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Short Communication

Lack of circulating *Candida* mannoprotein antigen in patients with focal hepatosplenic candidiasis

Corrado Girmenia,[1] Pietro Martino,[1] Flavia De Bernardis,[2] Maria Boccanera[2] and Antonio Cassone[2]

[1]Dipartimento di Biotecnologie Cellulari ed Ematologia, University 'La Sapienza', Via Benevento 6, 00161 Rome, Italy

[2]Dipartimento di Batteriologia e Micologia Medica, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Rome, Italy

Correspondence
Corrado Girmenia
girmenia@bce.uniroma1.it

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The significance of *Candida* mannoprotein serum detection in 15 patients with haematological malignancies and proven (six cases) or probable (nine cases) hepatosplenic candidiasis was retrospectively evaluated. Circulating mannoprotein antigen was detected in three of six and in one of two serum samples from two patients with probable infection. The antigen was not detected in 38 serum samples of 13 (87 %) patients. Thus, in contrast to other deep-seated *Candida* infections, mannoprotein is infrequently detectable during focal hepatosplenic candidiasis and does not appear to be of diagnostic value.

Introduction

Hepatosplenic disease involving *Candida* species is occurring with increasing frequency in cancer patients, particularly those with haematological malignancies, either as part of disseminated infection or confined to the liver and/or spleen (Anttila *et al.*, 1994, 1997; Haron *et al.*, 1987; Jones, 1981; Tashjian *et al.*, 1984; Thaler *et al.*, 1988). Clinical diagnosis of probable hepatosplenic candidiasis is usually made on the basis of multiple small, target-like, focal lesions in the liver or spleen demonstrated by ultrasound, computed tomography (CT) or magnetic resonance imaging (Ascioglu *et al.*, 2002). However, these specific clinical findings are usually detectable only after patients have recovered from chemotherapy-induced neutropenia. During neutropenia, the clinical syndrome of focal hepatic or hepatosplenic disease is non-specific, being characterized only by persistent fever and abnormal liver enzyme levels (Tashjian *et al.*, 1984; Thaler *et al.*, 1988).

As for other localizations of deep-seated invasive *Candida* infections, microbiological examinations often lack sensitivity, and invasive diagnostic techniques are not permitted by the underlying conditions of these patients. For these reasons, there is increasing interest in the use of serological tests for the diagnosis of these infections, including the detection of *Candida* cell-wall and cytoplasmic antigens, such as mannoprotein and enolase (Girmenia *et al.*, 1997, 1999; Reiss & Morrison 1993; Walsh *et al.*, 1991). We previously described a sensitive and highly specific dot immunobinding assay for the detection of a circulating immunodominant *Candida* mannoprotein (MP) antigen (De Bernardis *et al.*, 1993). This assay has been implemented in a pilot study of patients with focal hepatosplenic candidiasis to evaluate its usefulness in the diagnosis of invasive *Candida* infection.

Abbreviation: MP, mannoprotein.

Methods

Patients. All patients with hepatic or hepatosplenic candidiasis admitted to the Dipartimento di Biotecnologie Cellulari ed Ematologia of the University 'La Sapienza', Rome, during the period June 1990–December 1998 were considered retrospectively, provided that at least two serum samples taken around the time that the infection was documented were available.

Diagnostic criteria. Infections were defined according to recently published definitions (Ascioglu *et al.*, 2002). The infection was defined as proven candidiasis if imaging revealed multiple focal lesions in the liver and/or the spleen and if a specific diagnosis of yeast infection was established by means of microscopy of liver biopsy with or without positive culture for *Candida* species from the biopsy specimen. The presence of budding yeasts, whether or not accompanied by pseudohyphae at histopathological examination, was considered adequate confirmation of the diagnosis of candidiasis.

