risk factors associated with serological proven candidiasis (Table 1) and, probably, with hidden invasive Candida infection in the ICU population due to the absence of other microbiological positive tests. Only previous surgery was statistically more frequent in patients with CAGTA-positive results. Furthermore, in our study previous surgery is a determinant risk factor for candidemia as has been described by León et al. [12]; however, although diabetes mellitus has been previously described as a risk factor for developing IC in critically ill patients [37], our findings do not support the inclusion of diabetes mellitus in clinical scores to predict IC in ICU patients. Recently published clinical scores such as the "Candida score" [12] or the Ostrosky-Zeichner et al. rule [38] have not included this risk factor. Consequently, periodical CAGTA determination should be mandatory especially in ICU surgical patients to achieve an early diagnosis and, therefore, to improve the candidemia prognosis, especially in those patients with patterns of increasing CAGTA titres [20]. This assay could improve the predictor value of these previously described prediction scores and rules.

The rate of highly colonized patients was very high as has been previously described [39] and no statistical differences were found between CAGTA-positive and -negative patients at the end of the study. This finding could corroborate the CAGTA technique as a key biomarker to distinguish between colonization and infection in these patients.

In our study, the decision to add empiric antifungal therapy for patients with suspected IC was at the discretion of the prescribing physician based on clinical criteria, but was never guided by CAGTA results. The no difference observed neither the global administration of antifungal treatment nor antifungal agent used between CAGTA-positive and -negative give more value to this technique avoiding false-negative results in presence of any antifungal therapy.

Several limitations must be noted in this study. First, the small number of patients due to the difficulty to enrol this kind of patients with predefined criteria. Moreover, there was no possibility to establish the sensitivity and specificity of CAGTA technique due to the absence of microbiological and histological documentation of IC in this study.

Conclusions
This study identified previous surgery as the principal clinical factor associated with CAGTA-positive results (serologically proven candidiasis) and emphasises the utility of this promising technique, which was not influenced by high Candida colonization or antifungal treatment. For these reasons the addition of the CAGTA technique to the armamentarium for the diagnosis of IC in surgical patients admitted in ICU would be a great advantage as it could help to identify hidden invasive Candida infection in the surgical ICU population with a long stay in this setting.

Acknowledgements
The present work is dedicated to Dr. José Pontón, a great friend and better researcher, who passed away on July 2010. Besides the authors, in the CAGTAUCI study group also collaborated: Angel Viudes; María D. Gómez from Hospital Universitario La Fe, Valencia; Susana Sancho, Rosa González from Hospital Universitario Dr. Peset, Valencia; José Ramón Irueta from Hospital Cruces, Barakaldo and José Suárez from Hospital Severo Ochoa, Leganés. This study has been supported by a Pfizer research grant, which has been used for logistic reasons and for supplying the laboratory tests. This work was partially presented in the 20th ECCMID 2010 (Vienna). We thank Miriam Phillips for revision of the English in the manuscript. Authors’ have obtained permission from above named people for being cited in this section.

Author details
1. Department of Microbiology, Hospital Universitario La Fe, (Av. Campanar, 21), Valencia, (46009), Spain. 2. Intensive Care Unit, Hospital Universitario Dr. Peset, (Gaspar Aguilar, 90), Valencia, (46017), Spain. 3. Department of Immunology, Microbiology and Parasitology, Facultad de Medicina y Odontología, Universidad del País Vasco, (Apdo 699), Bilbao, (48080), Spain. 4. Department of Microbiology, Hospital de Zumarraga, (Arkuxoa Taldea s/n), Zumarraga, (20700), Spain. 5. Department of Microbiology, Hospital Severo Ochoa, (AV Orellana s/n), Leganés, (28911), Spain. 6. Department of Microbiology, Hospital Universitario Dr. Peset, (Gaspar Aguilar, 90), Valencia, (46017), Spain. 7. Intensive Care Unit, Hospital Universitario La Fe, (Av Campanar, 21), Valencia, (46009), Spain. 8. Department of Microbiology Hospital N.S. de Valme, (Ctra. Cádiz, km 548), Seville, (41014), Spain. 9. Department of Microbiology, Facultad de Medicina, (Av Menéndez Pidal s/n), Córdoba, (14004), Spain.

