

# Assessment of Detection of *Candida* Mannoproteinemia as a Method To Differentiate Central Venous Catheter-Related Candidemia from Invasive Disease

CORRADO GIRMENIA,<sup>1</sup> PIETRO MARTINO,<sup>1\*</sup> FLAVIA DE BERNARDIS,<sup>2</sup>  
AND ANTONIO CASSONE<sup>2</sup>

Hematology, Department of Human Biopathology, University "La Sapienza,"<sup>1</sup> and Department of Bacteriology and Medical Mycology, Istituto Superiore di Sanità,<sup>2</sup> Rome, Italy

Received 15 October 1996/Returned for modification 21 November 1996/Accepted 14 January 1997

The proper management of candidemic patients is controversial because of the difficulties of an early differentiation of central venous catheter (CVC)-related candidemia from deep-seated invasive *Candida* infection. In particular, more information on possible markers of invasive disease is needed. We performed a retrospective, pilot investigation to assess the diagnostic potential of a dot immunobinding assay for *Candida* mannoprotein antigen in serial serum samples from 31 candidemic patients in the setting of hematologic malignancy. Mannoproteinemia (antigenemia) was detected in 1 of 14 (7.1%) patients with transient or CVC-related candidemia and in 13 of 17 (76.5%) patients with non-CVC-related persistent candidemia. Of the 11 subjects of this latter group with documented tissue invasion, 10 (91%) were antigenemic. The patients belonging to the different categories did not significantly differ in the duration of candidemia, nor was there any significant difference among the different groups of subjects either in the number of serum samples examined or in their collection time during candidemia. The day of the first antigenemic sample during candidemia greatly varied among subjects with invasive infection, although on average mannoproteinemia was detectable by the first week of candidemia. In summary, our data demonstrate a correlation between mannoproteinemia and tissue invasion by *Candida* spp. in candidemic patients and suggest that mannoprotein detection by our method has a potential for the diagnosis of invasive candidiasis in these subjects.

The proper management of patients with candidemia, a disease now representing a common complication of immunocompromised patients, is a debated issue (7, 10, 11, 14). The rather frequent occurrence of transient and central venous catheter (CVC)-related candidemic episodes, coupled with the absence of pathognomonic signs of invasive infection and poor diagnostic tools, makes the correct clinical approach to the candidemic subject particularly difficult (5–7, 10–12, 14, 21). The high mortality rate observed in these patients has led most investigators to recommend the use of antifungal therapy and prompt CVC removal (6, 12, 21).

Others contend that some patients with candidemia may not require antifungal therapy, thus making it unjustified as a universal treatment (5). How to identify those who could be safely left untreated is, however, a matter of great concern.

Recently, we described a sensitive and highly specific dot immunobinding assay for the detection of a circulating immunodominant *Candida* mannoprotein (MP) antigen (4). This assay has now been implemented in a pilot study of candidemic patients with different clinical characteristics, prognoses, and outcomes.

## MATERIALS AND METHODS

All candidemic subjects admitted to the Hematology Clinic of the University of Rome "La Sapienza" during the period August 1988 to January 1996 were retrospectively considered in this pilot study, provided that a sufficient quantity of at least one serum sample taken during the candidemic episode was still available. The subjects (21 males and 10 females) had a mean age of 29 years (range, 6 to 62 years). The underlying condition was leukemia, lymphoma, or intestinal polyposis in 26, 4, and 1 patients, respectively. The patients were

divided into the following categories: (i) transient candidemia, (ii) CVC-related candidemia; (iii) persistent non-CVC-related candidemia without evidence of tissue invasion, and (iv) persistent non-CVC-related candidemia with tissue invasion. Transient candidemia (category i) was defined as a single blood culture positive for *Candida* spp. in a patient without any clinical sign of fungal infection. The candidemia was defined as CVC related (category ii) if the semiquantitative catheter tip culture yielded more than 15 colonies of the fungus and candidemia cleared within 24 h after CVC removal. Consequently, the definition of CVC-related candidemia implies the removal of CVC. Persistent non-CVC-related candidemia was defined as multiple *Candida* isolations from the blood of patients without indwelling catheters, including those with CVC removal (category iii), or from patients whose CVC was not removed but who had clinically or histologically documented invasive candidiasis (category iv). The clinical evidence of invasive infection was inferred from ultrasound documentation of heart valve vegetation or hepatosplenic lesions and multiorgan failure presumably related to a disseminated disease. All patients were under treatment with antifungals (mostly amphotericin B) during candidemia, and those with clinical signs of invasive diseases were treated until the clearance of candidemia and disappearance of clinical signs of the infection.