Infection was considered probable if the presence of multiple focal lesions in the liver and/or the spleen was demonstrated by imaging and if no specific histological or microbiological diagnosis could be established. Liver tissue specimens were also cultured on blood agar at 37 °C and Sabouraud's 2 % dextrose agar with 0·5 mg chloramphenicol ml^{-1} at 25 °C. Surveillance cultures of urine, stools and sputum and of nasal, oropharyngeal, rectal and vaginal swabs were performed once a week. Samples were plated on Sabouraud's 2 % dextrose agar with 0·5 mg chloramphenicol ml^{-1}, incubated at 25 °C and examined daily for at

least 2 weeks. Blood cultures were performed in trypticase soy broth bottles (Sygnal System, Oxoid; BBL Septi-Chek, Becton Dickinson) and were examined daily for at least 2 weeks.

Serum samples. Serum samples were considered that were collected between 2 weeks before and 1 month after the documentation of suspected *Candida* infection on hepatosplenic imaging. At least two serum samples were collected within 1 week before or after the diagnosis of infection. *Candida* MP was detected by the dot immunobinding assay described previously (De Bernardis *et al.*, 1993). To separate the circulating mannan antigen from antibodies, each serum sample was treated with alkali and heat (De Bernardis *et al.*, 1993). This assay is based on the use of a monoclonal antibody (mAb AF1) specific for a β-1,2-oligomannoside epitope of secretory MP of *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida guillermondii* and *Candida glabrata* (Cassone *et al.*, 1988).

Results

Fifteen patients with focal hepatic or hepatosplenic candidiasis were considered. The subjects (seven males and eight females) had a mean age of 32 years (range 15–48 years). The underlying condition was leukaemia in 13 patients and lymphoma in two patients. The median times of the first serum sample and of collection of all serum samples from infection-imaging documentation were respectively day −8 (range, −14 to +5) and day +6 (range −14 to +28).

Table 1 shows the clinical characteristics, histopathological and microbiological findings and MP detection results for the 15 patients. Focal hepatosplenic candidiasis was diagnosed by ultrasound or CT examination after a median of 12 days (range 4–35) from the recovery of neutropenia (neutrophil count $>1000\ mm^{-3}$). Liver biopsy was performed in seven patients (ultrasound-guided fine-needle aspiration in two patients and laparoscopy-guided biopsy in five patients) after a median of 12 days (range 5–30) from infection-imaging documentation; these patients had received systemic antifungal therapy for a median of 14 days (range 7–30) before tissue was obtained for histopathological examination. It allowed a diagnosis of proven *Candida* infection in six cases. In patient 7, the infection was considered a probable candidiasis even though the liver biopsy specimen showed a granulomatous reaction with no evident fungal infiltration and culture was negative. However, biopsy was performed after 1 month of antifungal therapy, and clinical and imaging improvement of the infection was observed after fluconazole treatment.

Cultures of liver tissue grew *Candida* species in only three cases (*C. albicans* in two and *C. tropicalis* in one). *Candida* gastrointestinal colonization was demonstrated in all patients. Fourteen of 46 (30·4 %) serum samples had been collected before the start of antifungal therapy from 11 patients. Circulating MP antigen was detected in two patients with probable hepatic candidiasis: in three of six serum samples from patient 14 (one positive sample was collected before imaging-infection documentation and before anti-

Table 1. Clinical characteristics, causative agents and mannoproteinaemia detection for 15 patients with focal hepatosplenic candidiasis

Abbreviations: HC, hepatosplenic candidiasis; MP, mannoprotein; AmB, amphotericin B; ABLC, amphotericin B lipid complex; NP, not performed.

