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Original Article

Evaluation of the Cica Fungi Test *Candida*, a Novel Serum *Candida* Mannan Antigen Kit, and Its Comparison with Cand-Tec in Candidemia Patients

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SUMMARY: The Cica Fungi Test *Candida* is a novel immunoassay test that is used in Japan to detect *Candida* mannan antigens. A total of 130 samples from 89 cases in which the β-D-glucan assay (MK method) was positive were collected between July 2007 and August 2008 at Nagasaki University Hospital, and the Cica Fungi Test *Candida* and Cand-Tec were performed. Diagnosis of candidemia was based on a positive culture for *Candida* spp. from blood or other sterile clinical specimens. A total of 19 samples from 16 cases with candidemia, and 111 samples from 73 cases without microbiological evidence of candidemia, were obtained. The sensitivity and specificity of the Cica Fungi Test and Cand-Tec were 63.2 and 95.5% and 52.6 and 50.5%, respectively. The Cica Fungi Test showed significantly higher specificity than Cand-Tec ($P < 0.01$). The β-D-glucan assay values were significantly higher in the candidemia samples than in the non-candidemia samples ($P = 0.0003$), a result that was well correlated with the Cica Fungi Test ($P = 0.0005$). The Cica Fungi Test was thus found to be more reliable and specific than Cand-Tec, and the combined evaluation with the β-D-glucan assay was more efficient for diagnosis of candidemia.

INTRODUCTION

Candida spp. are a major pathogen in nosocomial bloodstream infections (1,2). In particular, candidemia has been observed among patients exposed to antibiotics, immunosuppressive agents, parenteral nutrition, central venous catheter placement, and other multiple invasive medical procedures (3,4). Although invasive candidiasis is associated with a high attributable mortality (5), the early diagnosis and rapid initiation of appropriate antifungal therapy reduces mortality (6,7). Early diagnosis is, however, often difficult (8), as blood cultures lack sensitivity and require several days to produce a result (9,10). Various rapid, non-culture methods, including the detection of an anti-*Candida albicans* antibody (11) and *Candida* components such as mannan (12) and (1→3)-β-D-glucan (13), have therefore been developed for the diagnosis of invasive candidiasis. However, these non-culture methods exhibit numerous drawbacks in their sensitivity and specificity, and sometimes require the combination of two or more assay results for an accurate diagnosis of invasive candidiasis (12,14).

The Cand-Tec latex agglutination test (Ramco Laboratories, Stafford, Tex., USA), which was the first *Candida* antigen test to become commercially available, is still widely used in Japan for the diagnosis of candidemia. Despite its widespread use, a report by Reiss and Morrison demonstrated that the Cand-Tec has low sensitivity (49%) and specificity (43%) (15). The Cica Fungi Test *Candida* (Kanto Chemical Co., Tokyo, Japan and Unitika, Ltd., Osaka, Japan), which is a novel enzyme immunoassay test for the detection of mannan antigens of *Candida*, has been commercially available in Japan since 2005. A retrospective study of the Unimedi *Candida* Monotest, a different brand name for the Cica Fungi Test, which was performed with the reserved samples, revealed a relatively high sensitivity of 82% (73% per sera) and a specificity of 96% (16). In this study we used the β-D-glucan assays performed in clinical practice to collect candidemia patients efficiently, assessed the usefulness of the Cica Fungi Test *Candida* in the diagnosis of invasive candidiasis, and compared these results with those obtained using Cand-Tec. We also calculated the correlation of both tests with the β-D-glucan assay.

METHODS

Study design: Serum samples were collected from those patients with suspected invasive fungal infections at Nagasaki University Hospital in the period July 2007 to August 2008 and the β-D-glucan test (Fungitec G test MK; Seikagaku Corp., Tokyo, Japan) was performed. Those samples that tested β-D-glucan-positive (>20 pg/ml) were selected and the Cand-Tec and Cica Fungi Test were performed with them. Samples from those patients who did not

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undergo blood culture tests within 3 days before or after the β-D-glucan test were excluded. The diagnosis of candidemia was based on a positive culture for *Candida* spp. from blood or other sterile clinical specimens. Those patients who did not satisfy this criterion were considered to be non-candidemia patients.

