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Clinical significance of the detection of *Candida albicans* germ tube-specific antibodies in critically ill patients

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Abstract

The present study, comprising a prospective multicentre study including 53 non-neutropenic patients from intensive care units (ICU) in six Spanish tertiary-care hospitals, was carried out to determine the clinical significance and influence on mortality of *Candida albicans* germ tube-specific antibodies (CAGTA). There were 22 patients (41.5%) for whom the CAGTA results were positive, although none of had a blood culture positive for *Candida*. The intra-ICU mortality rate was significantly lower ($p = 0.004$) in CAGTA-positive patients (61.2% vs. 22.7%). Multivariate analysis confirmed that a positive CAGTA result was the only protective factor to be independently associated with ICU mortality (β coefficient = −0.3856; 95% confidence interval = −0.648 to −0.123).

Keywords: Antibodies, *Candida* germ tube, critically ill patients, ICU, invasive candidiasis, mortality, pre-emptive antifungal therapy

Original Submission: 27 June 2008; **Revised Submission:** 15 September 2008; **Accepted:** 16 September 2008
Editor: M. Arendrup

Clin Microbiol Infect 2009; 15: 592–595
10.1111/j.1469-0691.2009.02794.x

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The mortality rate of invasive candidiasis (IC) remains excessively high [1] and is associated with the difficulty of making a prompt microbiological diagnosis [2] and a delay in antifungal treatment [3,4]. To date, no single serological test has demonstrated widespread clinical acceptance [5]. An immunofluorescence assay (*Candida albicans* IFA IgG; Vircell, Granada, Spain) was recently commercialized for *C. albicans* germ tube-specific antibody (CAGTA) detection. The present study aimed to determine the positive result rate of CAGTA testing in critically-ill patients and to assess its diagnostic and prognostic usefulnesses in this setting.

A prospective observational multicentre study was conducted at six Spanish University hospitals over a 2-year period (2005 to 2006). Inclusion criteria were: (i) acute pancreatitis of >7 days of disease evolution; (ii) prolonged intensive care unit (ICU) stay (>14 days) in conjunction with ≥3 risk factors (e.g. diabetes mellitus, extra-renal depuration, parenteral nutrition, >7 days of broad-spectrum antibiotic

therapy, major abdominal surgery); (iii) liver transplant, (iv) neutropenia or bone marrow transplant (BMT); and (v) high-level *Candida* colonization. Exclusion criteria were: (i) pregnancy, (ii) age <18 years, (iii) previous IC, and (iv) life expectancy <7 days. A corrected colonization index (CCI) was used to assess the intensity of *Candida* colonization [6], and patients with CCI ≥0.4 were considered to be highly colonized. CAGTA detection (*C. albicans* IFA IgG; Vircell) was performed twice weekly, with a serum titre ≥1/160 in at least one sample considered to be a positive result. Blood cultures were processed with automated systems (BACTEC, Becton-Dickinson Biosciences, Franklin Lakes, NJ, USA; BacTAlert, bioMérieux, Barcelona, Spain). Identification of yeasts was performed with API 32C or Vitek systems (bioMérieux). At each institution, the decision to initiate antifungal therapy for patients with suspected IC was made at the discretion of the prescribing physician, based on clinical criteria, but it was not influenced by the results of the CAGTA

TABLE 1. Clinical and serological characteristics of patients included in the study

