Eur J Cancer Clin Oncol, Vol. 24, No. 10, pp. 1655–1659, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

Evaluation of a *Candida* Antigen Detection Test (Cand-Tec™) in the Diagnosis of Deep Candidiasis in Neutropenic Patients

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Abstract—*The diagnostic efficiency of a serum* Candida *antigen detection test (Cand-Tec™ test) was prospectively investigated in* 104 *leukemic patients treated by intensive chemotherapy or alogeneic bone marrow transplantation.* Candida *antigen titers were determined on admission and then weekly as long as patients remained neutropenic. Nine patients had a proven disseminated yeast infection (diagnosed only at autopsy in five cases). The highest* Candida *antigen titers were* 1:2 *in two patients and* 1:4 *or more in seven patients (sensitivity:* 76% *for this last titer). This highest titer was observed* 12 *days before to* 3 *days after the diagnosis. Seven out of the* 97 *patients without proven deep candidiasis had a maximum titer of* 1:4 *(specificity:* 93%*). The positive predictive value was* 50% *for a titer of* 1:4 *and* 24% *for a titer of* 1:2*, whereas the negative predictive value was* 100% *for a titer of* 1:4 *and* 97% *for a titer of* 1:2*. Patients with elevated titers were mostly treated by chemotherapy, were older and had a worse prognosis than those with negative titers, although the duration of neutropenia was similar. It is concluded that* Candida *antigen detection is a reliable method of diagnosis of deep candidiasis in neutropenic patients. The clinical interest in this test, with special regard to empiric antifungal therapy, is discussed.*

INTRODUCTION

SYSTEMIC CANDIDIASIS is a frequent infection after intensive chemotherapy for leukemia, or after bone marrow transplantation (BMT). In retrospective studies of large series of patients, the frequency ranged from 11 to 15.4% [1–3]. Moreover, a number of deep infections are probably not diagnosed before death. In systematic autopsies, De Gregorio *et al.* found up to 20% of deep candidiasis in patients dying with neutropenia [4]. Neutropenic patients are often colonized by *Candida* species, but early dissemination is difficult to detect, and systemic anti-fungal treatment is often delayed or given empirically.

The detection of circulating *Candida* antigens is an efficient method for the diagnosis of disseminated candidiasis. A variety of techniques have been developed. Enzyme-linked immunosorbent assay (ELISA) and radio-immuno assay (RIA) are very sensitive, but require special facilities and are difficult to use [5]. An agglutination test using antibody-coated latex particles has been proposed [6, 7]. Its efficiency has been assessed so far in retrospective studies of sera obtained from patients with known systemic candidiasis. To evaluate the interest of this test in the early diagnosis of deep candidiasis, we have performed a prospective study in leukemic patients receiving intensive chemotherapy or undergoing bone marrow transplantation.

Accepted 13 June 1988.
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MATERIALS AND METHODS

Patients

One hundred and four patients were studied (51 male, 53 female). Twenty-five patients (mean age: 31.6 years) received allogeneic bone marrow transplantation and a conditioning regimen of cyclosphosphamide and total body irradiation. These patients were treated in laminar air flow rooms and received sterile food. The mean duration of neutropenia in this group was 25.9 days for granulocytes less than

0.5×10^9/l and 16.9 days for granulocytes less than 0.1×10^9/l. Seventy-nine patients (mean age: 46.3 years) received intensive chemotherapy for acute myeloid leukemia (51 first induction therapy, 25 relapse therapy), or adult acute lymphoblastic leukemia (four patients in relapse). The respective durations of neutropenia of less than 0.5 and 0.1×10^9/l were 24.8 and 18.9 days. Those patients were nursed in conventional single rooms and received non-sterile food. All patients had a central catheter.

All patients received an oral decontamination by chlorhexidine and nystatin mouth-washes (4–6 times a day) and a gastro-intestinal decontamination by gentamicin (40 mg/day), colistine sulfate (4 million Units/day) and nystatin (6 million Units/day). When they became febrile, they received an empiric antibiotic treatment by ticarcillin (5 g/8 h) (or piperacillin 3 g/8 h) and moxalactam (1 g/8 h). When this treatment remained unsuccessful for more than 72 h, or when a second febrile episode occurred, intravenous amphotericin B (0.5 mg/kg/day) was added empirically. The daily dose was increased to 1 mg/kg/day in case of disseminated fungal infection. Sixty-five patients received amphotericin B. The mean duration of treatment was 5.7 days in BMT patients and 9.8 days in chemotherapy patients. The amphotericin B treatment was not modified according to the result of the Cand-Tec™ test alone.