Blood culture samples were processed for isolation of *Candida* spp. using the BacT/Alert system (bioMeriux, Marcy l'Etoile, France) and aerobic culture bottles. *Candida* spp. were identified on the basis of morphology, pigmentation pattern on CHROM agar, or by VITEK (bioMeriux). Plasma levels of (1→3)-β-D-glucan were measured using the Fungitec MK assay according to the manufacturer's instructions.

Clinical information, including the existence of underlying diseases, risk factors for candidemia, prior use of antibiotics or antifungal drugs, and the number of days from which the culture tests were performed, was obtained from each patient's clinical records. The clinical information and the results of both tests were analyzed retrospectively. Use of both the Cand-Tec and Cica Fungi Test has been previously approved in Japan. As these tests were required for diagnosis in the clinical practice, they were performed with the patient's consent. This study was approved by the institutional ethics committee of Nagasaki University hospital.

Cand-Tec: The Cand-Tec was performed according to the manufacturer's instructions. Briefly, sera were diluted 1:2 with the supplied diluent, then the diluted specimens (20 μl) were combined with the same volume of latex particles coated with anti-*Candida* antibody. The mixture was then spun at 100–140 rpm for 10 min and immediately assessed for agglutination. Positive sera at a dilution of 1:2 were then further titrated in two-fold serial dilutions to determine the maximum dilution that achieved positive agglutination.

Cica Fungi Test *Candida*: The Cica Fungi Test *Candida* was performed according to the manufacturer's instructions. Briefly, 300 μl of serum was combined with an equal volume of extraction buffer in an extraction tube, and heated at 100°C using a workstation heater (Kanto Chemical) for 10 min or boiled for 4 min. The mixture was then centrifuged at 4,000 × *g* for 10 min. The supernatant (200 μl) was transferred to a reaction tube and 100 μl of alkaline phosphatase-conjugated mannan antibody was added and incubated for 30 min at room temperature (20–30°C). A 250-μl aliquot of the reaction mixture was applied to a reaction cassette containing fixed mannan antibodies using a sample applicator. After complete absorption of the solution, the applicator was removed from the cassette and 10 drops of wash buffer were added to the sample window on the cassette, followed by 4 drops of substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate and nitrotetrazolium blue. After incubating for 10 min, 4 drops of stop solution were added to terminate the color development reaction. The test was interpreted as positive when a purple color was observed in the center of the sample window.

Statistical analysis: Categorical and continuous variables were analyzed using the chi-square test or Fisher's exact test, and Student's *t* test, respectively. A *P*-value of <0.05 was considered to indicate statistical significance.

RESULTS

Candidemia patients: A total of 19 samples from 16 cases with candidemia were obtained. In one of these patients, a different kind of *Candida* spp. was detected in the culture tests 2 months after the patient had recovered from the first episode. This particular patient was assessed as two separate cases. A total of 111 samples were obtained from 73 patients who had not previously been diagnosed with candidemia by culture tests. The clinical characteristics, culture results, and antigen tests for the 16 cases with candidemia are summarized in Table 1. The median age of the candidemia patients was 55.8 years. Central venous catheters had been placed in 14 of the 16 (87.5%) cases. Other conditions known to be associated with candidemia, including prior antibiotic therapy (43.8%), the presence of malignancy (25%), immunosuppressive agent administration (25%), and previous abdominal operations (18.8%), were observed in the definite candidemic patients.

The Cica Fungi Test and Cand-Tec: The Cand-Tec was positive in 10 of the 19 samples from candidemia cases. However, this test was also positive for 55 of the 111 samples collected from non-candidemia (culture negative) cases. In contrast, the Cica Fungi Test was positive in 12 of the 19 samples from the candidemia cases but was only positive in 5 of the 111 samples from non-candidemia cases. In terms of results per sample, the sensitivity, specificity, and positive predictive values of the Cand-Tec were 52.6, 50.5, and 15.4%, respectively, whereas these values for the Cica Fungi Test were 63.2, 95.5, and 70.6%, respectively. The Cica Fungi Test therefore demonstrated significantly higher specificity and positive predictive values than the Cand-Tec ($P < 0.01$).