Patient number	Age	Sex	Inclusion criteria*	Highest CCI	Serum samples (No.)		Highest CAGTA titre	Antifungal treatment	CAGTA + titre kinetics	Outcome
					Total	Positive				
1	74	M	2	0.3	1	0	<1/80	No		Death
2	52	F	2	0.0	2	0	<1/80	Yes		Survival
3	51	M	2	0.3	3	0	<1/80	No		Survival
4	71	M	2	0.3	4	0	<1/80	No		Survival
5	77	F	2/5	0.8	2	1	1/160	Yes	↓	Death
6	78	F	1/2	0.8	6	6	1/640	Yes	↓	Survival
7	75	M	2	0.3	2	2	1/640	No	↔	Survival
8	82	M	5	0.5	4	0	<1/80	Yes		Death
9	74	M	5	0.6	1	0	<1/80	No		Survival
10	64	M	2	0.3	1	1	1/160	No	NA	Death
11	45	M	4	0.5	3	0	<1/80	No		Survival
12	69	F	4	0.8	3	0	<1/80	No		Death
13	63	F	4	0.3	5	0	<1/80	No		Death
14	75	M	2	0.0	3	0	<1/80	Yes		Death
15	83	M	2	0.0	2	2	1/2560	Yes	↑	Survival
16	79	F	5	0.8	3	0	<1/80	No		Survival
17	71	M	2	0.0	9	9	1/2560	Yes	↓	Death
18	51	M	3	1.0	3	1	1/320	Yes	↑	Survival
19	53	F	2	0.0	2	1	1/160	No	↑	Survival
20	59	M	5	0.4	8	0	<1/80	Yes		Death
21	53	M	5	0.6	6	0	<1/80	Yes		Survival
22	70	F	2	0.0	1	0	<1/80	No		Death
23	61	M	2	0.0	1	0	<1/80	No		Death
24	78	M	2	0.0	3	0	<1/80	Yes		Death
25	62	M	1	0.0	1	0	<1/80	Yes		Death
26	80	F	5	0.4	2	2	1/160	Yes	↔	Death
27	45	F	5	0.5	4	1	1/160	Yes	↓	Survival
28	62	M	2	0.3	2	2	1/320	Yes	↓	Death
29	50	F	2	0.0	4	1	1/160	No	↓	Survival
30	76	M	5	1.0	3	0	<1/80	Yes		Survival
31	71	F	2	0.0	2	2	1/1280	Yes	↑	Survival
32	67	M	2	0.0	1	0	<1/80	No		Death
33	78	F	2	0.0	3	3	1/160	No	↔	Survival
34	62	F	2	0.0	3	1	1/320	No	↑	Survival
35	81	F	2	0.0	1	0	1/80	No		Survival
36	81	M	2	0.0	3	0	1/80	Yes		Death
37	31	M	2	0.0	2	2	1/1280	No	↑	Death
38	26	M	2	0.0	2	1	1/160	Yes	↑	Survival
39	60	M	2/3/4	0.8	2	0	1/80	No		Death
40	55	F	2	0.5	5	1	1/160	No	↓	Survival
41	35	M	1/2	0.4	3	3	1/160	Yes	↔	Survival
42	101	M	2	0.5	3	2	1/320	No	↓	Survival
43	79	F	2	0.0	1	1	1/1280	No	NA	Survival
44	64	M	2/5	0.8	1	0	1/80	Yes		Death
45	32	M	3	0.3	2	2	1/320	No	↔	Survival
46	46	F	2	0.5	2	0	1/80	Yes		Death
47	71	M	2/5	0.5	5	0	1/80	Yes		Death
48	18	M	2	0.6	3	0	1/80	Yes		Survival
49	79	M	2/5	0.8	2	0	1/80	Yes		Survival
50	73	F	2	0.0	6	0	1/80	Yes		Death
51	59	M	2/5	0.6	6	0	<1/80	Yes		Survival
52	64	F	2	0.0	1	0	1/80	Yes		Death
53	61	M	2	0.3	3	0	1/80	No		Survival

M, male; F, female; CCI, corrected colonization index.
Inclusion criteria: *1: acute pancreatitis of more than seven days of evolution; *2: prolonged ICU stay (>14 days) and three or more risk factors (diabetes mellitus. extra-renal depuration, parenteral nutrition, >7 days of broad spectrum antibiotic therapy, and major abdominal surgery); *3: liver transplant; *4: neutropenia or bone marrow transplant; and *5: high *Candida* colonization.
↑: increasing titres; ↔: no change; ↓: decreasing titres; NA, not applied.

assay. Chi-square or Fisher's exact tests were used to compare categorical variables. Multivariate analysis was performed to determine independent predictors of related intra-ICU candidaemia mortality using the stepwise method. $p < 0.05$ was considered statistically significant.

Fifty-three critically ill patients were included in the study (Table 1). The most frequent inclusion criteria were prolonged ICU stay and the presence of ≥3 IC risk factors (60%), followed by CCI ≥0.4 (23%) and neutropenia or BMT (7.5%). Age and APACHE II score averages were 71.5 ± 18.4 years, and 14.9 ± 5.4 points, respectively. The major reasons for ICU admission were septic shock (28.3%) and respiratory failure (28.3%). Patient characteristics did not differ among the participating centres. Culture-based methods yielded negative results for yeasts in all patients.

Twenty-two patients (41.5%) had CAGTA-positive results (ten patients had one positive sample, eight patients had two, and four patients had greater than or equal to three). CAGTA titres were in the range of 1/160 to 1/2560. The presence of acute renal failure (ARF) was more frequent in CAGTA-negative patients. The APACHE II score was statistically higher in this group.

Intra-ICU mortality was 45.2%, being significantly lower ($p = 0.025$) in CAGTA-positive patients (22.7% vs. 61.2% in CAGTA-negative patients). Higher APACHE II score, ARF, and CAGTA-negativity were also associated with ICU mortality in the univariate analysis (Table 2). Conversely, surgical patients were significantly associated with a better outcome.

