

Comparison of Antibody, Antigen, and Metabolite Assays for Hospitalized Patients with Disseminated or Peripheral Candidiasis

MARIE-ELISABETH BOUGNOUX,^{1,2} CATHERINE HILL,³ DIDIER MOISSENET,⁴ MARTINE FEUILHADE DE CHAUVIN,¹ MARC BONNAY,⁴ ISABELLE VICENS-SPRAUEL,² FRANÇOISE PIETRI,³ MICHAEL McNEIL,⁵ LEO KAUFMAN,⁵ JEAN DUPOUY-CAMET,² CLAUDE BOHUON,⁴ AND ANTOINE ANDREMONT^{4*}

Laboratoire de Parasitologie-Mycologie, Hôpital Henri-Mondor, Créteil,¹ Département de Statistique Médicale³ and Département de Biologie Clinique,⁴ Institut Gustave-Roussy, Villejuif, and Service de Parasitologie-Mycologie, Hôpital Cochin, Paris,² France, and Division of Mycotic Diseases, Centers for Disease Control, Atlanta, Georgia 30333⁵

Received 28 June 1989/Accepted 5 January 1990

Repeat serum samples from 22 patients with proven disseminated candidiasis and 42 with simple peripheral colonization were assayed for *Candida* antibodies by coelectrosynthesis, immunoprecipitation, and A and B immunofluorescence, for metabolites by D-arabinitol measurement, and for antigens by the mannan immunoassay and Cand-tec latex agglutination (mean number of samples tested, 2.5 per patient). For the antibody and metabolite assays, the results showed no statistical difference between the two groups. By contrast, the results of both antigen assays were positive for a significantly larger number of patients with disseminated candidiasis than of those with simple peripheral colonization. Results were similar regardless of whether the patients were neutropenic. They were not predictive of death. We calculated that the mannan antigen assay had 29% sensitivity and 97% specificity for the diagnosis of disseminated candidiasis. Likelihood ratios of a positive and a negative result of this test were 9.2 and 0.7, respectively, for this diagnosis. In the latex agglutination test, likelihood ratios were 2.5, 1.5, 1.6, and 0.3 when the test was positive for dilutions of 1/8, 1/4, and 1/2 and was negative, respectively.

Disseminated candidiasis is observed with increasing frequency in hospitalized patients (11, 18) and is associated with high mortality, even when first-line therapy with intravenous amphotericin B is used (11, 16). Epidemiological studies (13) and experimental studies (5, 10, 17) have shown that disseminated candidiasis follows intestinal colonization by *Candida* species. It is difficult to differentiate between these two conditions, however, because most clinical signs and symptoms of disseminated candidiasis are nonspecific (4, 16, 20). Premortem histological diagnosis of the disease is difficult in that surgical biopsies of infected sites are often impossible to perform and blind-needle biopsies are unrewarding (3). Blood cultures often become positive too late, and delays in initiating therapy might therefore account for part of the poor prognosis. In a search for early indicators of disseminated candidiasis, we recently showed in a case-control study of cancer patients that severe neutropenia, central catheterization, and candidal colonization of at least one peripheral site were each associated with a significantly increased risk of candidemia (13). The specificities and sensitivities of these risk factors for the diagnosis of candidemia, however, were too low for reliable diagnosis at an early stage of systemic candidiasis. This explains why empirical treatment with systemic amphotericin B has been recommended for patients at high risk of disseminated candidiasis, i.e., immunocompromised patients or those with fever of unknown origin which persists under appropriate antibacterial chemotherapy (20). However, this treatment led to the administering of amphotericin B, which causes discomfort and potentially severe side effects, to certain patients who did not have disseminated candidiasis (4). That is why several methods for rapid serologic diagnosis of significant *Candida* infections were subsequently investigated, including assays for detecting *Candida* antigens,

antibodies, or metabolites in the serum of patients. The published results of evaluations of these assays were recently reviewed (6, 7, 10). They were also compared in an animal model of systemic and gastrointestinal candidiasis (10). As far as we know, however, no direct comparison of the usefulness of these assays has been reported for hospitalized patients. Therefore, in the present study, we compared the results of antigen, antibody, and metabolite assays for patients with disseminated or peripheral candidiasis.

