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Original Article

Diagnostic surveillance by *Candida albicans* germ tube antibody in intensive care unit patients

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Abstract *Background:* The diagnosis of Invasive Candidiasis (IC) presents serious problems, mainly associated with the absence of pathognomonic symptoms of the disease and the difficulty of isolating the fungus in blood culture. *Candida albicans* germ tube antibody (CAGTA) provides a rapid and simple test for diagnosis of IC. The aim of this study was to evaluate the diagnostic role of the CAGTA in the monitoring of critically-ill patients at risk of developing IC.
Methods: During diagnostic surveillance in the intensive care units (ICU) CAGTA was performed twice a week if predetermined risk factors were present and a positive result was considered when a serum titer $\geq$1/160 was detected in at least one sample.
Results: Seventy critically ill patients were included in the study. Twenty-three patients with proven/probable IC were identified. The sensitivity, specificity, PPV, and NPV of CAGTA for the diagnosis of proven/probable IC in all 70 patients were 91.3%, 68.1%, 58.3%, and 94.1%, respectively. Statistically significant highest titers were found in patients with proven/probable IC as well as increasing titers more than 1/160.
Conclusions: Our results suggest that detection of CAGTA could be a useful biomarker for the diagnosis of proven and probable IC in critical patients during prolonged ICU stay. During the monitoring it is opportune to evaluate the titers kinetics since the clinical diagnosis of proven/probable IC coincided with increase titer from negative ($<$1/160) to more than 1/160.

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Introduction

The incidence of invasive candidiasis (IC), particularly candidemia, has increased significantly in recent years and *Candida* spp. is the fourth most common pathogen isolated in blood cultures in the US.[1] In Europe it ranks among the ten most frequently isolated pathogens.[2] Candidemia is a life-threatening infection with high morbidity and mortality.[3,4] Even in the most recent studies, crude mortality rates reached 50—60% in critically ill patients, although attributable mortality can be substantially lower. Immunocompromised patients are at high risk of developing *Candida* infection.[5,6] *Candida albicans* remains the predominant strain although non-*albicans* species are increasingly common and in some adult intensive care units (ICU) they are responsible for over 50% of candidemias.[7,8] Blood cultures remain the mainstay for the diagnosis of candidemia, although sensitivity is not optimal and the time from the blood sample collection to the microbiological response of a growing yeast is long.[9] New scores and laboratory tests have been developed to make an early therapeutic intervention in an attempt to reduce the high mortality associated with invasive fungal infections.[10,11] Substantial progress has been made in the diagnosis of IC with the development of a variety of methods for the detection of antibodies and antigens. An immunofluorescence test for *C. albicans* germ tube antibody (CAGTA) detection has been marketed to help with the IC diagnosis. Indeed the test provides a rapid and simple diagnosis of IC in the clinical microbiology laboratory (84.4% sensitivity and 94.7% specificity).[12,13] The performance of the test has been studied in haematological patients (87.5% sensitivity and 95.2% specificity),[14] and in ICU patients in which a significant decrease in mortality was observed in those with a CAGTA positive result, especially in those with increasing CAGTA values who had been treated with antifungals.[15,16] Recently the CAGTA detection assay has been used in combination with (1 → 3)-β-D-Glucan (BDG), C-reactive protein and procalcitonin for discriminating between *Candida* spp. colonization and IC in non-neutropenic critically ill patients with severe abdominal conditions and to establish a model for the prediction of IC.[17] This study shows that BDG levels greater than 259 pg/mL combined with at least 1/160 for positive CAGTA results accurately discriminate *Candida* spp. colonization from IC in non-neutropenic critically ill patients with severe abdominal conditions.[18] Finally, it was shown that the presence of a positive CAGTA test in a serum sample from a patient with candidemia suggests deep-seated candidiasis.[19] The utility of laboratory biomarkers in the field of fungal infections has become the object of intensive investigation. The aim of this study was to evaluate the diagnostic role of the CAGTA in the monitoring of critically-ill patients at risk of developing invasive candidiasis.