Authors’ contributions
JaP, RZ, QQ and JoP designed the study. RZ, PR and EMM collected the data; MA, MSC, JJC, MJG, EMM, MJL performed the technique. JaP, RZ made the input from all the authors who each approved the final version. JaP, RZ, GQ and JoP designed the study. RZ, PR and EMM collected the data; MA, MSC, JJC, MJG, EMM, MJL performed the technique. JaP, RZ made the input from all the authors who each approved the final version.

Competing interests
The authors declare that they have no competing interests.

Received: 17 April 2010 Accepted: 9 March 2011

References
1. Zaragoza R, Pemán J. Invasive fungal infections in critically ill patients: different therapeutic options and a uniform strategy. Rev Iberoam Micol 2006, 23:59-63.
2. Zaragoza R, Pemán J. Diagnostic and therapeutic approach to fungal infections in critical care settings: different options but the same strategy. J Invasive Fungal Infect 2007, 15:50-58.
3. Mora-Duarte J, Betes R, Ratstein C, Colombo AI, Thompson-Moya L, Smetana J, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med 2002, 347:2020-2029.
4. Kullberg BJ, Sobel JD, Ruhnke M, Pappas PG, Viscoli C, Rex JH, et al. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidaemia in non-neutropenic patients: a randomised non-inferiority trial. Lancet 2005, 366:1435-1442.
5. Pachl J, Svboda P, Jacoby F, Vanekovoucky K, van der HB, Spronk P, et al: A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. Clin Infect Dis 2006, 42:1-404-1413.

6. Rebolí AC, Rotstein C, Pappas PG, Chapman SW, Kett DH, Kumar D, et al: Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med 2007, 356:2472-2482.

7. Kuse ER, Chetchothiasit P, da Cunha CA, Ruhnke M, Barrios C, Raghunadharao D, et al: Micafungin versus liposomal amphotericin B for candidemia and invasive candidiasis: a phase III randomised double-blind trial. Lancet 2007, 369:1519-1527.

8. Morell M, Fraser VJ, Kollef MH: Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother 2005, 49:3640-3645.

9. Garey KV, Rege M, Pai MP, Mingo DE, Suda KI, Turpin RS, et al: Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis 2006, 43:25-31.

10. Gamacho-Montero J, Garcia-Garmendia JL, Barrero-Almodovar A, Jimenez-Jimenez FJ, Perez-Paredes C, Ortiz-Leyba C: Impact of adequate empirical antibiotic therapy on the outcome of patients admitted to the intensive care unit with sepsis. Crit Care Med 2003, 31:272-273.

11. Parkins MD, Sabuda DM, Elsayed S, Laupland KB, et al: Anidulafungin versus fluconazole for invasive candidiasis: a randomized, blinded, multicenter trial of lipid-associated amphotericin B for the prevention of candidemia and invasive candidiasis. J Clin Microbiol 1999, 34:217-219.

12. Pittet D, Monod M, Suter PM, Frenk E, Auckenthaler R: Simplified adsorption method for detection of antibodies to Candida albicans germ tubes. J Clin Microbiol 1994, 32:217-219.

13. Ostrosky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, et al: Detection of antibodies to Candida albicans germ tubes in two intensive care patients with invasive candidiasis. Rev Iberoam Micol 2000, 179:91-96.

14. Zaragoza R, Pemán J, Cisterna R: Use of germ-tubes in the serodiagnosis of systemic candidosis. Enferm Infecc Microbiol Clin 2006, 24:103-109.

15. Moragues MD, Ortiz N, Iruretagoyena JR, Garcia-Ruiz JC, Amutio E, Rojas A, et al: Germ Tube Antibody (CAGTA) as an aid to diagnosis of fungal infections in humans. Clin Infect Dis 2005, 41:654-659.

16. Osterky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, et al: Multicenter clinical evaluation of the (1→3)-beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clin Infect Dis 2005, 41:654-659.

17. Quindós G, Pontón J, Cisterna R: Detection of antibodies to Candida albicans germ tubes in two intensive care patients with invasive candidiasis. J Clin Microbiol Infect Dis 1990, 9:178-183.