MATERIALS AND METHODS

Sera and patients. We retrospectively included in the study frozen sera obtained from patients suspected of candidiasis at the time when the suspicion was based on clinical grounds. The clinical basis for the suspicion of candidiasis was the association of signs of sepsis and of a positive culture for *Candida* sp. These sera were sent to our laboratories for various diagnostic procedures between October 1985 and July 1987. Sera were immediately tested for antibodies in one hospital and for Cand-tec antigen in the other. In both hospitals what remained of the samples was aliquoted and stored frozen at -80°C until the other assays (see below) were performed. To determine the diagnosis, we reviewed clinical charts, results of surveillance and blood cultures, and biopsy data. Patients with disseminated candidiasis were defined as those who had either two or more blood cultures positive for the same *Candida* sp. within a 72-h time period or a biopsy-proven deep *Candida* infection. Patients with simple peripheral colonization had peripheral cultures positive for *Candida* sp., no positive blood culture or positive biopsy, and had not been treated by systemic antifungal therapy with flucytosine, ketoconazole, or intravenous amphotericin B. A colonized patient who had received such treatment, or who had only one blood culture positive for *Candida* sp. was considered to have an uncertain diagnosis and was not included in the study.

* Corresponding author.

Antibody assays. Immunoprecipitation was performed with commercially available antigens (Diagnostic Pasteur, Paris). The number of lines of precipitation was recorded after staining with Coomassie blue.

Co-counter-electrophoresis was performed as previously described (21) with a commercially available kit (BioMérieux, Charbonnières-les-Bains, France). In this test, the serum of the patient and a rabbit control serum specific for the germ-free tubes of *Candida albicans* were run in parallel against antigens of *C. albicans* prepared from blastoconidia obtained by growing *C. albicans* VW32. The test was considered positive when a common line of precipitation was found between the rabbit control serum and the serum of the patient.

Indirect immunofluorescence was assayed as described earlier (14) with blastoconidia obtained by growing on Sabouraud glucose agar *C. albicans* HM1 (serotype A) or HM2 (serotype B). These strains were originally isolated by M. Biguet (Institut National de la Santé et de la Recherche Médicale, Lille, France) and kept since 1972 in our laboratory in liquid nitrogen. After being prepared, the slides were kept frozen at -20°C until immunofluorescence assays were performed.

Antigen assays. The presence of an unidentified candidal antigen was detected by Cand-tec latex agglutination with a commercial test kit (Ramco Laboratories, Houston, Tex.) (1, 8, 12). Mannan polysaccharide, a major heat-stable component of the yeast cell wall, was detected in a simplified enzyme immunoassay, as previously described (6). The serum mannan was considered positive when 2 or more ng of mannan per ml was detected. Sensitivity and specificity of the test for invasive candidiasis have been reported to be 65 and 100%, respectively (6).

Creatinine and D-arabinitol measurement. Creatinine was measured with an automated creatinine analyzer (Creatinine Analyzer 2; Beckman Instruments, Inc., Fullerton, Calif.). D-Arabinitol was measured by kinetic spectrofluorometry chromatography (23) with an inducible D-arabinitol dehydrogenase from *Aerobacter aerogenes* (15).

Statistical analysis. Data were managed and checked by the PIGAS system (24). Sensitivities, specificities, likelihood ratios, and predictive values were calculated as previously described (22). Likelihood ratios are the ratios of the proportions of patients with a given characteristic in the disseminated candidiasis group to those of patients in the peripheral candidiasis group. They express the odds that this characteristic would be expected in a patient with disseminated candidiasis as opposed to one with peripheral disease only.

We used analysis of variance for comparison of means and the chi-square or Fisher exact test for comparison of proportions.

