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

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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.

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
Candida tropicalis/C226 based on the use of a monoclonal antibody (mAb AF1) specific for a suspected between 2 weeks before and 1 month after the documentation of candidiasis treated with alkali and heat (De Bernardis et al., 1993). To separate the circulating mannantigen 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 guillermontii 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>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis of HC</th>
<th>Culture of hepatic biopsy (Candida species)</th>
<th>Candida colonization</th>
<th>Treatment</th>
<th>Outcome of infection</th>
<th>MP-positive serum samples (n)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Proven</td>
<td>Positive (C. tropicalis)</td>
<td>C. tropicalis</td>
<td>AmB, fluconazole</td>
<td>Improvement†</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>2</td>
<td>Proven</td>
<td>Positive (C. albicans)</td>
<td>C. albicans</td>
<td>AmB, fluconazole</td>
<td>Cure</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td>3</td>
<td>Proven</td>
<td>Positive (C. albicans)</td>
<td>C. albicans</td>
<td>Fluconazole</td>
<td>Cure</td>
<td>0/3 (0/1)</td>
</tr>
<tr>
<td>4</td>
<td>Proven</td>
<td>Negative</td>
<td>C. krusei</td>
<td>AmB, fluconazole</td>
<td>Cure</td>
<td>0/3 (0/1)</td>
</tr>
<tr>
<td>5</td>
<td>Proven</td>
<td>Negative</td>
<td>C. albicans</td>
<td>AmB</td>
<td>Cure</td>
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</tr>
<tr>
<td>6</td>
<td>Proven</td>
<td>Negative</td>
<td>C. albicans</td>
<td>Fluconazole</td>
<td>Cure</td>
<td>0/4 (0/2)</td>
</tr>
<tr>
<td>7</td>
<td>Probable†</td>
<td>Negative</td>
<td>C. albicans</td>
<td>Fluconazole</td>
<td>Improvement</td>
<td>0/2 (0/1)</td>
</tr>
<tr>
<td>8</td>
<td>Probable</td>
<td>NP</td>
<td>C. albicans</td>
<td>AmB</td>
<td>Not evaluated</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td>9</td>
<td>Probable</td>
<td>NP</td>
<td>C. albicans</td>
<td>AmB</td>
<td>Cure</td>
<td>0/4 (0/2)</td>
</tr>
<tr>
<td>10</td>
<td>Probable</td>
<td>NP</td>
<td>C. albicans</td>
<td>AmB, fluconazole</td>
<td>Cure</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>11</td>
<td>Probable</td>
<td>NP</td>
<td>C. albicans</td>
<td>ABLC</td>
<td>Cure</td>
<td>0/3 (0/1)</td>
</tr>
<tr>
<td>12</td>
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<td>C. albicans</td>
<td>Fluconazole</td>
<td>Cure</td>
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</tr>
<tr>
<td>13</td>
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<td>C. albicans</td>
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<td>Cure</td>
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</tr>
<tr>
<td>14</td>
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<td>Fluconazole</td>
<td>Cure</td>
<td>3/6 (1/2)</td>
</tr>
<tr>
<td>15</td>
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<td>C. albicans</td>
<td>Fluconazole</td>
<td>Cure</td>
<td>1/2 (0/1)</td>
</tr>
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*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 antifungal agents. A definite diagnosis of hepatosplenic candidiasis is based on positive results of tissue cultures and/or the candidal agents. A definite diagnosis of hepatosplenic candidiasis could be associated in cancer patients with other infectious 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 mannoproteinemia (De Bernardis et al., 1993; Girmenia et al., 1999). Furthermore, considering that 14 serum samples 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.

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 mannoproteinemia 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 mannoproteinemia (De Bernardis et al., 1993; Girmenia et al., 1999). Furthermore, considering that 14 serum samples 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 mannoproteinemia 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.

References