The Cica Fungi Test and Cand-Tec were positive in 11 of the 16 (68.8%) and 8 of the 16 (50%) candidemic cases, respectively. Of the 5 cases (7 samples) for which *Candida* spp. were isolated from sterile clinical samples other than blood, the Cica Fungi Test was positive in only 3 of 7 samples. In our study, 4 of the cases included as candidemia cases gave a positive result in the Cica Fungi Test but were negative in the Cand-Tec. There were no apparent differences in the underlying disease, risk factors, strain isolated, or the timing of the culture tests performed in these 4 cases.

False-negative cases in the Cica Fungi Test: A total of 7 samples gave false-negative results in the Cica Fungi Test. Table 2 shows the characteristics of the false-negative samples in comparison with the true positive samples. Although the numbers of sample were small, false-negative samples tend to indicate lower β-D-glucan levels than true positive samples. Samples obtained some time after the onset of candidemia tend to result in false-negatives in the Cica Fungi Test.

False-positive cases in the Cica Fungi Test: The clinical characteristics of the 5 cases (5 samples) in which the Cica Fungi Test was positive but the culture test negative are summarized in Table 3. All these cases exhibited underlying diseases that are known risk factors for candidemia and had received central venous catheter placement. In addition, antifungal drug therapy had been initiated prior to performance of the blood culture tests in 3 of the 5 cases. In contrast, only 2 of the 41 cases (55 samples) in which the Cand-Tec was positive but the culture tests were negative had started treatment with antifungal drugs before the blood culture tests were performed.

The positivity rates in *Candida* spp.: The positivity rates for both tests for the *Candida* spp. detected in this study are presented in Table 4. The total positivity rate for the Cica Fungi Test and Cand-Tec were 63.1 and 52.6%, respectively.

Table 1. Clinical characteristics of the 16 cases (19 samples) diagnosed with candidemia at Nagasaki University Hospital (July 2007 to August 2008)

Patient (sample) no.	Age	Sex	Underlying disease	Central venous catheter	Site of isolation	*Candida* sp. isolated	Cica Fungi Test	Cand-Tec	β-D-glucan (pg/ml)	Days from the latest culture test
1 (1)	77	M	Hepatocellular carcinoma	+	Blood	*C. albicans*	+	× 2	376	0
1 (2)							+	× 4	70	1
2	83	M	Miliary tuberculosis	+	Blood	*C. albicans*	+	−	63	1
3	1	M	Virus associated hemophagocytosis	+	Endotracheal aspirated sputum	*C. albicans*	+	−	930	0
4	25	M	Peritonitis	−	Ascites	*C. albicans*	+	× 2	70	0
5	76	M	Aspiration pneumonia	+	Blood	*C. albicans*	+	× 2	347	2
6	65	M	Peritonitis	−	Abscess	*C. albicans*	−	−	72	2
7	1	M	Hepatoblastoma	+	Blood	*C. famata*	−	−	42	1
8	58	M	Acute renal failure	+	Blood	*C. glabrata*	+	−	202	2
9	66	M	Peritonitis	+	Blood	*C. glabrata*	−	× 2	220	0
10	24	M	Glioblastoma	+	Blood	*C. parapsilosis*	+	× 2	240	0
11	80	F	Aortic aneurysm	+	Blood	*C. parapsilosis*	+	× 4	1,070	1
12	72	M	Miliary tuberculosis	+	Blood	*C. parapsilosis*	−	−	139	0
13	45	F	Ulcerative colitis	+	Blood	*C. tropicalis*	+	× 2	919	0
14 (1)	81	F	Acute interstitial pneumonia	+	Catheter tip	*C. glabrata*	+	−	124	0
14 (2)							−	−	44	3
14 (3)							−	× 2	34	4
15	81	F	Acute interstitial pneumonia	+	Blood	*C. tropicalis*	+	× 4	46	−1
16	83	F	Cervical cancer	+	Catheter tip	*C. albicans*	−	−	318	0

The Cica Fungi Test demonstrated a relatively higher positivity rate (75%) than the Cand-Tec (50%) for *C. albicans* detection.