Serum samples were collected starting from the initial documentation of candidemia to 1 week after fungal clearance or disappearance of clinical signs of infection. *Candida* MP was detected by the dot immunobinding assay described elsewhere (4). This assay is based on the use of a monoclonal antibody (MAB), AF1, specific for a novel  $\beta$ -1,2-oligomannoside epitope of secretory MP of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata*, i.e., the most pathogenic *Candida* species and those most commonly found as agents of candidemia in our setting (9, 14). Four candidemia cases due to *C. krusei* and one due to *C. famata* were excluded from the investigation because MP from these two fungi is not reactive with MAB AF1.

## RESULTS

Table 1 shows the agent of candidemia and the MP detection results in the four categories of patients examined. The antigen was detected neither in the subjects with transient nor (with one exception) in those with CVC-related candidemia. Conversely, it was detected in at least one serum sample of 13 of 17 patients with non-CVC-related persistent candidemia, namely, in three of the 6 without clinical evidence of tissue

\* Corresponding author. Mailing address: Ematologia, Dipartimento di Biopatologia Umana, Via Benevento 6, 00161 Rome, Italy. Phone: 06/857951. Fax: 06/44241984.

TABLE 1. Clinical characteristics, causative agent, and mannoproteinemia detection for 31 candidemic patients

Candidemia category	Patient no.	<i>Candida</i> sp. <sup>a</sup>	Invasive disease	Mannoproteinemia	No. of antigenemia-positive samples over total no. of serum samples	
Transient	1	<i>C. albicans</i>	— <sup>b</sup>	Negative	0/1	
	2	<i>C. pseudotropicalis</i>	—	Negative	0/1	
CVC related	3	<i>C. parapsilosis</i>	—	Negative	0/2	
	4	<i>C. parapsilosis</i>	—	Negative	0/2	
	5	<i>C. parapsilosis</i>	—	Negative	0/3	
	6	<i>C. parapsilosis</i>	—	Negative	0/2	
	7	<i>C. parapsilosis</i>	—	Negative	0/5	
	8	<i>C. parapsilosis</i>	—	Negative	0/2	
	9	<i>C. parapsilosis</i>	—	Negative	0/4	
	10	<i>C. parapsilosis</i>	—	Negative	0/1	
	11	<i>C. albicans</i>	—	Negative	0/3	
	12	<i>C. albicans</i>	—	Positive	1/1	
	13	<i>C. guilliermondii</i>	—	Negative	0/3	
	14	<i>C. guilliermondii</i>	—	Negative	0/3	
	Non-CVC related persistent without invasive infection	15	<i>C. albicans</i>	—	Negative	0/2
		16	<i>C. albicans</i>	—	Negative	0/3
17		<i>C. albicans</i>	—	Negative	0/2	
18 <sup>c</sup>		<i>C. tropicalis</i>	—	Positive	1/1	
19		<i>C. tropicalis</i>	—	Positive	5/5	
20		<i>C. parapsilosis</i>	—	Positive	2/3	
Non-CVC related persistent with invasive infection	21	<i>C. parapsilosis</i>	Endocarditis	Positive	3/5	
	22	<i>C. parapsilosis</i>	Endocarditis	Positive	1/5	
	23	<i>C. parapsilosis</i>	Skin lesions	Negative	0/3	
	24	<i>C. parapsilosis</i>	Skin lesions	Positive	1/2	
	25 <sup>c</sup>	<i>C. parapsilosis</i>	MOF <sup>d</sup>	Positive	2/3	
	26 <sup>c</sup>	<i>C. parapsilosis</i>	MOF <sup>d</sup>	Positive	2/5	
	27	<i>C. albicans</i>	Endocarditis <sup>e</sup>	Positive	2/3	
	28	<i>C. albicans</i>	Enterocolitis	Positive	1/2	
	29	<i>C. tropicalis</i>	Skin lesions	Positive	1/3	
	30	<i>C. guilliermondii</i>	Skin lesions	Positive	1/2	
	31	<i>C. glabrata</i>	Cystopyelitis	Positive	1/3	

<sup>a</sup> Four cases of *C. krusei* candidemia and one of *C. famata* candidemia were excluded because these fungi are not reactive with MAb AF1.

<sup>b</sup> —, absent.

<sup>c</sup> Dead.

<sup>d</sup> MOF, multiorgan failure.