Methods

Patients and methods

A retrospective observational study was conducted at the University hospitals "Policlinico-Vittorio Emanuele", Catania, Italy, over a 1-year period (January 2015—December 2015) in the medical, surgical and respiratory ICU setting in routine clinical practice. Inclusion criteria were as follows: prolonged ICU stay (>4 days) and one or more risk factors: >7 days of broad-spectrum antibiotic therapy, any surgery under general anaesthesia, parenteral nutrition, use of steroids or use of other immunosuppressive agents, *Candida* colonization index (CI) value ≥ 0.5.[20] Severity of illness at inclusion in the study was calculated with the Simplified Acute Physiology Score II (SAPS II). In the study has been enrolled also the patients who had proven/probable IC at the initial screening.

For all eligible patients, a CAGTA detection assay (*C. albicans* immunofluorescence assay immunoglobulin G; Vircell, Spain) was performed twice a week, and a positive result was when a serum titer ≥1/160 was detected in at least one sample. Surveillance cultures for *Candida* colonization were performed once a week. Surveillance cultures for *Candida* spp. using samples obtained from bronchial aspirates, urine and rectal swabs. The colonization index was calculated as the ratio of the number of culture-positive surveillance sites to the total number of sites cultured.[20] Blood cultures were obtained at the discretion of the attending physician and were processed using the automated BACTEC system (Becton Dickinson).

The different *Candida* isolates were identified at the species level using the ID32C kit (bioMérieux, Marcy l'Étoile, France). 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 since they were not known.

Definitions of proven and probable disease

Proven disease was determined on the basis of the European Organization of the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria,[21] modified such that they could be applied with greater relevance to non-neutropenic adults.[22] Proven IC was defined by: i) histological evidence of yeast cells or hyphae or pseudohyphae from a normally sterile site, ii) isolation of *Candida* species from at least one blood specimen obtained from a peripheral vein, or iii) positive culture result for a sample from any other sterile site (excluding urine, sputum, bronchoalveolar lavage fluid, mucous membrane swabs, and specimens from skin sites).[22] Probable IC was defined by one of the following: Fever (temperature > 38 °C)

persisting despite use of broad-spectrum antibacterial therapy for 96 h, or temperature <36 °C or >38 °C, plus use of immunosuppressive therapy for at least 7 of the preceding 30 days (excluding corticosteroids), or symptomatic AIDS, or use of corticosteroid therapy for at least 21 days in the previous 60 days; plus one of the following: culture positive for the same *Candida* species from at least 2 non-contiguous (including non-sterile) sites, or bulls-eye lesions in liver and/or spleen on ultrasound or CT imaging accompanied by an elevated serum alkaline phosphatase level.[22]

Statistical analysis

Data were analysed using the MedCalc Statistical Software version 17.9.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Log-normally distributed variables are reported as geometric mean (GM; 95% CI) and compared using Student's t test. Medians with ranges were used to describe non-normally distributed continuous variables and compared using the Mann–Whitney *U*-test. Categorical variables are reported as percentages and compared using the two-tailed χ^2 test or Fisher's exact test, as appropriate. The following parameters of diagnostic performance and their 95% confidence intervals (CIs) were calculated: sensitivity, specificity, positive and negative predictive value (PPV, NPV).

Results

Seventy critically ill patients were included in the study and a total of 361 serum samples (mean 5.1 per patient, range 2–20) were collected for the CAGTA detection assay. The characteristics and risk factors of patients are shown in Table 1. In particular, ICU stay ($P < 0.01$), Simplified Acute Physiology Score ($P < 0.05$), Continuous Renal Replacement Therapy ($P < 0.05$) as well as the colonization index ≥ 0.5 ($P < 0.05$) and the antifungal treatment ($P < 0.001$) were significantly higher in the group of proven/probable IC.