RESULTS

Serum samples were available from 164 patients. In 100 cases, the diagnosis was uncertain, either because patients had only one blood culture positive for a *Candida* sp. (11 patients) or because they had been given ketoconazole (35 patients), flucytosine (3 patients), or intravenous amphotericin B (80 patients). A total of 51 (80%) patients included in the analysis had cancer. Disseminated candidiasis was diagnosed for 22 patients. One of them had a positive deep-tissue biopsy, and the other 21 had positive blood cultures (*C. albicans*, nine patients; *C. tropicalis*, eight patients; *C. krusei* plus *C. albicans*, one patient; and *Torulopsis glabrata*, three patients). Forty-two patients had simple periph-

TABLE 1. Characteristics and description of the type of colonization by *Candida* species in 64 patients with candidiasis

Characteristics	Results with the following type of candidiasis:	
	Disseminated (n = 22)	Peripheral only (n = 42)
Patient data (% of patients)		
Male	73	64
Cancer	67	85
Severe neutropenia	24	23
Type of ward		
Surgical	41	31
Medical	45	38
Intensive care	14	31
Age (yr; mean [range])	50 (6-84)	49 (6-83)
Length of hospital stay (days; mean [range])	55 (7-153)	31 (1-93)
No. of serum samples studied per patient (mean [range])	2.9 (1-12)	2.1 (1-11)
Site (no. of patients with positive site/no. tested)		
Blood	21/22	0/42
Oropharynx	13/17	23/31
Feces	12/19	21/33
Genital mucosa	6/10	12/24
Urine	10/20	12/38
Surgical drainage	5/5	11/16
Central catheter	9/14	1/22
Species (% of patients colonized)		
<i>C. albicans</i>	64	81
<i>C. tropicalis</i>	36	7
<i>C. pseudotropicalis</i>	0	10
<i>C. krusei</i>	5	10
<i>T. glabrata</i>	18	12
More than one species	23	19

eral colonization. Fifty-one (80%) of the 64 patients included in the analysis had cancer.

The characteristics of the patients with disseminated candidiasis and of those with simple peripheral colonization were not statistically different (*t* test, chi-square test, and Fisher exact test; Table 1). The intestinal tract was the most frequently colonized site in both groups (Table 1). *C. albicans* was isolated in more than half the patients. Eighteen of the 21 patients (86%) with disseminated candidiasis and candidemia had at least one peripheral site colonized by the same *Candida* species as that isolated from the blood.

The results of antibody assays, including assays of co-counter-electrophoresis, immunoprecipitation, and A and B immunofluorescence were not significantly different in the patients with disseminated and peripheral candidiasis. Similarly, there was no significant difference between these two groups for the results of the metabolite assays, in which we measured the D-arabinitol and creatinine levels and calculated the ratio of the former to the latter (Table 2). In the mannan antigen assay and in the Cand-tec agglutination antigen assay, positivity was significantly associated with disseminated candidiasis ($P = 0.05$, by the Fisher exact test for the mannan antigen assay; $P < 0.05$, by the chi-square test for heterogeneity for the Cand-tec assay).

TABLE 2. Results of antibody, antigen, and metabolite assays in 64 patients with candidiasis^a

Assay and methods	Results with the following type of candidiasis:	
	Disseminated (n = 22)	Peripheral only (n = 42)
Antibody		
Co-counterelectrophoresis (no. of patient positive/total)	5/10	7/30
Immunoprecipitation		
Mean no. of precipitation lines (SD)	1.4 (0.8)	1.3 (1.2)
n	11	13
Immunofluorescence A		
Mean score ^b (SD)	2.8 (1.3)	2.4 (1.5)
n	10	13
Immunofluorescence B		
Mean score (SD)	2.9 (1.2)	2.5 (1.4)
n	11	13
Metabolite (D-arabinitol):		
No. of patients positive/total	10/22	10/42
Range of D-arabinitol/creatinine ratio	1-13	1-55
Antigen (mannan)		
No. of patients positive/total with Cand-tec	4 ^c /14	1 ^d /32 ^e
No. (%) of patients with titer of:		
0	3 (14)	21 (50) ^f
1/2	8 (36)	9 (21)
1/4	7 (32)	9 (21)
1/8	4 (18)	3 (7)
n	22	42

^a When several serum samples were assayed for the same patient, the maximum value for each assay in any of the samples was used to tabulate the data.

^b Scores were 0 for a negative result, and 1, 2, 3, 4, 5, and 6 for assays positive at dilutions of 1/200, 1/400, 1/800, 1/1,600, 1/3,200, and 1/6,400, respectively.

