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Clostridium difficile Colitis in Leukemia Patients*

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Abstract—*Leukemia patients with diarrhea or other abdominal symptoms have been investigated for the presence of* Clostridium difficile *and its cytotoxin in stools. Of the patients studied* 19% *had* C. difficile, *in most cases together with cytotoxin. All patients but one had received antibiotics, while one had been treated with cytotoxic agents only. Symptoms of colitis were most often abdominal pain and distension rather than diarrhea. Owing to the not infrequent fatal evolution, it is recommended that routine search for* C. difficile *in leukemia patients with abdominal symptoms be performed and appropriate therapy started immediately.*

INTRODUCTION

DIARRHEAL diseases following antibiotic administration is one of the most common adverse drug reactions [1]. The spectrum of the disease may vary widely, from the life-threatening pseudomembranous colitis (PMC) to a mild form of colitis with scarce, if any, pathological changes of the colonic mucosa [2].

In recent years *C. difficile* has been implicated as being the etiological agent in most cases of antibiotic-associated PMC and in approximately 20% of antibiotic-associated diarrhea without pseudomembrane formation [3, 4].

Clostridium difficile is a non-invasive organism and colonic lesions and diarrhea are due to the production of at least two different toxins [5]. The presence of one of these, a cytotoxin, in the feces of patients, is of diagnostic value [6]. A recent report demonstrated that *C. difficile* produces colitis in experimental animals following administration of an antineoplastic drug (methotrexate) [7] and there is also some evidence of the role of antineoplastic chemotherapy in inducing *C. difficile* colitis in man [8]. It follows that patients exposed to both antibiotic and antineoplastic chemotherapy appear to be at high risk of developing *C. difficile* colitis.

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Patients with leukemia often receive not only extensive cytotoxic therapy for their underlying disease, but also antibiotic therapy for prophylaxis and/or therapy of infectious episodes. In this patient population bacterial diseases often have a subtle presentation, owing to impaired host defence and neutropenia. Moreover, *C. difficile* colitis has been reported to occur with unusual clinical and histological findings [9]. The present study was designed to assess if, in leukemia patients, (1) *C. difficile* colitis can represent a clinical problem; (2) if neutropenia or usage of antineoplastic drugs, besides antibiotics, can be a risk factor for colitis; and (3) what are the characteristic features of the disease in this group of patients.

MATERIALS AND METHODS

Patients studied

All patients admitted to the leukemia unit in the period March 1982–December 1983 who developed diarrhea and/or abdominal tenderness or distension, entered the study. On two occasions fecal specimens were obtained from all patients in the ward.

Cultures

Stools were cultured for *C. difficile* on a selective medium containing cycloserine and cefoxitin (CCFA) [10]: plates were incubated in an anaerobic cabinet for up to 5 days. Colonies with

typical morphology were further identified by gas-liquid chromatography [11] and biochemical tests [12]. Common enteric pathogens were excluded by conventional cultures of the specimens [13].

Toxin assay

Fecal specimens were assayed for the presence of cytotoxin using HeLa cell tissue culture [6]. Two-fold dilutions of fecal extracts, obtained by centrifugation at 12,000 *g* for 30 min and filtration through a 45 μm millipore membrane, were inoculated in cell cultures grown in 92-well flat bottom microtiter trays and incubated at 37°C in 5% CO_2 for 24 hr. *Clostridium sordelli* antitoxin (Wellcome) was used for the neutralization test. The assay was considered positive when actinomorphic changes affecting the monolayer were present and these were neutralized by *C. sordelli* antitoxin.

Cytotoxic titers were read as the greatest dilution affecting at least 50% of the cells.

RESULTS

During the period of the study, 247 patients were admitted to the unit for a total of 304 admissions. Ninety-eight (40%) were affected by acute myeloid leukemia (AML), 77 (31%) by acute lymphoblastic leukemia (ALL), 28 (11%) by lymphoma, ten (4%) by the blastic crisis of chronic myeloid leukemia (CML) and 34 (14%) by other hematological malignancies. Almost all the patients received cytotoxic drugs for remission induction therapy.

One hundred and ninety-three patients became neutropenic (PMN $< 1 \times 10^9/l$). One hundred and nineteen patients had one or more febrile episodes and were treated with antibiotics in combination, mainly cefotaxime + amikacin.