The prevalence of proven fungal disease was 5.7% and specifically four candidemias (2 with *C. albicans*, 1 with *Candida glabrata*, 1 with *Candida parapsilosis*). All these patients had CAGTA positive results. In particular, in one patient a CAGTA titer of 1/1280 was observed eighteen days after enrolment and six days before positive blood culture for *C. albicans*. In another patient a CAGTA titer of 1/640 was observed twenty-two days after enrolment and seven days before positive blood culture for *C. albicans*. In this patient, the same day as the positive blood culture for *C. albicans*, the CAGTA titer was 1/2560. In the patient with positive blood culture for *C. parapsilosis* the CAGTA titer was 1/320, observed twenty-eight days after enrolment and the same day of the positive blood culture. Finally, the last patient CAGTA titer was 1/160 observed ten days after enrolment and two days before positive blood culture for *C. glabrata*.

Nineteen patients with probable IC were identified on the basis of modified EORTC/MSG criteria. Particularly, seventeen patients had fever (temperature > 38 °C) persistent despite use of broad-spectrum antibacterial therapy for 96 h and cultures positive for the same *Candida* species from at least two non-contiguous sites, and two patients had a temperature > 38 °C, had received immunosuppressive therapy for more than 7 of the preceding 30 days and cultures were positive for the same *Candida* species from at least two non-contiguous sites. Three of these nineteen patients had a probable IC at enrolment. In these patients who had initial titers >1/160 the titers remained the same during monitoring. The features of patients with proven/probable IC are summarized in Table 2. Thirty-six patients (51.4%) had CAGTA positive results (9 patients had one positive, 5 had two, and 22 had ≥3 positive samples). Patients with proven/probable IC were compared to patients who had no identified IC according to the modified EORTC/MSG criteria. Twenty-one of the 23 proven/probable IC patients yielded CAGTA positive

Table 1 Characteristics and risk factors of patients included in the study.

Patients and risk factors	Total ($n = 70$)	Proven IC ($n = 4$)	Probable IC ($n = 19$)	No IC ($n = 47$)	P[a]
Male sex (%)	48 (68.6)	4 (100)	13 (68.4)	31 (65.9)	0.682
Age, median years (range)	70 (19–91)	79 (50–84)	69.5 (42–86)	70 (19–91)	0.914
ICU stay, median days (range)	14.5 (4–108)	41.5 (21–53)	18 (8–108)	12 (4–57)	<0.01
SAPS II[b], median (range)	39 (14–90)	69 (39–90)	42 (23–83)	38 (14–84)	<0.05
Broad spectrum antibiotics (no., %)	67 (95.7)	3 (75)	19 (100)	45 (95.7)	1.000
Any surgery under general anaesthesia (no., %)	41 (58)	2 (50)	13 (68.4)	26 (55.3)	0.213
Total parenteral nutrition (no., %)	58 (82)	3 (75)	18 (94.7)	37 (78)	0.311
Steroids (no., %)	23 (32)	2 (50)	9 (47.3)	12 (25.5)	0.114
CRRT[c] (no., %)	32 (45)	3 (75)	12 (63.1)	17 (36.1)	<0.05
No. of patients (%) with a					
Positive CAGTA	36 (51.4)	4 (100)	17 (89.5)	15 (31.9)	<0.001
Colonization index ≥ 0.5	37 (52.8)	3 (75)	19 (100)	15 (31.9)	<0.05
Antifungal treatment	34 (48.5)	4 (100)	19 (100)	11 (23.4)	<0.001

[a] *P* values were calculated summing the proven and probable IC.
[b] Simplified Acute Physiology Score.
[c] Continuous Renal Replacement Therapy.

Table 2 Twenty-three patients with proven/probable candidiasis and the *Candida albicans* germ tube antibody results.

