

Note

Detection of *Candida* Mannoproteinemia in Patients with Neutropenic Enterocolitis

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Abstract To assess the role of *Candida* spp. in the etiology of neutropenic enterocolitis complicating aggressive cytotoxic chemotherapy, a dot immunobinding assay for an immunodominant *Candida* mannoprotein antigen was employed in 20 patients with hematologic malignancies. *Candida* antigen was detected in at least one serum sample from 12 (60%) patients. Eleven (92%) patients were cured when an antifungal agent was added to the antibacterial treatment. In eight patients a selective anticandidal therapy with fluconazole was administered on the basis of positive *Candida* mannoproteinemia, and treatment was successful in all cases but one. Detection of *Candida* mannoproteinemia seems to be a useful diagnostic tool in patients with neutropenic enterocolitis and represents an additional tool for selecting a less empiric, low toxic antifungal treatment with fluconazole.

Introduction

Neutropenic enterocolitis (NE), a term that is synonymous with typhlitis, is a recognized cause of morbidity and mortality in neutropenic cancer patients, particularly in patients with hematologic malignancies who are undergoing intensive chemotherapy [1–4]. Fungal pathogens, particularly *Candida* spp., have been frequently implicated in the etiology of NE, essentially on the basis of autopsy findings. In fact, antemortem diagnosis of fungal NE is rarely achieved because the clinical criteria are nonspecific, it is difficult to differentiate gastrointestinal commensalism and colonization from infection, and blood cultures are rarely positive [2]. To assess the role of *Candida* spp. in the etiology of

the syndrome, we employed a sensitive and specific dot immunobinding assay for the detection of an immunodominant *Candida* mannoprotein antigen [5] in addition to conventional microbiologic investigations.

Patients and Methods

We studied 20 consecutive patients with hematologic malignancies who developed NE between February 1994 and May 1995. Nineteen of these patients had been treated with intensive polychemotherapeutic protocols that included cytarabine. One patient underwent autologous bone marrow transplantation.

The patients were nursed in two-bed rooms with reverse isolation precautions. When patients exhibited neutropenia, anti-infective prophylaxis was provided with ciprofloxacin (500 mg b.i.d.) plus oral amphotericin B (2000 mg/day) in adults and with amphotericin B alone in children. Surveillance cultures of urine, stool, and sputum and nasal, oropharyngeal, rectal, and vaginal swabs were performed once a week. In the event of fever and granulocytopenia [<500 polymorphonuclear leukocytes (PMN)/ μL], empiric antibiotics, usually a beta-lactam agent plus amikacin, were administered. Upon persistence of fever after 72 h, teicoplanin was added, followed by empiric intravenous amphotericin B (1 mg/kg/day) if the fever was resistant to teicoplanin after 48–72 h without any microbiologic finding.

A clinical diagnosis of NE was made when fever, diarrhea (at least 4 episodes/day), abdominal pain, and peritoneal irritation [6] were associated with one of the following signs: ileus, defined as the presence of a distended abdomen with alteration in bowel sounds and distended bowel loops on ultrasound examination or plain abdominal radiograph; necrotizing colitis, documented at echography and computerized tomography as thickening (more than 7 mm) of the bowel wall and/or pneumatosis intestinalis; or lower gastrointestinal bleeding requiring blood transfusions. Most patients diagnosed with NE were treated with total parenteral nutrition; the empiric antimicrobial therapy was eventually modified on the basis of laboratory results.

In patients with a clinical diagnosis or suspicion of NE, blood cultures and stool cultures for *Clostridium difficile*, *Salmonella* spp., *Pseudomonas* spp., and fungi were performed. Stool specimens were inoculated onto Sabouraud-chloramphenicol, cetrimide, and salmonella-shigella agar plates for aerobes and onto cycloserine-cefoxitin fructose agar incubated in an anaerobic holding jar for *Clostridium difficile*. Direct detection of qualitative *Clostridium difficile* toxin A in fecal samples by rapid immunoassay was also performed. Serial serum samples at the onset

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and during the course of the clinical syndrome were examined for *Candida* mannoprotein antigen using the dot immunobinding assay described elsewhere [5].

Results and Discussion

The demographic and disease characteristics of patients with NE are summarized in Table 1. All patients presented with abdominal pain and persistent diarrhea for an average of 12 days (range, 6–20 days) after institution of chemotherapy. The results of microbiological and serological investigations, the antimicrobial therapy administered, and outcome are shown in Table 2. *Candida* mannoprotein antigen was detected in at least one serum sample of 12 (60%) patients [26 of 39 (66.6%) total serum samples]. No antigenemia was detected in any of the five subjects without isolation of *Candida* from stool, whereas 12 of the 15 subjects with *Candida* stool isolation were antigenemic.