Twenty-three patients died during the treatment. The main cause of death was bacterial infection in eight patients, fungal infection in nine patients (yeast infection in five patients and aspergillosis in four patients). One patient died of drug toxicity. The direct cause of death was not found in five patients. Permission for autopsy was obtained in 10 cases.

Mycology

The detection of *Candida* antigen was performed by the Cand-Tec™ test (Ramco Inc, Houston, Texas, U.S.A., distributed in France by Eurobio, Paris). This test is based on the agglutination, in the presence of antigen, of latex particles coated with rabbit anti-*Candida* antibodies. Briefly, 100 µl of the patient serum were mixed with 100 µl of sample diluent and placed on a glass slide. 20 µl of antibody-coated latex suspension were added and mixed. The agglutination was observed immediately after a 10 min rotation of the slide. Controls were performed with positive and negative serum provided with the test. Intra-assay reproducibility tests did not show any modification of the results. Inter-assay tests using two different kits showed a one-dilution (1:4 to 1:8) variation in one out of 10 samples. The reproducibility of the test was therefore considered very satisfactory.

The test was performed on admission and then weekly throughout the neutropenic phase. A total of 495 tests were performed in the 104 patients.

Surveillance cultures for fungi were obtained from oropharynx, stools and urine on admission and weekly. A minimum of three blood cultures were taken through the central catheter in case of febrile episode before initiation of antibiotic or antifungal therapy, and from then on every day with pyrexia. Samples were cultured on Sabouraud medium. Culture samples were also obtained from broncho-alveolar lavage in case of pulmonary infection. Colonization was not quantified.

Evaluation of the results

The diagnosis of superficial colonization was carried out when *Candida* were grown from oropharynx, broncho-alveolar lavage (BAL), urine or stool samples. A systemic candidiasis was diagnosed when *Candida* were grown from at least two blood cultures, or when the biopsy or autopsy specimens of deep organs revealed the presence of *Candida* by histology. In six cases a *Candida* sp. was grown from one blood culture only. These cases did not meet the criteria for systemic candidiasis. One patient of this group died. No evidence of disseminated candidiasis was found at autopsy. For the evaluation of sensitivity and specificity of the test, these cases were not considered as systemic candidiasis, but as fungemia.

The following statistical tests were used: the Kruskal–Wallis one-way analysis of variance for comparing the mean age of different groups, analysis of variance for comparing the mean durations of neutropenia or antifungal treatment, and the chi-square test for comparing proportions.

The sensitivity, the specificity, and the predictive value of the Cand-Tec™ test were calculated as described by Kozinn *et al.* [8].

RESULTS

The patients were classified into three groups according to their highest antigen titer. *Candida* antigen detection was negative in 67 patients. The maximum titer was 1:2 in 23 patients and 1:4 or more in 14 patients.

Correlations between Candida *antigen and clinical findings*

The distribution of *Candida* antigen titer was significantly different ($P = 0.02$) between chemotherapy patients (negative titer: 45/79 patients; 1:2 = 21/79; 1:4 = 13/79) and BMT patients (negative titer: 22/25 patients; 1:2 = 2/25; 1:4 = 1/25). The mean durations of neutropenia did not differ according to antigen titers either in whole group of patients or in chemotherapy patients.

Patients with titers of 1:2 or 1:4 or more were significantly older than patients with negative titers (mean age: 38.3 vs. 48.1 and 55.6 years respectively, $P = 0.001$). This was the case for all patients, but also for patients treated only by chemotherapy ($P = 0.002$).

The number of patients who received amphotericin B was higher in the groups with titers equal to or greater than 1:2. Among the 65 patients treated with this drug, the duration of treatment was longer in those with elevated titers (Table 1). The clinical outcome was different according to antigen titers. The mortality was higher in the patients with titers of 1:2 (eight deaths/23 patients) or 1:4 and 1:8 (8/14 patients) as compared with patients with negative titers (7/67). The difference was also significant for patients treated only by chemotherapy (Table 1).