<sup>e</sup> Associated with endophthalmitis and folliculitis.

invasion and 10 of the 11 with documented tissue invasion. All three patients with *C. tropicalis* infection were mannoproteinemic. Table 1 also shows that *C. parapsilosis* was the most frequent agent of both CVC-related candidemias (8 of 12 episodes) and non-CVC-related persistent candidemias (7 of 17 episodes). However, *C. parapsilosis* MP was never detected in the serum of the eight subjects of the former group, whereas it was detected in at least one serum sample of six subjects of the latter group ( $P < 0.01$ , Fisher exact test).

MP detection was also analyzed according to individual serum samples. Overall, only 0 of 2 or 1 of 31 (3.2%) serum samples from patients with transient or CVC-related candidemia, respectively, was positive. Among patients with non-CVC-related persistent candidemia, 8 of 16 (50%) and 15 of 36 (42%) serum samples from patients without and with proven invasive infection, respectively, were positive. In particular, seven of nine serum samples from *C. tropicalis*-infected subjects were antigenemic. A total of 3 of 14 (21%) antigenemic patients died, whereas none of 17 nonantigenemic patients died during the infectious episode ( $P < 0.05$ , Fisher exact test).

Table 2 shows that there were no significant differences among the categories of patients either in the candidemia duration or in the number of serial serum samples examined.

Rather comparable also in all categories was the time of sample collection with respect to the candidemia duration. In the category with the highest number of antigenemic subjects, the first positive serum sample could occur either early or late during candidemia although, on average, antigenemia became detectable by the first week of candidemia.

## DISCUSSION

The questions (i) whether all patients with candidemia require therapy, (ii) whether CVCs should be removed in all cases, and (iii) what the optimum treatment regimen is for candidemia are all still controversial.

In a recent editorial (6), Edwards suggested that “until methods to stratify patients more accurately are developed, it is prudent to treat all patients who have candidemia, regardless of whether they are neutropenic or whether the candidemia is associated with the presence of catheter.” This opinion is in agreement with the conclusions drawn by Lecciones et al. (12) from a retrospective study on vascular catheter-associated fungemia in cancer patients, as well as with those reached by Rex et al. (21) in a study of antifungal therapy in nonneutropenic, candidemic patients.

TABLE 2. Some candidemia and antigenemia parameters in the different categories of patients<sup>a</sup>

Parameter	Value for candidemia category:		
	CVC related	Non-CVC related without invasive infection	Non-CVC related with invasive infection
Candidemia duration <sup>b</sup>	10.2 (4–20)	9 (4–17)	9.4 (2–27)
No. of serum samples <sup>c</sup>	2.6 (1–5)	2.7 (1–5)	3.3 (2–5)
Time of the first serum sample collection <sup>d</sup>	5.6 (1–14)	4.2 (1–7)	3.9 (1–8)
Time of the first antigenemic serum sample <sup>d</sup>	— <sup>e</sup>	3.3 (2–4)	6.2 (1–27)
Time of all serum sample collections <sup>f</sup>	9.2 (1–23)	10.3 (1–19)	12.6 (1–35)
Antigenemic subjects <sup>g</sup>	1/12 (83)	3/6 (50)	10/11 (91)

<sup>a</sup> The two subjects with transient candidemias were not considered.

<sup>b</sup> In days; mean (range).

<sup>c</sup> Mean (range).

<sup>d</sup> Mean days (range), starting from the onset of candidemia.

<sup>e</sup> —, not shown, as only one subject (one serum sample) was antigenemic.

<sup>f</sup> Mean days (range), by cumulating all days of all sample collections and starting from the onset of candidemia.

<sup>g</sup> Number positive over total number (percent).

Of a different opinion is Di Nubile (5), who contends that (i) selected patients, with candidemia quickly clearing after CVC removal, may not require therapy and (ii) the subsequent possible relapse with a focal infection can still probably be treated successfully.

The management of catheters in patients with candidemia is another debated issue. The frequent failure of the antifungal therapy in the treatment of CVC-related candidemia leads to removal of the intravascular device in most patients with candidemia (9, 12, 20). However, CVC removal is a problematic practice in severely ill patients requiring intensive supportive therapy.

Basic problems remain owing to the difficulties in stratifying patients with candidemia, in diagnosing a deep invasive infection at the first *Candida* isolation from blood, and in identifying those patients who could avoid antifungal treatment. In this context, the availability of a marker capable of differentiating a candidemia with at least a strong suspicion of deep-seated infection from a CVC-related candidemia would greatly help in making a clinical decision about the nature and duration of the treatment.

There is a rather wide consensus that antigen detection methods might be potentially useful for diagnosing invasive candidiasis and for monitoring treatment response (1, 13, 15, 23). Immunodiagnosis of invasive *Candida* infections by antigen detection methods has indeed been evaluated by several investigators with patients with candidemia. However, this was done more with the aim of validating a laboratory method than as an aid to a clinical decision, which requires a careful and controlled definition and stratification of the patients (2, 8, 16–19).