^c Individual results: 4, 6, 6, and 29 ng/ml.

^d Individual result: 7 ng/ml.

^e $P = 0.05$ (Fisher exact test).

^f $P < 0.05$ (chi-square test for heterogeneity).

The same results were found when the analysis was restricted to results from patients without severe neutropenia (data not shown).

We found no statistically significant differences in the results of any of the assays studied between the nine patients with disseminated candidiasis caused by *C. albicans* and the eight patients in whom the disease was caused by *C. tropicalis* (data not shown).

From the data in Table 2, we calculated that the mannan antigen assay had 29% sensitivity and 97% specificity for the diagnosis of disseminated candidiasis. The likelihood ratios for positive and negative results for this diagnosis were 9.2 and 0.7, respectively. In the latex agglutination test, likelihood ratios for the same diagnosis were 2.5, 1.5, 1.6, and 0.3, respectively, when the test was positive at dilutions of 1/8, 1/4, or 1/2 or was negative.

Nine of the 22 patients (41%) with disseminated candidiasis died during the hospital stay in which this disease was diagnosed. The results of the Cand-tec and mannan antigen assays were not significantly different for the patients who died and those who survived (data not shown).

TABLE 3. Results of Cand-tec and mannan antigen assays in 90 serum samples from 46 patients with disseminated or peripheral candidiasis

Mannan assay result	No. of Cand-tec assay results			
	Negative	Positive at titer of:		
		1/2	1/4	1/8
Negative	45	23	12	4
Positive	2	2	1	1

Table 3 shows the results of the mannan and Cand-tec antigen assays for 90 serum samples from 46 patients tested by both methods. When the cutoff limits of positivity in the Cand-tec assay were 1/2, 1/4, and 1/8, concordance between the two tests was 54, 78, and 90%, respectively.

Creatinemia increased significantly (Pearson correlation coefficient; $P = 0.002$) with increased Cand-tec titers (Fig. 1). By contrast, we found no correlation ($r = 0.013$) between serum levels of creatinine and mannan (89 samples tested in both assays; individual data not shown).

DISCUSSION

Due to the retrospective design of the study, the results of all assays were not available for each serum sample. Our results, however, show that significantly more serum samples from patients with disseminated candidiasis than from those with simple peripheral colonization yielded positive antigen assays. This significant relationship was found in both the Cand-tec agglutination assay and the mannan anti-

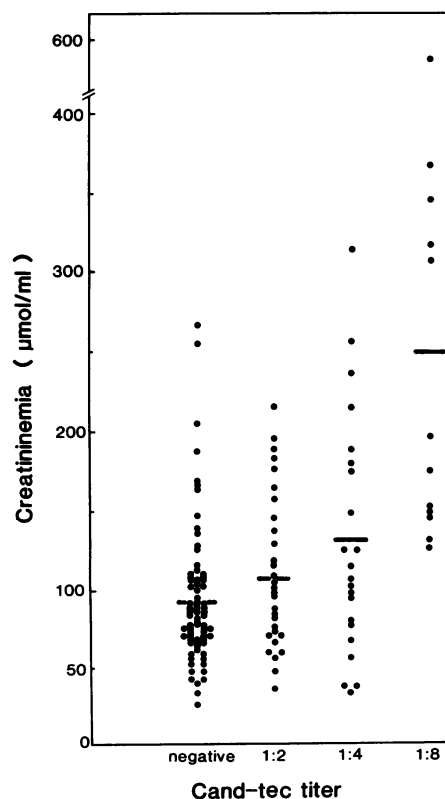


FIG. 1. Relationship between creatinemia and Cand-tec titers in 150 serum samples from 64 patients with disseminated candidiasis.

gen assay. By contrast, no significant difference between the two groups of patients could be found in the results of any of the antibody or metabolite assays. All these results were similar, regardless of whether the patients were severely neutropenic. These findings are in agreement with what was found in a rat model of gastrointestinal colonization with subsequent invasive infection after induction of deep leukopenia (10). At the same time, the results of several studies suggest that a significant association may exist between positive antibody assays (2, 7) or metabolite (9, 25) assays on the one hand and disseminated candidiasis on the other.