Eleven (92%) of the 12 patients with at least one serum sample positive for *Candida* mannoprotein were cured with the addition of the antifungal therapy. Disappearance of fever and improvement of clinical conditions were observed at a mean of 7.5 days (median, 6 days; range, 25 days before and 7 days after) before recovery from neutropenia (PMN $>500/\text{mm}^3$); nine patients were still neutropenic at that time. In eight of these 12 patients, a selective anticandidal therapy with intravenous fluconazole (400–1000 mg/day) was administered on the basis of positive *Candida* mannoproteinemia. The other four antigenemic patients who were

Table 1 Demographic and disease characteristics of patients with neutropenic enterocolitis

Characteristic	
Mean age in years	37 (range, 6–63)
Gender, M/F	16/4
Mean days with PMN $<100/\text{mm}^3$	17 (range, 6–33)
Underlying disease	
Acute myeloid leukemia	14
Acute lymphoid leukemia	3
Chronic myeloid leukemia ^a	2
Non-Hodgkin's lymphoma	1
No. (%) with gastrointestinal signs and symptoms	
Diarrhea	20 (100)
Abdominal pain	20 (100)
Ileus ^b	10 (50)
Necrotizing colitis ^b	15 (75)
Peritonitis	4 (20)
Bleeding	4 (20)

^a One patient underwent autologous bone marrow transplantation. The other 19 patients were treated with intensive remission-induction cytotoxic chemotherapy

^b Determined by abdominal radiograph examination and/or echography
PMN, polymorphonuclear leukocytes

undergoing empiric amphotericin B at the time of the first positive mannoproteinemia assay continued with amphotericin B therapy. In the eight patients who received intravenous fluconazole, the antifungal drug was administered at a mean of 6.5 days (range, 4–11 days) and 4 days (range, 2–7 days) from the onset of fever and abdominal symptoms, respectively. The treatment was successful in seven cases, with disappearance of fever and improvement of gastrointestinal infection after a mean of 4 days of therapy (range, 2–11 days).

Table 2 Microbiological investigations, therapy, and outcome of 20 patients with neutropenic enterocolitis

Patient no.	Blood ^a and stool ^b cultures	<i>Candida</i> intestinal colonization	<i>Candida</i> MP (positive/total)	Therapy	Outcome
1	negative	<i>C. albicans</i>	1/2	AT+FCZ	cured
2	negative	<i>C. albicans</i>	6/7	AT+FCZ	cured
3	negative	<i>C. glabrata</i>	1/2	AT+FCZ	cured
4	<i>Pseudomonas aeruginosa</i> ^a	<i>C. glabrata</i>	0/2	AT	cured
5	negative	<i>Candida</i> spp	2/2	AT+AmB	cured
6	negative	<i>C. albicans</i>	3/3	AT+FCZ	cured
7	negative	negative	0/2	AT	died
8	negative	<i>C. albicans</i>	2/3	AT+FCZ	died
9	<i>Clostridium difficile</i> ^b	<i>C. albicans</i>	1/3	AT+AmB	cured
10	negative	<i>C. albicans</i>	4/6	AT+FCZ	cured
11	negative	negative	0/2	AT+AmB	cured
12	negative	<i>C. albicans</i>	2/3	AT+FCZ	cured
13	negative	<i>Candida</i> spp.	1/3	AT+AmB	cured
14	<i>Salmonella</i> spp. ^b	negative	0/1	AT	cured
15	negative	<i>C. albicans</i>	1/2	AT+FCZ	cured
16	negative	<i>C. albicans</i>	2/3	AT+AmB	cured
17	negative	<i>Candida</i> spp.	0/3	AT	cured
18	negative	<i>C. albicans</i>	0/2	AT+AmB	cured
19	negative	negative	0/2	AT	died
20	<i>Staphylococcus aureus</i> ^{a,b}	negative	0/1	AT	died

^a Blood cultures for bacteria and fungi

^b Only gastrointestinal bacterial pathogens were considered. Normal enteric microbial flora was not considered

MP, mannoproteinemia (antigenemia); AT, antibacterial therapy; AmB, amphotericin B; FCZ, fluconazole

One patient died of multiorgan failure after 4 days of therapy; an autopsy was not performed.