In our pilot study, no patient with transient candidemia and only 1 of the 12 (8%) patients with CVC-related candidemia was antigenemic. On the contrary, MP was detected in 76% of the patients with non-CVC-related, persistent candidemia and in 91% of the cases with documented tissue invasion. The sensitivity of our test was considerably lower if MP detection was analyzed according to individual serum samples. However, it is rather clear that antigenemia may occur only transiently during an invasive infection and that multiple, serial specimens are necessary to achieve optimum diagnostic sensitivity.

In our investigation, the number of serum samples examined per category of patients was practically the same and the sera were collected with comparable timing and frequency, relative to candidemia duration. Thus, antigenemia detection was not grossly biased by differences in serum sampling in the different

categories of patients. On the other hand, there was a marked spread in the timing of the first antigenemic serum among the patients with invasive disease, although on average, the first positive sample occurred by the first week of infection. These data should be taken cautiously because of the retrospective nature of the study and the absence of a preplanned serum sampling scheme equal for all patient categories. At any rate, our results emphasize still more the need for serial and prolonged serum sample collection for antigenemia assay. A firm conclusion on all these important aspects must necessarily await future prospective studies with an appropriate number of patients.

Despite the above limitations, our findings suggest the existence of a specific correlation between detectable mannoproteinemia and tissue invasion by *Candida* spp. in patients with candidemia. Despite persistent fungemia, the antigen seems not to be released by the yeast when the source of the infection is a contaminated CVC. It seems logical to conclude that a rather massive and/or persistent tissue burden of *Candida* spp. must be present for the detection of circulating MP. Thus, positive mannoproteinemia would give a clear indication for a particularly aggressive chemotherapeutic treatment. In this context, it is quite remarkable that all patients with non-CVC-related *C. tropicalis* fungemia had positive antigenemia (for one patient, five consecutive serum samples contained MP). The high pathogenicity (in terms of propensity to deep-seated infection) of *C. tropicalis* has been previously emphasized (24).

In conclusion, our data support the assumption that MP detection could be a useful diagnostic tool for an invasive candidiasis. In the event of positive antigenemia in a candidemic patient with indwelling CVC, the presence of a deep-seated infection should be seriously hypothesized and the patient should be treated consequently. Our results encourage further prospective studies for a full verification of the clinical relevance of the MP assay in the management of the candidemic patient. In further studies, a quantitative assessment of serum MP as a further discriminatory diagnostic tool will also be attempted.

#### ACKNOWLEDGMENTS

This work was supported by the National Research Council, Special Project F.A.T.M.A. (94.00683.41); the National AIDS Project (920/U); and ACRO Project CNR Italy "Applicazioni cliniche nella ricerca oncologica" (920221939).

We are grateful to F. Girolamo for help in the preparation of the manuscript.