Correlations between Candida *antigen and mycologic results*

A systemic candidiasis was diagnosed in nine patients. The diagnosis was obtained at autopsy in five cases and by blood cultures in four cases (three cases also presenting with skin lesions positive for *Candida* in culture and histology). In seven cases, the titer was 1:4 or more. Details of these nine patients are given in Table 2.

In six cases, *Candida* was found in one blood culture. Details on the antigen titers are given in Table 2. One out of these six patients died (with a titer of 1:2). No evidence of deep candidiasis was found at autopsy.

Eighty-nine patients were diagnosed as having superficial colonization (62 patients) or sterile surveillance cultures (27 patients). The *Candida* antigen titer was negative in 64 patients, 1:2 in 19 patients and 1:4 in six patients (Table 3). One out of the six patients with a titer of 1:4 died. No autopsy was performed.

Statistical evaluation

Sensitivity, specificity of the test and predictive value are presented in Table 4. Because patients with fungemia (one positive blood culture) were difficult to assess, the evaluation was made in two different ways considering these cases either as disseminated infections or not.

DISCUSSION

Our results confirm the high frequency of systemic candidiasis in severely neutropenic patients. A systemic candidiasis was proven in nine out of 104 patients (8.7%). Five cases were not diagnosed before death, which confirms the results obtained by De Gregorio *et al.* [4] in systemic autopsies. On the other hand, because of the risk of undiagnosed fungal infection, 65 patients (64%) received intravenous amphotericin B. These findings demonstrate the need for an early and reliable diagnosis of deep fungal infections.

In our series, of the nine patients with proven systemic candidiasis, seven had *Candida* antigen titer of 1:4 or greater and two a titer of 1:2. Moreover, in the five patients in whom the diagnosis was performed only at autopsy, the titer was 1:4 in four cases and 1:2 in one case. Interestingly, a cross-reactivity was observed between *C. albicans* and other *Candida* species (the titer was 1:4 in patients and 1:2 in two patients with deep *C. tropicalis* infections). No false negative result was encountered when using a titer of 1:2 as diagnostic of systemic candidiasis (sensitivity: 100%). There were two false negatives when using a diagnostic titer of 1:4 (sensitivity: 78%). This sensitivity is similar to that observed by Gentry *et al.* (91%) [6] and Burnie and William (67%) [7] with the same diagnostic titer.

With regard to specificity, 21 patients without deep candidiasis had a titer equal to or greater than 1:2. Three of these patients had a fungemia (two with *C. albicans*, one with *C. pseudotropicalis*) and

Table 1. *Relationships of death and amphotericin B treatment to* Candida *antigen titer*

Patients studied	*Candida* antigen titer Negative	1:2	1:4	*P*
Death rate				
all patients	7/67	8/23	8/14	0.0001*
chemotherapy patients	4/45	8/21	7/1	0.0001*
Proportion of patients receiving amphotericin B	32/67	19/23	14/14	0.0001†
Mean duration of amphotericin B treatment (mean in days ± S.D.)	6.8 ± 9.3	13.6 ± 9.8	11.9 ± 6.8	0.005‡

*P calculated by chi-square test after grouping patients with titers of 1:2 and 1:4.
†P calculated by chi-square test.
‡P calculated by analysis of variance.

Table 2. Candida *antigen titer in patients with systemic candidiasis and with fungemia*

Patients number	Localization of infection	Yeasts	Interval (days) between first titer to* 1:2	1:4	Diagnosis and amphotericin B therapy*
1	Pleural effusion Lung†	*C. albicans*	−10	−4	−15
2	Lung†	*C. albicans*	N.E.	−10	−11
3	Lung†	*C. krusei*	N.E.	−3	0
4	Lung† Cerebrospinal fluid	*T. glabrata*	−12	N.E.	−18
5	Lymph nodes†	*C. albicans*	N.E.	−12	−11
6	Blood‡ Skin	*C. tropicalis*	+11	N.E.	0
7	Blood‡ Skin	*C. tropicalis*	−14	−4	+1
8	Blood‡ Skin	*C. tropicalis*	+1	+3	+1
9	Blood‡	*C. tropicalis*	0	+3	+1
10	Blood§	*C. albicans*	N.E.	N.E.	−7
11	Blood§	*C. albicans*	−1	N.E.	+1
12	Blood§	*C. albicans*	+2	+9	+1
13	Blood§	*C. tropicalis*	N.E.	N.E.	+1
14	Blood§	*C. krusei*	N.E.	N.E.	+1
15	Blood§	*C. pseudotropicalis*	−3	N.E.	−3

*Considered as − when the date of first titer or amphotericin B therapy preceded the date of diagnosis.
†Diagnosis on autopsy samples.
‡Diagnosis by two blood culture as least.
§Diagnosis by one blood culture (fungemia).
N.E. = Non-evaluable.