These discrepancies might be linked to differences between patient populations (2) or case definitions. We therefore took care here to exclude from the analysis all patients with a doubtful diagnosis. Patients with peripheral colonization only were those with positive surveillance cultures. However, colonized patients who were being empirically treated with systemic antifungal therapy were excluded from the group with limited peripheral colonization, because their blood cultures might have been sterilized by the treatment. We choose the two blood culture rule as a marker for disseminated candidiasis because it has been accepted as such in a previously published study (13). Patients with only one blood culture positive for a *Candida* sp. were excluded from the group with disseminated candidiasis, because transient candidemia has been suggested to occur in colonized patients in the absence of disseminated infection (3).

Concerning detection of D-arabinitol, D-mannitol is also a substrate for the enzyme used (23). Because of the retrospective design of the study, it was not possible to ascertain with certainty whether patients had been exposed to D-mannitol. Therefore, one should be very cautious in interpreting this part of the data. More specific methods (9, 25) to detect D-arabinitol are more complex and were not available for the present study.

We confirmed a significant association between high Cand-tec titers and high serum creatinine values. It has been suggested that the antigens detected in Cand-tec assay accumulate in sera from patients with renal failure (19). We did not find such an association for the results of serum mannan levels.

Our results can be used to quantify the probability of disseminated candidiasis in patients with peripheral colonization. For instance, the prevalence of disseminated candidiasis was estimated at 60% in leukemic patients with fever of unknown origin who are treated empirically with systemic amphotericin B (4). On the basis of this prevalence, it can be calculated from the data in Table 3, by means of simple formulas (22), that in such leukemic patients a positive mannan antigen assay will be associated with a 93% positive probability of disseminated candidiasis; on the other hand, a Cand-tec antigen assay that is positive at dilutions of 1/8, 1/4, or 1/2 will be associated with a positive probability of only 79, 69, or 70%, respectively. When the mannan antigen assay is negative in these patients, they have a 58% probability of being free of disseminated candidiasis. When the Cand-tec assay is negative, this probability rises to 69%. These results are in agreement with those published recently for Cand-tec results in a prospective analysis of immunocompromised patients, in which the prevalence of invasive disease was 5% (19). Taken together, they suggest that the mannan antigen assay is more promising than the Cand-tec assay for detecting of antigenemia in disseminated candidiasis.

However, our data were obtained in a retrospective fashion, and assays were not performed on all samples after an

equivalent period of storage. Since no data pertaining to the stability of the Cand-tec antigen, mannan, or arabinitol under the conditions used for collection and long-term storage are available, our results should be confirmed in a more standardized prospective study. We feel, however, that the reliability of the tests presently available should be improved before they can be used for clinical practice.

ACKNOWLEDGMENT

We thank David McLaughlin for technical assistance.