No	SAPS II	Underlying conditions	Blood culture	CAGTA[a]	CAGTA[b]	Colonization index	*Candida* sp. colonization	LOS[c] ICU (days)
1	42	Cardiac failure	Negative	1/160	1/1280	>0.5	*C. albicans*	57
4	83	Haematoma post angioplasty	Negative	1/40	1/320	>0.5	*C. parapsilosis*	8
6	37	Respiratory failure	Negative	<1/20	1/640	>0.5	*C. glabrata*	42
8	51	Hepatocellular carcinoma	Negative	1/80	1/160	>0.5	*C. parapsilosis*	10
12	29	Colorectal cancer	Negative	1/80	1/320	>0.5	*C. albicans*	10
14	66	Duodenal cancer	Negative	1/40	1/80	>0.5	*C. albicans*	8
15	40	Postoperative Peritonitis	Negative	1/40	1/320	>0.5	*C. albicans*	32
16	23	Kidney cancer, septic shock	Negative	1/80	1/640	>0.5	*C. albicans*	108
17[d]	44	Respiratory failure	Negative	1/320	1/320	>0.5	*C. tropicalis*	51
18	30	Respiratory failure	Negative	<1/20	1/640	>0.5	*C. albicans*	12
19[d]	43	Gastrointestinal perforation	Negative	1/640	1/640	>0.5	*C. albicans*	8
20	45	Solid cancer	Negative	1/40	1/80	>0.5	*C. glabrata*	24
25	75	Renal transplant	*C. parapsilosis*	1/40	1/320	<0.5	*C. parapsilosis*	53
31	72	Chronic heart failure	Negative	1/160	1/1280	>0.5	*C. albicans*	24
40	68	Cardiac failure	Negative	1/80	1/320	>0.5	*C. albicans*	18
43	42	Respiratory failure	Negative	<1/20	1/160	>0.5	*C. glabrata*	8
45	80	Chronic renal failure	Negative	1/160	1/1280	>0.5	*C. albicans*	49
48[d]	75	Abdominal surgery	Negative	1/320	1/320	>0.5	*C. albicans*	18
49	39	Respiratory failure	Negative	1/160	1/640	>0.5	*C. albicans*	27
52	39	Intestinal occlusion	*C. glabrata*	1/80	1/160	>0.5	*C. glabrata*	21
59	64	Intraparenchymal haemorrhage	*C. albicans*	1/20	1/2560	>0.5	*C. albicans*	43
60	28	Chronic renal failure	Negative	1/320	1/640	>0.5	*C. albicans*	8
70	90	Haematological malignancy	*C. albicans*	1/80	1/1280	>0.5	*C. albicans*	40

[a] At enrolment time.
[b] Highest titer observed during monitoring.
[c] Length of stay.
[d] Patient with probable IC at enrolment.

results. Among the patients who had no IC, 15 of the 47 patients tested had CAGTA-positive results. The sensitivity, specificity, PPV, and NPV of CAGTA for the diagnosis of proven/probable IC in all 70 patients were 91.3%, 68.1%, 58.3%, and 94.1%, respectively. The CAGTA titers observed in all the serum samples of the patients with proven/probable IC ranged from <1/20 to 1/2560 (median, 160) while for patients without IC CAGTA titers ranged from <1/20 to 1/640 (median, 40). To determine any change in the patients' titers, both with proven/probable IC and without IC, three patterns in CAGTA positive patients were detected: increasing titers up to 1/160 (13% vs 4.3%), increasing titers more than 1/160 (65.2% vs 4.3%), and no change in titers kinetics (13.2% vs 23.4%) (Table 3). The increasing titers more than 1/160 were significantly higher ($P < 0.001$) in patients with proven/probable IC, as well as no change in titer kinetics in patients without IC ($P < 0.001$). In patients with CAGTA positive results no decreasing titers were observed.