Correlations with the β-D-glucan assay: The correlations between the β-D-glucan assay, Cica Fungi Test, and Cand-Tec obtained in this study are presented in Fig. 1. The mean concentration of β-D-glucan in samples isolated from candidemia cases (mean, 288.3 pg/ml; range, 33.8–1,069.5 pg/ml) was significantly higher than that observed in samples from non-candidemia cases (mean, 95.6 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.0003$). Likewise, the mean concentration of β-D-glucan in those samples that were positive using the Cica Fungi Test (mean, 290.9 pg/ml; range, 22.4–1,069.5 pg/ml) was also significantly higher than that for the negative samples (mean, 98.6 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.0005$). There were no significant differences in the mean value of β-D-glucan between the Cand-Tec-positive (mean, 139.2 pg/ml; range, 21.5–1,069.5 pg/ml) and -negative samples (mean, 108.3 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.4192$).

DISCUSSION

The Cand-Tec is still frequently used as one of serological screening tests for candidemia in Japan, although it is no longer used in the U.S. or Europe. The sensitivity and specificity results achieved for the Cand-Tec in this study were roughly the same as those reported previously (15). Fujita et al. retrospectively evaluated the Unimedi *Candida* Monotest and compared the results with the Cand-Tec, Platelia *Candida*, Unimedi *Candida*, and β-D-glucan assays. The Unimedi *Candida* Monotest, which is equivalent to the Cica Fungi Test, demonstrated a similar specificity but a 10% higher sensitivity (per sera) compared to our study data (16). Although there are various differences between the study of Fujita et al. and ours, including study design (criteria for patient inclusion), the number of cases investigated, and the timing of the blood culture tests performed, it remains unclear as to why these differences in sensitivity were observed.

All false-positive cases for the Cica Fungi Test in our study had various risk factors for blood stream infections. Furthermore, 3 of the 5 cases had already started antifungal therapy before the blood culture tests. The use of antifungals is therefore likely to have influenced the results of the culture tests. Despite the possibility that antifungal drug administration may have eliminated *Candida* cells, the Cica Fungi Test might be able to detect residual antigen and there-

Table 2. Clinical characteristics of false-negative samples of Cica Fungi Test

	Site of isolation	*Candida* sp. isolated	β-D-glucan (pg/ml)	Days from the latest culture test	Antifungal treatments before the Cica Fungi Test
True positive sample	Blood	*C. albicans*	376	0	+
	Blood	*C. albicans*	70	1	−
	Blood	*C. albicans*	63	1	+
	Endotracheal aspirated sputum	*C. albicans*	930	0	+
	Ascites	*C. albicans*	70	0	−
	Blood	*C. albicans*	347	2	−
	Blood	*C. glabrata*	202	2	−
	Blood	*C. parapsilosis*	240	0	−
	Blood	*C. parapsilosis*	1,070	1	+
	Blood	*C. tropicalis*	919	0	−
	Catheter tip	*C. glabrata*	124	0	+
	Blood	*C. tropicalis*	46	−1	+
		C. albicans 50%	Ave. 371.4	Ave. 0.5	50%
False negative sample	Abscess	*C. albicans*	72	2	−
	Blood	*C. famata*	42	1	−
	Blood	*C. glabrata*	220	0	+
	Blood	*C. parapsilosis*	139	0	−
	Catheter tip	*C. albicans*	318	0	−
	Catheter tip	*C. glabrata*	44	3	−
	Catheter tip	*C. glabrata*	34	4	+
		C. albicans 28.6%	Ave. 124.1	Ave. 1.43	28.6%

Table 3. Clinical characteristics of the 5 cases with positive Cica Fungi Test but negative culture test

Patient no.	Age	Sex	Underlying disease	*Candida* sp. isolated	Cica Fungi Test	Cand-Tec	β-D-glucan (pg/ml)	Antifungal treatments before the blood culture test	Central venous catheter
1	80	F	Peritonitis	−	+	−	191	−	+
2	70	M	Pancreatitis	−	+	× 2	22	−	+
3	61	F	Liver transplantation	−	+	−	30	+	+
4	73	M	Hepatic failure	−	+	× 4	27	+	+
5	49	F	Acute myeloid leukemia	−	+	−	67	+	+

fore have higher sensitivity than the blood cultural test.