Forty patients (19% of all patients) had diarrhea or other abdominal symptoms. *Clostridium difficile* was found in the feces of 11 patients. Table 1 summarizes the clinical data of patients with positive stool cultures. All patients but two had received both antineoplastic and antibiotic therapy, one patient had received antibiotics alone and the other patient antineoplastics alone. No antibiotic had been administered to this patient for the 2 months prior to the onset of the disease.

Toxin assays were positive in all patients but two, with titer $\geq$1:1280.

Six patients were highly neutropenic (PMN $< 0.1 \times 10^9/l$) at the time of the onset of diarrhea or other intestinal disturbances.

Diarrhea, if present, was generally mild. Abdominal pains and severe distension were the more common developments. Diagnosis of colitis was done on clinical grounds only as sigmoidoscopy was not performed due to the thrombocytopenic state of the patients. In three fatal cases (Nos. 3, 5 and 10) lesions of the colonic mucosa were found on postmortem examination. Case No. 3 had typical pseudomembranes scattered all over the surface of the colon and rectum, while in case No. 5 the most striking finding was an enormous distension of the large bowel, consistent with the diagnosis of toxic megacolon. In cases Nos. 3 and 10 *C. difficile* was isolated also from a necropsy specimen of washed colonic mucosa.

Cultures of rectal swabs obtained from all the patients in the unit showed that two asymptomatic patients had *C. difficile* in their stools. Both patients were in the same room; no toxin was detected in their feces.

DISCUSSION

Diarrhea is not an infrequent complication of antineoplastic chemotherapy [14]. It is usually ascribed to an arrest in the rapid turnover of the intestinal epithelium due to cytotoxic drugs. Moreover, in leukemia patients a particular form of 'necrotizing enterocolitis' with pseudomembrane formation has been described in association with neutropenic states [15].

Our study demonstrates that some diarrheal complications and pseudomembranous enterocolitis in leukemia patients can be due to an infectious agent: *C. difficile*. Positivity for cytotoxin in most patients confirms the pathogenic role of the organism. The failure to demonstrate toxin in two symptomatic patients may be due to a low titer in feces not detectable by our method. On the other hand, two asymptomatic patients had *C. difficile* but no cytotoxin in their stools; this is in accordance with the fact that they were colonized by the organism but did not have the disease. Cases Nos. 3 and 5 were also bacteremic concurrently with *C. difficile* infection. *Staphylococcus aureus* was isolated from the blood of patient No. 3 and *Streptococcus fecalis* and *Bacillus* sp. in patient No. 5. Only one patient, No. 7, developed ascites and jaundice; this patient subsequently died and at necropsy systemic fungal infection with involvement of the liver was diagnosed.

We observed 11 cases of *C. difficile* colitis in 13 months, which represents 3.5% of all patients admitted to the unit during that period. If we consider only patients who received antibiotic treatment, ten patients out of 119 developed *C. difficile* colitis, that is, approximately 19%. This figure is probably much higher than for antibiotic-treated general medical patients, even if precise data are not available. If we consider

Table 1. Patients with Clostridium difficile *- associated colitis*

Patients No.	Sex	Age	Diagnosis	PMN/mm³*	Antibiotics	Antineoplastic therapy	Symptoms	*Clostridium difficile* in stools: Culture	Cytotoxin
1	F	23	AML	<100	cefotaxime amikacin	cytarabine L-asparaginase	mild diarrhea	+	+
2	F	5	AML	<100	cefotaxime amikacin	daunomycin cytarabine	mild diarrhea	+	NT
3	F	59	ALL	100-200	cefotaxime amikacin	methotrexate prednisone vincristine adriamycin	abdominal distension diarrhea	+	NT
4	F	40	CML (blastic crisis)	300	—	daunomycin L-asparaginase vincristine	abdominal distension no diarrhea	+	+
5	M	29	AML	<100	piperacillin amikacin amphotericin B	daunomycin cytarabine	abdominal distension diarrhea with blood	+	+
6	F	22	ALL in Hodgkin's disease	<100	cefotaxime cefamandole tobramycin amikacin amphotericin B	daunomycin cytarabine	abdominal pain no diarrhea	+	+
7	M	43	AML	11,000	cefotaxime amikacin amphotericin B cephalotin, gentamycin, colistin (per os)	daunomycin cytarabine	abdominal pain mild diarrhea with blood	+	-
8	M	44	CML (blastic crisis)	200	cefotaxime amikacin	—	severe diarrhea	+	+
9	M	13	AML	<100	cefotaxime piperacillin amikacin	daunomycin cytarabine	mild diarrhea	+	+
10	F	43	CML (blastic crisis)	<100	cefotaxime amikacin	daunomycin hydroxiurea	abdominal distension no diarrhea	+	-
11	M	13	ALL	350	cefotaxime piperacillin amikacin	daunomycin cytarabine	abdominal distension	+	+