## REFERENCES

- Buckley, H. R., M. D. Richardson, E. G. V. Evans, and L. J. Wheat. 1992. Immunodiagnosis of invasive fungal infection. *J. Med. Vet. Mycol.* **30** (Suppl.):249–260.
- Cabezudo, I., M. Pfaller, T. Gerarden, F. Koontz, R. Wenzel, R. Gingrich, K. Heckman, and C. P. Burns. 1989. Value of the Cand-tec *Candida* antigen assay in the diagnosis and therapy of systemic candidiasis in high-risk patients. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**:770–777.
- Cassone, A., A. Torosantucci, M. Boccanera, G. Pellegrini, C. Palma, and F. Malavasi. 1988. Production and characterization of a monoclonal antibody to a cell surface, glucomannoprotein constituent of *Candida albicans* and other pathogenic *Candida* species. *J. Med. Microbiol.* **27**:233–238.
- De Bernardis, F., C. Girmenia, M. Boccanera, D. Adriani, P. Martino, and A. Cassone. 1993. Use of a monoclonal antibody in a dot immunobinding assay for detection of a circulating mannoprotein of *Candida* spp. in neutropenic patients with invasive candidiasis. *J. Clin. Microbiol.* **31**:3142–3146.
- Di Nubile, M. J. 1995. The treatment of candidemia. *N. Engl. J. Med.* **332**:1100. (Letter.)
- Edwards, J. E., Jr. 1992. Should all patients with candidemia be treated with antifungal agents? *Clin. Infect. Dis.* **15**:422–423.
- Fraser, V. J., M. Jones, J. Dunke, S. Storfer, G. Medoff, and W. C. Dunagan. 1992. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. *Clin. Infect. Dis.* **14**:414–421.
- Fujita, S., and T. Hashimoto. 1992. Detection of serum *Candida* antigens by enzyme-linked immunosorbent assay and a latex agglutination test with anti-*Candida albicans* and anti-*Candida krusei* antibodies. *J. Clin. Microbiol.* **30**:3132–3137.
- Girmenia, C., P. Martino, F. De Bernardis, G. Gentile, M. Boccanera, M. Monaco, G. Antonucci, and A. Cassone. 1996. Rising incidence of *Candida parapsilosis* fungemia in patients with hematologic malignancies: clinical aspects, predisposing factors, and differential pathogenicity of the causative strains. *Clin. Infect. Dis.* **23**:506–514.
- 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.
- Komshian, S. V., A. K. Uwaydah, J. D. Sobel, and L. R. Crabe. 1989. Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patients: frequency, characteristics, and evaluation of factors influencing outcome. *Rev. Infect. Dis.* **11**:379–390.
- Lecciones, J. A., J. W. Lee, E. E. Navarro, F. G. Witebsky, D. Marshall, S. M. Steinberg, P. A. Pizzo, and T. J. Walsh. 1992. Vascular catheter-associated fungemia in patients with cancer: analysis of 155 episodes. *Clin. Infect. Dis.* **14**:875–883.
- Martino, P., G. Meloni, and A. Cassone. 1990. Candidal endocarditis and treatment with fluconazole and granulocyte-macrophage colony-stimulating factor. *Ann. Intern. Med.* **112**:966–967. (Letter.)
- Martino, P., C. Girmenia, A. Micozzi, R. Raccach, G. Gentile, M. Venditti, and F. Mandelli. 1993. Fungemia in patients with leukemia. *Am. J. Med. Sci.* **306**:225–232.
- Matthews, R., and J. Burnie. 1988. Diagnosis of systemic candidiasis by an enzyme-linked dot-immunobinding assay for a circulating immunodominant 47-kilodalton antigen. *J. Clin. Microbiol.* **26**:459–463.
- Meunier-Carpentier, F., T. E. Kiehn, and D. Armstrong. 1981. Fungemia in the immunocompromised host. Changing pattern, antigenemia, high mortality. *Am. J. Med.* **71**:363–370.
- Mitsutake, K., T. Miyazaki, T. Tashiro, Y. Yamamoto, H. Kakeya, T. Otsubo, S. Kawamura, M. A. Hossain, T. Noda, Y. Hirakata, and S. Kohno. 1996. Enolase antigen, mannan antigen, Cand-tec antigen, and  $\beta$ -glucan in patients with candidemia. *J. Clin. Microbiol.* **34**:1918–1921.
- Ness, M. J., W. P. Vaughan, and G. Woods. 1989. *Candida* antigen latex test for detection of invasive candidiasis in immunocompromised patients. *J. Infect. Dis.* **159**:495–502.
- Reboli, A. C. 1993. Diagnosis of invasive candidiasis by a dot immunobinding assay for *Candida* antigen detection. *J. Clin. Microbiol.* **31**:518–523.
- Rex, J. H. 1996. Catheters and candidemia. *Clin. Infect. Dis.* **22**:467–470.
- Rex, J. H., J. E. Bennett, A. M. Sugar, P. G. Pappas, C. M. van der Horst, J. E. Edwards, R. G. Washburn, W. M. Scheld, A. W. Karchmer, A. P. Dine, M. J. Levenstein, and C. D. Webb. 1994. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. *N. Engl. J. Med.* **331**:1325–1330.
- Venditti, M., F. De Bernardis, A. Micozzi, P. Pontieri, A. Chirletti, A. Cassone, and P. Martino. 1992. Fluconazole treatment of catheter-related right-sided endocarditis caused by *Candida albicans* and associated with endophthalmitis and folliculitis. *Clin. Infect. Dis.* **14**:422–426.
- Walsh, T. J., J. W. Hathorn, J. D. Sobel, W. G. Merz, V. Sanchez, S. M. Maret, H. R. Buckley, M. A. Pfaller, R. Schaufele, C. Sliva, E. Navarro, J. Lecciones, P. Chandrasekar, J. Lee, and P. A. Pizzo. 1991. Detection of circulating *Candida* enolase by immunoassay in patients with cancer and invasive candidiasis. *N. Engl. J. Med.* **324**:1026–1031.
- Wingard, J. R., W. G. Merz, and R. Saral. 1979. *Candida tropicalis*: a major pathogen in immunocompromised patients. *Ann. Intern. Med.* **91**:539–543.