As far as the positivity rates for *Candida* spp. are concerned, although the number of cases included in the study was small, the Cica Fungi Test had a relatively higher positivity rate than the Cand-Tec for *C. albicans* detection. This result may be due to differences in the antigen targeted in the two tests. The antigen targeted in the Cica Fungi Test is the *C. albicans* mannan antigen, whereas the targeted antigen is a currently uncharacterized, heat-labile fungal antigen that circulates in the sera of patients with invasive infections caused by *C. albicans*, *C. tropicalis*, and *C. parapsilosis* in the Cand-Tec (17).

In this study we also assessed the correlation of the Cica Fungi Test and the Cand-Tec with the β-D-glucan assay. The measurement of β-D-glucan concentration has been widely used in Japan as a primary serological diagnostic procedure for invasive fungal infections (IFIs) as it exhibits a high sensitivity for the diagnosis of major IFIs (13,18). Furthermore, the β-D-glucan assay has recently been employed for the diagnosis of IFIs by the European Organization for Research and Treatment of Cancer (19). In agreement with the positive correlation between the β-D-glucan assay and mannan antigen (Platelia) reported previously (20), the Cica Fungi Test also showed a better correlation with the β-D-glucan assay in our study. As the Cica Fungi Test showed high specificity, its combination with the β-D-glucan assay, which possesses high sensitivity, may improve the overall diagnostic efficiency for the diagnosis of candidemia.

Table 4. Positivity rate of the Cica Fungi Test and Cand-Tec for *Candida* spp. (per sample)

Strain	Cica Fungi Test	Cand-Tec
C. albicans	6/8	4/8
C. glabrata	2/5	2/5
C. parapsilosis	2/3	2/3
C. tropicalis	2/2	2/2
C. famata	0/1	0/1
Total	12/19 (63.1%)	10/19 (52.6%)

In conclusion, this study suggests that the novel Cica Fungi Test is more reliable and specific for the diagnosis of candidemia than the Cand-Tec and that additional evalua-

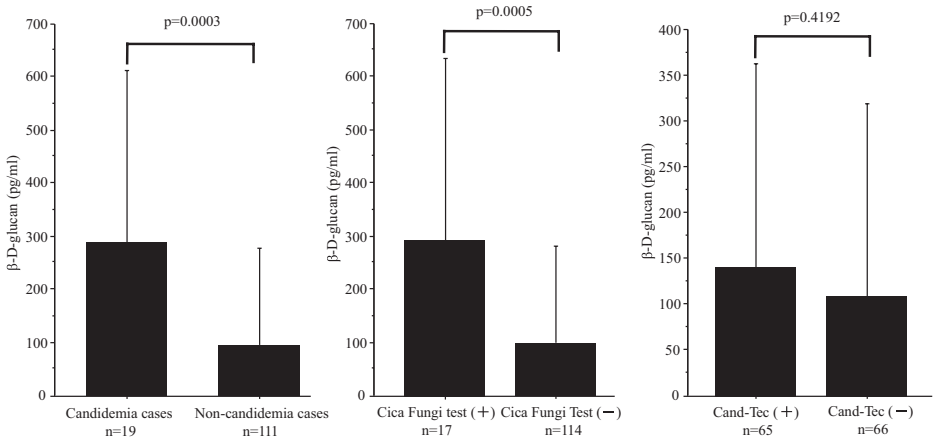

Fig. 1. Correlations between the values of the β-D-glucan test, Cica Fungi Test and Cand-Tec. Data are presented as the mean ± SD. The mean value of samples from the candidemia cases (mean, 288.3 pg/ml; range, 33.8–1,069.5 pg/ml) was significantly higher than that from the non-candidemia cases (mean, 95.6 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.0003$). The mean value of the β-D-glucan in the positive samples for the Cica Fungi Test (mean, 290.9 pg/ml; range, 22.4–1,069.5 pg/ml) was also significantly higher than that for the negative samples (mean, 98.6 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.0005$). There were no significant differences in the mean value of β-D-glucan between the Cand-Tec-positive (mean, 139.2 pg/ml; range, 21.5–1,069.5 pg/ml) and -negative samples (mean, 108.3 pg/ml; range, 20.4–1,451.8 pg/ml, $P = 0.4192$).

tion of the β-D-glucan assay may be beneficial for the diagnosis of candidemia.

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Conflict of interest None to declare.

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