Patient	Diagnosis of HC	Culture of hepatic biopsy (*Candida* species)	*Candida* colonization	Treatment	Outcome of infection	MP-positive serum samples (*n*)*
1	Proven	Positive (*C. tropicalis*)	*C. tropicalis*	AmB, fluconazole	Improvement†	0/3 (0)
2	Proven	Positive (*C. albicans*)	*C. albicans*	AmB, fluconazole	Cure	0/2 (0)
3	Proven	Positive (*C. albicans*)	*C. albicans*	Fluconazole	Cure	0/3 (0/1)
4	Proven	Negative	*C. krusei*	AmB, fluconazole	Cure	0/3 (0/1)
5	Proven	Negative	*C. albicans*	AmB	Cure	0/3 (0/1)
6	Proven	Negative	*C. albicans*	Fluconazole	Cure	0/4 (0/2)
7	Probable‡	Negative	*C. albicans*	Fluconazole	Improvement	0/2 (0/1)
8	Probable	NP	*C. albicans*	AmB	Not evaluated	0/2 (0)
9	Probable	NP	*C. albicans*	AmB	Cure	0/4 (0/2)
10	Probable	NP	*C. albicans*	AmB, fluconazole	Cure	0/4 (0)
11	Probable	NP	*C. albicans*	ABLC	Cure	0/3 (0/1)
12	Probable	NP	*C. albicans*	Fluconazole	Cure	0/3 (0/1)
13	Probable	NP	*C. albicans*	Fluconazole	Cure	0/2 (0/1)
14	Probable	NP	*C. glabrata*	Fluconazole	Cure	3/6 (1/2)
15	Probable	NP	*C. albicans*	Fluconazole	Cure	1/2 (0/1)

*Values in parentheses indicate samples taken before start of antifungal therapy.

†Early death due to other causes.

‡Histopathological examination of the liver biopsy performed after 1 month of antifungal therapy showed granulomatous infiltration but did not reveal fungi.

fungal treatment) and in one of two serum samples from patient 15. The antigen was not detected in 38 serum samples of 13 (87 %) patients with probable (seven patients) or proven (six patients) *Candida* infection. Except for one early death due to other causes and one patient lost to follow up, all patients recovered during antifungal treatment.

Discussion

Hepatic and splenic focal lesions in cancer patients detected by imaging after recovery from chemotherapy-induced neutropenia are considered diagnostic of probable *Candida* infection, although hepatic infection by other micro-organisms can occasionally mimic these findings (Martino *et al.*, 1990; Pagano *et al.*, 1992). During neutropenia, diagnosis may be difficult, owing to the absence of specific clinical and imaging findings and the low sensitivity of microbiological methods. Focal hepatosplenic candidiasis in a neutropenic patient is frequently associated with persistent fever, nausea, vomiting, abdominal pain, hepatomegaly and high alkaline phosphatase. However, all of these signs and symptoms could be associated in cancer patients with other infectious and non-infectious complications and are often of limited value in differential diagnosis and in therapeutic decisions, e.g. empirical broad-spectrum antifungal or specific anti-candidal agents. A definite diagnosis of hepatosplenic candidiasis is based on positive results of tissue cultures and/or the histological demonstration of *Candida* organisms in the liver and/or spleen. However, invasive diagnostic techniques are rarely permitted by the underlying conditions of these patients; consequently, in most cases, diagnosis is presumptive on the basis of clinical, biochemical and instrumental findings.

There is a wide consensus that antigen detection methods are potentially useful for diagnosing invasive candidiasis; however, no method has been rigorously standardized and the results achieved with their use have not reached unequivocal clinical significance (Reiss & Morrison, 1993). The utility of mannoproteinaemia detection by our method has been recently evaluated in different settings of invasive *Candida* infections in patients with haematological malignancies (Girmenia *et al.*, 1997, 1999). A correlation between mannoproteinaemia and deep-tissue fungal invasion in candidaemic patients has been observed (Girmenia *et al.*, 1997). In fact, mannoproteinaemia was detected in a minority of patients with transient or central venous catheter-related fungaemia and in most patients with documented tissue invasion. Despite persistent *Candida* isolation from blood, the antigen seems not to be released by the yeast when the source of the infection is a contaminated catheter. In a further study, focused on the value of mannoproteinaemia detection in patients with neutropenic enterocolitis, the test seemed to be a useful diagnostic tool to suggest the involvement of *Candida* in the development of this severe complication occurring increasingly in patients with haematological malignancies (Girmenia *et al.*, 1999).