In the light of these results the sensitivity, specificity, PPV and NPV were evaluated in two scenarios. In the first scenario considering a positive result when a serum titer ≥1/320 was observed during monitoring. In this case the sensitivity, specificity, PPV and NPV were 78.3%, 82.3%,

Table 3 Dynamic patterns of CAGTA-positive results in patients with proven/probable IC and without IC.

Dynamic pattern	No. of patients (%) with proven/probable IC and without IC			
	Total	Proven/probable IC (23)	Without IC (47)	*P* value
CAGTA positive	36 (100)	21(91.3)	15 (31.9)	–
Increasing titers up to 1/160	5 (13.9)	3 (13.0)	2 (4.3)	0.994
Increasing titers more than 1/160	17 (47.2)	15 (65.2)	2 (4.3)	<0.001
No change	14 (38.9)	3 (13.2)	11 (23.4)	<0.001

69.2% and 88.6% respectively. In the second scenario, considering a positive result when a serum titer ≥1/320 during monitoring or with a pattern of an increasing titer up to 1/160 was observed (a serum titer of 1/160 at enrolment without dynamic change during monitoring it was not included). In this case the sensitivity, specificity, PPV and NPV were 91.3%, 78.7%, 67.7% and 94.9% respectively.

Empiric antifungal treatment was applied in 48.5% of cases during the study; the regimens most frequently prescribed were fluconazole (52.9%), caspofungin (23.5%) and fluconazole + caspofungin in sequential treatment (17.6%). There was difference in the global administration of antifungal treatment between CAGTA positive and negative patients (67.6% vs 32.4%; $P < 0.01$). The use of fluconazole showed a tendency to be more frequent in CAGTA negative patients (66.7% vs 37%; $P < 0.01$) (Table 4).

Discussion

The diagnosis of IC presents serious problems, mainly associated with the absence of pathognomonic symptoms of the disease and the difficulty of isolating the fungus in blood culture. A prompt and accurate diagnosis for the establishment of an early fungal treatment is essential, since a delay of 48 h is associated with a significant increase in mortality.[23]

The detection of antibodies against antigens of the mycelium phase by means of a previous absorption of samples with *C. albicans* yeasts in order to eliminate other antibodies typical of colonized but not infected patients has been proposed as a useful biomarker for the diagnosis of IC in different groups of patients, including ICU patients.[17,24] Although the titers found in immunocompromised patients are lower than those found in immunocompetent patients, the overall performance of the test is similar.[17] It has been reported that detection of CAGTA in patients with invasive infections caused by *Candida* species other than *C. albicans* (*Candida tropicalis*, *C. parapsilosis*, *C. glabrata*, *Candida dubliniensis*, *Candida guilliermondii* and *Candida krusei*) may also be positive, although titers are lower than in candidiasis by *C. albicans*.[19,25–27] In our experience, all patients with proven IC had CAGTA positive results, although the titers found in the patients with infection by *C. glabrata* and *C. parapsilosis* were lower, as well as the response of positive antibodies respect to positive blood culture was more late. In particular, in the three patients with proven IC caused by *C. albicans* and *C.glabrata* the CAGTA positive results were observed before the positive blood culture (six and seven days vs two days), while in the patient with proven IC caused by *C. parapsilosis* the CAGTA positive results were observed the same day of the positive blood culture. The reason for this apparent lower earliness of positive results CAGTA remains to be investigated.