CAGTA positivity, APACHE II score, and the use of antifungal therapy were introduced into the multivariate statistical model as dependent variables. The stepwise method confirmed a CAGTA-positive result as a protective factor and the only independent variable associated with intra-ICU mortality (β coefficient = −0.3856; 95% confidence interval = −0.648 to −0.123).

TABLE 2. Univariate analysis of intensive care unit mortality

	Number of patients (%)		
	Survival	Died	p value
Age (years)	68.6 ± 21.7	75 ± 15.3	0.20
Male/Female (ratio)	1.57	2	0.56
APACHE II (score)	12.9 ± 5.2	17 ± 4.7	0.005
Septic shock	6 (20.7)	9 (37.5)	0.17
Respiratory failure	9 (31)	6 (25)	0.62
Coma	2 (6.9)	3 (12.5)	0.48
Renal failure	8 (27.6)	16 (66.7)	0.004
Extra-renal depuration	6 (20.7)	10 (41.7)	0.09
Hepatic failure	8 (27.6)	9 (37.5)	0.44
Neutropenia	1 (3.4)	3 (12.5)	0.21
Antifungal treatment	13 (44.8)	14 (58.3)	0.32
CAGTA+	17 (58.6)	5 (20.8)	0.006
Diabetes mellitus	4 (13.8)	8 (33.3)	0.09
Previous surgery	16 (55.2)	4 (16.7)	0.004
Total	29 (100)	24 (100)	–

CAGTA+, Detection of *Candida albicans* germ tube antibodies.

Ponton *et al.* [7–9] have developed an indirect immunofluorescence assay to detect CAGTA, which proved useful in the diagnosis of IC in different groups of patients [10–13]. The test has shown an overall sensitivity of 77–89% and a specificity of 91–100%. CAGTA has been detected in patients with IC due to *Candida* species other than *C. albicans* [9,12–15]. The *C. albicans* IFA IgG test has been compared with the standard test in a retrospective study using 172 sera from 51 haematology and ICU patients [13]. The commercially available test was similar to the standard test and provided a faster and easier diagnosis of IC.

The sensitivity and specificity of CAGTA detection have not been established in the present study because all blood cultures performed for *Candida* spp. were negative. The high prevalence of CAGTA-positive results obatained (41.5%) corroborates the adequacy of the inclusion criteria used in the present study as a predictive biomarker of *Candida* infection and the need to consolidate data concerning CAGTA detection in a well-defined ICU population.

Although the global sensitivity rate of blood culture is not too high (approximately 50%) in the hospital population [16], this procedure remains the reference standard for candidemia diagnosis. However, this ratio is considerably lower in the ICU setting (5.7%), as previously observed in a *Candida* score study [17].

The univariate analysis confirmed the APACHE II score, ARF, and diabetes mellitus as mortality risk factors. To our knowledge, no association between a positive serological result and mortality has been reported previously in a prospective study in ICU patients with IC. However, the significance of antibody responses to other antigens (especially heat shock proteins) has been reported previously in animal models [18,19].

Intra-ICU mortality was significantly lower in CAGTA-positive patients. Although CAGTA-positive and -negative patients received antifungal treatment based on clinical data, the lower mortality observed in the CAGTA-positive group might be related to correct empirical treatment. Moreover, immunological responses could also play a role in the high mortality of CAGTA-negative patients.

Several limitations of the present study should be noted. First, the small number of patients is a limitation as a result of the difficulty of enrolling this kind of patient with predefined criteria. Moreover, it was not possible to establish the sensitivity and specificity of the CAGTA technique as a result

of the absence of proven IC. The possible bias due to the APACHE II score mismatch between groups needs to be evaluated with tested specimens.

One-third of non-survivors had only a single specimen tested, whereas 90% of survivors had serial specimens tested. One possible explanation of this mismatch could be that the higher APACHE scores resulted in an earlier mortality, before serial samples could be collected and before antibody positivity had developed. However, its effect on mortality was not confirmed in the multivariate analysis.

In conclusion, the rate of positive CAGTA results recorded in a selected group of ICU patients was markedly higher than theoretically expected. Based on these results, a strategy of early antifungal treatment after CAGTA detection might reduce the ICU mortality of those individuals with risk factors for the development of IC. Further studies are warranted to confirm these initial findings.

Acknowledgements

This work was partially presented at ICAAC 2006 (San Francisco). We thank M. Phillips for revision of the English.

Transparency Declaration

This study was financially supported by an independent investigation grant from Pfizer. All authors declare that they have no potential conflicts of interest.

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