18. Garcia-Ruiz JC, del Carmen AM, Regulez P, Quindós G, Alvarez A, Pontón J: Indications for antifungal treatment in intensive care unit patients. Enferm Infecc Micr Biol Clin 2004, 22:279-285.

19. Parkins MD, Sabuda DM, Elsayed S, Laupland KB, et al: Anidulafungin versus fluconazole for invasive candidiasis: a randomized, blinded, multicenter trial of lipid-associated amphotericin B for the prevention of candidemia and invasive candidiasis. J Clin Microbiol 1999, 34:217-219.

20. Zaragoza R, Pemán J, Cisterna R: Use of germ-tubes in the serodiagnosis of systemic candidosis. Enferm Infecc Microbiol Clin 2006, 24:103-109.

21. Zaragoza R, Pemán J, Cisterna R: germ-tubes for diagnosis of two germ tube specific cell wall antigens of Candida albicans. Infect Immun 1986, 54:864-868.

22. Quindós G, Alvarez M, Regulez P, Pontón J, Cisterna R: Diagnosis of systemic candidiasis in parenteral drug addicts by detection of antymycelial antibodies. Med Clin (Barc) 1988, 90:451-455.

23. Irurtagoyena JR, Regulez P, Quindós G, Pontón J: Antibodies to Candida albicans germ tubes in intensive care patients with invasive candidiasis. Rev Iberoam Micol 2000, 179:91-96.

24. Zaragoza R, Pemán J, Cisterna R, Mackenzie DW: Value of detection of antibodies to Candida albicans germ tube in the diagnosis of systemic candidiasis. Eur J Clin Microbiol Infect Dis 1990, 9:178-183.

25. Ibañez-Nolla J, Nolla-Salas M, León MA, García F, Marrugat J, Soria G, et al: Clinical significance of Candida albicans germ tube antibody detection in critically ill patients. J Clin Microbiol Infect Dis 2001, 20:884-870.

26. Zaragoza R, Moragues MD, Pontón J: Is there a role for antibody testing in the diagnosis of invasive candidiasis? Rev Iberoam Micol 2004, 21:10-14.

27. Moragues MD, Ortiz N, Irurtagoyena JR, Garcia-Ruiz JC, Amutio E, Rojas A, et al: Evaluation of a new commercial test (Candida albicans IFA IgG) for the serodiagnosis of invasive candidiasis. Enferm Microbol Clin 2004, 22:83-88.

28. Zaragoza R, Pontón J, Cisterna R: Detection of antibodies to Candida albicans germ-tubes in the serodiagnosis of systemic candidosis. Eur J Clin Microbiol Infect Dis 1987, 6:142-146.

29. Garcia-Ruiz JC, del Carmen AM, Regulez 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, 35:3284-3287.

30. Zaragoza R, Pemán J, Quindós G, Irurtagoyena JR, Cuetara M, Ramirez P, et al: Clinical significance of Candida albicans germ tube antibody detection in critically ill patients. J Clin Microbiol Infect Dis 2009, 15:592-595.

31. Zaragoza R, Pemán J, Quindós G, Irurtagoyena JR, Cuetara M, Ramirez P, et al: Kinetic Patterns of Candida albicans Germ Tube Antibody (CAGTA) in Critically Ill Patients: Influence on Mortality. Clin Vaccine Immunol 2009, 16:1527-1528.

32. Pitter D, Monod M, Suter PM, Frenk E, Auckenthaler R: Candida colonisation and subsequent infections in critically ill surgical patients. Aro Surg 1994, 200:751-758.

33. Eggimann P, Garino J, Pitter D: Management of Candida species infections in critically ill patients. Lancet Infect Dis 2003, 3:772-785.

34. Piarroux R, Grenouillet F, Balvay P, Tran V, Blasco G, Million L, et al: Assessment of preemptive treatment to prevent severe candidiasis in critically ill surgical patients. Crit Care Med 2004, 32:2443-2449.

35. Bikandi J, San Millán R, Regulez P, Moragues MD, Quindós G, Pontón J: Detection of antibodies to Candida albicans germ tubes during experimental infections by different Candida species. Clin Diagn Lab Immunol 1998, 5:369-374.

36. Zaragoza R, Pemán J, Salavert M, Vilades A, Sole A, Jarque I, et al: Multidisciplinary approach to the treatment of invasive fungal infections.