There are not many clinical studies on the use of this test and few published reports correlate the results of CAGTA with the *Candida* blood stream infection. In a publication, Martínez-Jiménez M.C. and colleagues evaluated the potential role of CAGTA in the diagnosis of deep-seated candidiasis.[19] The study was conducted from 2003 to 2012 with 50 patients diagnosed as having candidemia and the authors concluded that the presence of a positive CAGTA test in a sample from a patient with candidemia suggests deep-seated candidiasis. In our study we evaluated the diagnostic role of the CAGTA in the monitoring of critically-ill patients at risk of developing invasive candidiasis. A prevalence of proven fungal disease of 5.7% was observed, while, on the basis of modified EORTC/MSG criteria, nineteen patients with probable IC but with blood cultures negative were identified. For proven/probable IC the CAGTA technique had sensitivity, specificity, PPV and NPV of 91.3%, 68.1%, 58.3% and 94.1%, respectively. Compared to prior studies,[12,13] overall CAGTA assay sensitivity was slightly higher in our cohort, while the specificity was lower, probably because in our study 15 patients without IC had titers ≥1/160. However, 78.6% (11/15) of these patients had titers ≥1/160 at the enrolment time in the study, although they did not develop proven/probable IC during monitoring. In addition, no changes in titer kinetics were observed in these patients. Only in four patients without proven/probable IC, change in the titer kinetics was observed during the monitoring and in particular two patients had an increase titers up to 1/160 and two patients had an increase titers more than 1/160. In the light of these results and considering the median value titers observed in the patients with proven/probable IC, it might be appropriate to consider positive results when titers more than 1/160 was detected during the monitoring. Just as be it could be useful for the diagnosis of proven/probable IC, during the prolonged ICU stay, evaluate the kinetic patterns of CAGTA only in the patients presenting at the first determination titer <1/160 considering that in our study the clinical diagnosis of proven/probable IC coincided with increase titer from negative (<1/160) to more than 1/160. In the patients with titers at the enrolment ≥1/160 in order to discriminate between a condition of candidiasis in progress and a condition of previous IC, one or more laboratory markers (e.g. BDG) must be associated. Probably, the high

Table 4 Distribution of antifungal treatment administered according to CAGTA results.

	No. of patients (%)			
	Total	CAGTA+	CAGTA−	*P* value
Fluconazole	18 (53)	10 (38.5)	8 (100)	0.003
Fluconazole + caspofungin[a]	6 (17.6)	6 (23)	0	0.297
Caspofungin	8 (23.5)	8 (30.8)	0	0.152
Liposomal amphotericin B + caspofungin[a]	2 (5.9)	2 (7.7)	0	1.000
Total	34 (100)	26 (100)	8 (100)	—

[a] Sequential treatment.

percentage of CAGTA positive results at enrolment in the patients without IC could be linked to the different setting of patients admitted to intensive care.

Peman suggested that systematic CAGTA serum determinations from critically ill patients at risk of invasive candidiasis were good markers for administration of empirical antifungal drugs.[17] Two published mortality analyses of a study group of *C. albicans* Germ Tube Antibody Detection in Critically Ill Patients (CAGTAUCI) showed a significant decrease of mortality in those ICU patients with a CAGTA positive result,[15,16] especially in those with patterns of increasing CAGTA titers who had been treated with antifungal agents. Thus, antifungal treatment should be considered when CAGTA titers increase in critically ill patients.[16] In our study, 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 never guided by CAGTA results. The total administration of antifungal treatment was significantly more frequent in CAGTA positive patients ($P = 0.01$) and, in particular, in those patients with increasing titers more than 1/160 (85.7%) compared to the patients with increasing titers up to 1/160 (44.4%) and no change in titers during monitoring (41.7%).

Several limitations must be noted in this study. First of all, the group of patients with positive blood cultures was not sufficiently large for a reliable analysis of the performance of the CAGTA in proven IC. Moreover, the use of modified EORTC/MSG criteria to identify patients with probable IC may have influenced the results of the analysis evaluated between the two groups.

Nevertheless, we believe that CAGTA could be a useful biomarker for the diagnosis of proven and probable IC in critical patients in ICUs when a serum titer $\geq$1/320 during monitoring or with a pattern of an increasing titer up to 1/160 was observed. Moreover, one advantage of this technique, when compared with other nonculture diagnostic methods, is its reduced cost for determination and its relatively rapid turnover (3 h total, with 20 min of hands-on time). However, the combination with other biomarkers may improve diagnostic performance and the efficiency of management of IC in critically ill patients.

Declarations of interest

None.

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