LITERATURE CITED

- Bailey, J. W., E. Sada, C. Brass, and J. E. Bennett. 1985. Diagnosis of systemic candidiasis by latex agglutination for serum antigen. *J. Clin. Microbiol.* 21:749-752.
- Bisbe, J., J. M. Miro, J. M. Torres, X. Latorre, C. Alia, M. Amaral, D. Estivill, J. Mallolas, A. Trilla, and E. Soriano. 1989. Diagnostic value of serum antibody and antigen detection in heroin addicts with systemic candidiasis. *Rev. Infect. Dis.* 11:310-315.
- Bodey, G. P. 1984. Candidiasis in cancer patients. *Am. J. Med.* 74:13-19.
- Degregorio, M. W., W. M. F. Lee, C. A. Linker, R. A. Jacobs, and C. A. Ries. 1982. Fungal infections in patients with acute leukemia. *Am. J. Med.* 73:543-548.
- De Maria, A., H. Buckley, and F. von Lichtenberg. 1976. Gastrointestinal candidiasis in rats treated with antibiotics, cortisone, and azathioprine. *Infect. Immun.* 13:1761-1770.
- de Repentigny, L., L. D. Marr, J. W. Keller, A. W. Carter, R. J. Kuykendall, L. Kaufman, and E. Reiss. 1985. Comparison of enzyme immunoassay and gas-liquid chromatography for the rapid diagnosis of invasive candidiasis in cancer patients. *J. Clin. Microbiol.* 21:972-979.
- de Repentigny, L., and E. Reiss. 1984. Current trends in immunodiagnosis of candidiasis and aspergillosis. *Rev. Infect. Dis.* 6:301-312.
- Gentry, L. O., I. D. Wilkinson, A. S. Lea, and M. F. Price. 1983. Latex agglutination test for detection of *Candida* antigen in patients with disseminated disease. *Eur. J. Clin. Microbiol.* 2:122-128.
- Gold, J. W. M., B. Wong, E. M. Bernard, T. E. Kiehn, and D. Armstrong. 1983. Serum arabinitol concentrations and arabinitol/creatinine ratios in invasive candidiasis. *J. Infect. Dis.* 147:504-513.
- Greenfield, R. A., D. L. Troutt, R. C. Richard, and D. H. Altmiller. 1988. Comparison of antibody, antigen, and metabolite assays in rat models of systemic and gastrointestinal candidiasis. *J. Clin. Microbiol.* 26:409-417.
- Horn, R., B. Wong, T. E. Kiehn, and D. Armstrong. 1985. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. *Rev. Infect. Dis.* 7:646-655.
- Kahn, F. W., and J. M. Jones. 1986. Latex agglutination tests for detection of *Candida* antigens in sera of patients with invasive candidiasis. *J. Infect. Dis.* 153:579-585.
- Karabinis, A., C. Hill, B. Leclercq, C. Tancrede, D. Baume, and A. Andreumont. 1988. Risk factors for candidemia in cancer patients: a case-control study. *J. Clin. Microbiol.* 26:429-432.
- Lehner, T. 1970. Serum fluorescent antibody and immunoglobulin estimations in candidiasis. *J. Med. Microbiol.* 3:475-481.
- Lin, E. C. C. 1961. An inducible D-arabinitol dehydrogenase from *Aerobacter aerogenes*. *J. Biol. Chem.* 236:31-36.
- Maksymiuk, A. W., S. Thongprasert, R. Hopfer, M. Luna, V. Fainstein, and G. P. Bodey. 1984. Systemic candidiasis in cancer patients. *Am. J. Med.* 77(Suppl. 4D):20-27.
- Myerowitz, R. L. 1981. Gastrointestinal and disseminated candidiasis. An experimental model in the immunosuppressed rat. *Arch. Pathol. Lab. Med.* 105:138-143.
- Myerowitz, R. L., G. J. Pazin, and C. M. Allen. 1977. Disseminated candidiasis. Changes in incidence, underlying diseases, and pathology. *Am. J. Clin. Pathol.* 68:29-38.

19. **Ness, M. J., W. P. Vaughan, and G. L. Woods.** 1989. *Candida* antigen latex test for detection of invasive candidiasis in immunocompromised patients. *J. Infect. Dis.* **159**:495-502.
20. **Pizzo, P. A., K. J. Robichaud, F. A. Gill, and F. G. Witebsky.** 1982. Empiric antibiotics and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am. J. Med.* **72**:101-111.
21. **Poulain, D., and J. M. Pinon.** 1986. Diagnosis of systemic candidiasis: development of co-counterimmunoelectrophoresis. *Eur. J. Clin. Microbiol.* **5**:420-426.
22. **Sackett, D. L., R. B. Haynes, and P. Tugwell (ed.).** 1985. Clinical epidemiology. The interpretation of diagnostic data, p. 59-138. Little, Brown, & Co., Boston.
23. **Soyama, K., and E. Ono.** 1985. Enzymatic fluorometric method for the determination of D-arabinitol in serum by initial rate analysis. *Clin. Chim. Acta* **149**:149-154.
24. **Wartelle, M., A. Kramar, P. Jan, and D. Kruger.** 1983. PIGAS: an interactive statistical database management system, p. 124-132. *In* Proceedings of the Second International Workshop on Statistical Database Management. National Technical Information Service, U.S. Department of Commerce, Springfield, Va.
25. **Wong, B., and K. L. Brauer.** 1988. Enantioselective measurement of fungal D-arabinitol in the sera of normal adults and patients with candidiasis. *J. Clin. Microbiol.* **26**:1670-1674.