Only few data are available in the literature on the role of serological tests in the diagnosis of hepatosplenic candidiasis. An increase in antibody levels against cell-wall mannan and cytoplasmic antigens was observed in two leukaemic patients with *C. albicans* granulomatous hepatitis (Jones, 1981). In a multicentre study on the utility of *Candida* enolase serum detection in the diagnosis of deep-seated candidiasis, 10 of 11 patients who were serum enolase-positive had hepatic involvement (Walsh *et al.*, 1991). In view of the high sensitivity and specificity for enolase antigenaemia in hepatosplenic candidiasis, the authors concluded that a cancer patient with hepatic and splenic lesions consistent with hepatosplenic candidiasis on CT scanning might be spared a biopsy to confirm the diagnosis by this laboratory method. In contrast, the value of cell-wall MP serum antigen detection as part of the diagnostic strategy in patients with hepatosplenic candidiasis has not been evaluated. To our knowledge, only one case of a leukaemic patient with proven hepatic candidiasis but with negative mannanaemia has so far been reported (Tashjian *et al.*, 1984).

Our study shows the limited usefulness of MP serum detection by our method in the diagnosis of focal hepatosplenic candidiasis, contrasting with the positive correlation between mannoproteinaemia and other invasive diseases caused by *Candida* (De Bernardis *et al.*, 1993; Girmenia *et al.*, 1997, 1999; Reiss & Morrison, 1993). In fact, only two of the 15 patients included in the study had detectable serum antigen. It could be hypothesized that some of the patients might have a hepatosplenic infection due to a species of *Candida* with an MP that is not reactive with mAb AF1, such as patient 4, who was diagnosed with a probable *Candida* infection but who was colonized by *Candida krusei*. However, this seems to be unlikely in most of the other cases, considering that three patients had a microbiologically proven infection with *C. albicans* or *C. tropicalis* and that all but one of the other patients were colonized in the gastrointestinal tract by mAb AF1 MP-reactive *C. albicans* or *C. glabrata* (Cassone *et al.*, 1988; De Bernardis *et al.*, 1993). Incidentally, this clearly confirms that patients who are simply colonized, or at least non-neutropenic, are not mannoproteinaemic (De Bernardis *et al.*, 1993; Girmenia *et al.*, 1999). Furthermore, considering that 14 serum samples (only one MP-positive) from 11 patients had been collected before antifungal therapy was started, the possibility that the low rate of MP detection was due to the concurrent administration of antifungal drugs can be excluded.

It is quite clear that mannoproteinaemia may occur only transiently during an invasive infection and that multiple, serial specimens are necessary to achieve optimum diagnostic sensitivity. Reticuloendothelial cells in the liver and spleen have been shown to have an important role in the clearance of mannan antigen from *C. albicans* from the blood (Kappe & Muller, 1991). It could be hypothesized that the strong inflammatory reaction around the focal infection and the small number of fungal organisms inside the foci usually observed in hepatosplenic candidiasis could be responsible for efficient ingestion and local disposal of the secreted MP by liver/spleen phagocytes and inflammatory cells, thus avoid-

ing detectable spread in the bloodstream, a fact that also reflects the usual negativity of blood cultures in this clinical syndrome.

In conclusion, our clinical investigations on the use of detection of mannoproteinaemia by our method in different clinical entities of invasive *Candida* infection (deep infection with candidaemia, transient candidaemia, catheter-related candidaemia, neutropenic enterocolitis, hepatosplenic candidiasis) seem to show the variable diagnostic value of this laboratory test. In particular, the present data suggest a low diagnostic value in the setting of hepatosplenic candidiasis. We think that the stratification of the various *Candida* infections is required for better clarification of the possible clinical application of serological tests.

A new strategy consisting of the combined measurement of mannanaemia and an antibody response was recently developed, based on two standardized enzyme immunoassays, the Platelia Candida Ag and Platelia Candida Ab tests (Bio-Rad) (Sendid *et al.*, 1999, 2002, 2003). The combined tests proved to have increased sensitivity and specificity compared with the single assays. The availability of serial serum samples collected before and after clinical diagnosis of infection seems to be important, since the detection of mannoproteinaemia in sera from candidiasis patients is inversely correlated to the presence of anti-mannan antibodies. In fact, a decline of antigenaemia seems to correlate with rising titres of anti-mannan antibodies. The possible role of recovery from neutropenia in this setting is unknown. Further studies focused on laboratory diagnosis of hepatosplenic candidiasis as well as of other types of *Candida* infection during and after the neutropenic phase by using these promising serological tests are in progress at our centre.

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