Table 3. Candida *antigen titer in patients with superficial colonization and sterile surveillance culture (patient number)*

Yeast	*Candida* antigen titer Negative	1:2	1:4
C. albicans	36	6	4
C. tropicalis	3	3	1
C. pseudotropicalis	4	0	0
C. krusei	1	1	0
T. glabrata	2	1	0
Total with superficial colonization	46	11	5
Sterile	18	8	1
Total	64	19	6

10 a colonization (six with *C. albicans*). Thus the specificity was 70.5% with a diagnostic titer of 1:2. With a diagnostic titer of 1:4, there were seven false positive results (one fungemia, five superficial localizations and one case with sterile cultures), yielding a specificity of 90.5%. As some patients with titers of 1:2 or 1:4 died and had no *post mortem* examination, the number of these false positive results may be overestimated and the specificity better. Nevertheless, our results are again similar to those of Gentry *et al.* (specificity 100%) [6] and Burnie and William (94%) [7].

In the interpretation of the results, we did not classify patients with one positive blood culture as having deep candidiasis. The first reason is that blood cultures were taken through the central line. Secondly, these patients had no sign of dissemination such as skin lesions (unlike three of four patients with more than one positive blood culture). Moreover, the only patient who died in this group had no evidence of fungal dissemination at autopsy. The results presented in Table 4 show that the classification of these cases does not significantly modify the results anyway.

The clinical value of the test was assessed in terms of predictive value (that is the probability of yielding the right diagnosis). The predictive value of a negative test was 100% for a negative titer and still 97% for a titer of 1:2. The predictive value of a positive

Table 4. *Statistical evaluation of antigen* Candida *detection*

	Deep seated vs. fungemia and colonized and sterile		Deep seated + fungemia vs. colonized and sterile	
Number of patients	9/95		15/89	
Cutoff titer	1:2	1:4	1:2	1:4
Sensitivity(%)	100	77.8	80	53.3
Specificity(%)	70.5	90.5	71.9	91.4
Predictive value				
+	24	50	32.4	57.1
−	100	97.1	95.5	90.1

titer was much lower (50% for a titer of 1:4 and 24% for a titer of 1:2). The correlations between *Candida* antigen and clinical data were also an interesting point in our study. The titers were more often elevated in chemotherapy patients than in BMT recipients, probably because of the role of laminar air flow rooms and sterile food. The titers were also more elevated in older patients (even when BMT patients were excluded). This could be explained by a greater tendency to infection in older patients, due to more intense immune deficiency. As expected there was also a striking correlation between mortality and elevated titers. All these findings could be interpreted as an indirect confirmation of the value of the *Candida* antigen detection test.

Although our findings indicate a good diagnostic value of the *Candida* antigen test, its relevance to the management of antifungal treatment is not clear. In this study, the first positive titer preceded the diagnosis by 3–12 days in the cases diagnosed at autopsy and in one case of septicemia, but was concomitant to or followed the diagnosis in the three other cases with septicemia. Moreover, amphotericin B therapy had been initiated before the first positive result in six of nine patients with deep candidiasis. It must be stressed that the antigen detection tests were performed prospectively at weekly intervals, regardless of the clinical condition. These findings as well as the possibility of false positives, indicate that the antigen detection test should not be considered as a surveillance test. Nevertheless, it could be an interesting diagnostic tool in patients with otherwise non-documented infection. The problem of the management of antifungal treatment is also complex. First the potential severity of fungal infections necessitates early and prolonged therapy. Then, besides yeasts, other pathogens such as *Aspergillus* are the target of empiric antifungal therapy. The use of laminar air flow rooms could decrease the risk of aspergillosis. The excellent predictive value of a negative test should help identifying patients in whom empiric antifungal therapy could be delayed or at least shortened.

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