In order to evaluate the possible correlation between *Candida* intestinal colonization and antigenemia detection, we retrospectively studied 17 patients observed during the same period who had the following characteristics: (i) colonization of stool by *Candida*; (ii) absence of any sign of fungal infection and no empiric antifungal therapy; (iii) availability of at least one serum sample collected in the period of neutropenia following the cytotoxic treatment. Of 43 serum samples available (mean, 2.5; range, 1–9 serum samples/patient), all but three were negative for *Candida* mannoprotein. The three positive samples were collected from two patients during a self-limiting episode of diarrhea.

The pathogenesis of NE is not completely clear, and many aetiological agents have been implicated. Treatment with cytotoxic agents – especially cytarabine – which induce mucosal epithelial cell necrosis and are used to treat hematologic malignancies, is considered a risk factor for the development of NE [4]. The typical loss of epithelial integrity combined with neutropenia following cytoreductive chemotherapy makes the bowel susceptible to invasion by colonizing microorganisms with subsequent infection by single or multiple agents. Various enteric organisms have been implicated in the pathogenesis of neutropenic enterocolitis [7], and several studies have reported the frequent involvement of fungi [2–4]. In a recent review of children with acute leukemia and NE, only 16% of the organisms cultured from blood antemortem were fungi [2]. However, fungal pathogens accounted for 53% of microorganisms seen at autopsy, and *Candida* spp. was involved in one-third of the fatal episodes. In another study of 24 children with an antemortem diagnosis of NE, only three cases of fungal infection were documented, but empiric antifungal therapy was necessary in 15 subjects [3].

In a study of 35 consecutive patients with acute myelogenous leukemia who were undergoing intensive chemotherapy, we observed ten cases of gastrointestinal complications, five of which were due to *Candida* spp [4]. All of these studies show that *Candida* plays an important pathogenetic role in the setting of NE and emphasize the difficulties in making the correct microbiological diagnosis. Moreover, the isolation of *Candida* from stool is of doubtful significance, and there are difficulties in discriminating invasive candidiasis from extensive intestinal candidal colonization, although the latter is a risk factor for deep-seated infection in neutropenic subjects [9, 10].

In the present investigation, the use of a sensitive *Candida*-specific dot immunobinding assay to detect a circulating mannoprotein antigen [5] allowed us to presume that *Candida* was at least an associated cause,

if not the primary agent, of NE in 60% of patients (80% of those with *Candida* isolation from stools), even in the absence of a positive blood culture or other specific clinical signs. In addition, detection of *Candida* mannoproteinemia permitted us to administer fluconazole, a nontoxic effective anti-*Candida* agent, to eight patients. It also allowed us to choose the right antifungal therapeutic approach for four other patients who were undergoing empiric treatment with amphotericin B when mannoproteinemia was documented. Most antigenemic patients responded favorably and promptly to antifungal therapy despite persistent profound neutropenia, which strongly suggests that the supportive-therapy effect of fluconazole preceded recovery from neutropenia; thus its efficacy was substantially independent of recovery. Evaluation of the population of patients colonized by *Candida* without any sign or suspicion of invasive infection showed that mannoproteinemia almost never occurs in this setting even if an episode of diarrhea is associated with transitory spread of the antigen.

The detection of some *Candida* antigens (e.g., mannoprotein, enolase) has been proposed as a useful diagnostic marker of invasive disease [5,11–13]. However, the antigens are rapidly removed from the circulation by the formation of immune complexes and by endocytosis by Kupffer cells in the liver; thus, their presence in the serum is transient, and repeated serum sampling is usually required to improve sensitivity [11–14].

Contrary to the expected limited sensitivity of the antigenemia test owing to the fluctuating serum levels of mannoprotein antigen, we found that the ratio of positive to total samples was high, and some patients had persistent positive mannoproteinemia. This data is in agreement with the strong association between *Candida* mannoproteinemia and gastrointestinal disease observed in a rat model of neutropenic enterocolitis [15]. This could be explained by the extensive organ surface involved in invasive *Candida* infection with continuous antigen shedding.

Because we were able to document etiology in only a few cases in this series, we could not perform a statistical analysis or evaluate the sensitivity, specificity, positive, and negative predictive value of the test for NE as we have done for other kinds of invasive candidiasis [5]. Thus, our data can only be regarded as preliminary. We believe that because it is difficult to prove the cause of NE other than at autopsy, it is hard to obtain a sufficiently large series of cases of NE in which etiology can be documented.

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