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; NT, not tested.
*At the onset of symptoms.

only patients with abdominal symptoms, 9/40 (17%) had *C. difficile* in their stools. In leukemic patients antibiotic therapy appears to be a major factor in the development of *C. difficile* colitis, the same as in other groups of patients. In fact, all patients but one were receiving antibiotics when they developed intestinal symptoms. Patients were treated with at least two antibiotics, usually a combination of an aminoglycoside and a β-lactam, which proved to be highly effective against Gram-negative organisms, the principle cause of septicemia in the unit [16]. Only one patient had received oral non-adsorbable antibiotics for gut decontamination. As aminoglycosides have so far not been implicated in causing *C. difficile* colitis, cefotaxime and piperacillin appear the most likely responsible agents (Table 1). These antibiotics have a broad spectrum of action and are also very active against anaerobic bacteria, which represent the prevalent indigenous flora of the gut and are the principal factors of resistance to exogenous colonization [17]. Third-generation cephalosporins have already been reported to induce *C. difficile* colitis [18]. There is not enough evidence that neutropenia *per se* can represent a risk factor for *C. difficile* disease; usually it demands antibiotic usage and, therefore, it might cause *C. difficile* colitis indirectly.

In this study we have confirmed the observations of other authors, that *C. difficile* colitis has unusual features in neutropenic patients. Patients rarely presented with severe, bloody diarrhea, but more often only with abdominal discomfort or distension. At necropsy enormous abdominal distension was noticed twice, while typical pseudomembranes on the colonic mucosa were observed only in one patient who was not neutropenic at the onset of symptoms.

Clostridium difficile colitis, like other infections in the compromised host, can evolve rapidly and dramatically in spite of the apparently mild symptomatology. In three patients of our series, *C. difficile* colitis was at least a contributory factor to death.

Oral vancomycin, together with discontinuance of systemic antibiotics, is the treatment of choice for *C. difficile* colitis [19]. More often systemic antibiotics cannot be stopped in leukemic patients and this has been reported to increase mortality due to *C. difficile* colitis [20]; so the use of vancomycin is imperative. Our experience with vancomycin is not conclusive: so far we have treated six patients with oral vancomycin (250–500 mg four times a day): in two, symptomatology improved prompty and *C. difficile* and toxin disappeared from the feces. No relapse was observed. In one patient, despite an eight-day course of oral vancomycin, on post-mortem examination *C. difficile* was cultured from the small and large bowel mucosal specimens. The strain was susceptible *in vitro* to vancomycin (minimal inhibitory concentration $\leqslant 0.5$ μg/ml). In other patients, complications not related to colitis made it difficult to judge therapy efficacy. Nevertheless, we believe that vancomycin should be administered to leukemic patients with proven or suspected *C. difficile* colitis as soon as possible and support the opinion of others, that oral vancomycin should be reconsidered for protocols of gut decontamination [21, 22]. We believe that oral metronidazole, although less expensive than vancomycin and equally effective [23], should be used with caution in these patients, owing to its potential disruptive action on the anaerobic bowel flora, that is, the colonization resistance determinants.

We did not notice any outbreak of *C. difficile* colitis, as cases were scattered throughout the year, with a slight prevalence during the winter months. A nosocomial transmission of the disease has been demonstrated in some instances [24]; typing schemes for *C. difficile* are in development [25, 26] and will clarify this aspect. We can conclude that *C. difficile* colitis can be numbered among the infectious complications of leukemic patients and can have a fatal course. We therefore suggest that stool examination for *C. difficile* and its toxin should be performed in any suspect case and therapy promptly started.

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