The Search for a Better Treatment for Recurrent *Clostridium difficile* Disease: Use of High-Dose Vancomycin Combined with *Saccharomyces boulardii*

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Recurrent *Clostridium difficile* disease (CDD) is a difficult clinical problem because antibiotic therapy often does not prevent further recurrences. In a previous study, the biotherapeutic agent *Saccharomyces boulardii* was used in combination with standard antibiotics and was found to be effective in reducing subsequent recurrences of CDD. In an effort to further refine a standard regimen, we tested patients receiving a regimen of a standard antibiotic for 10 days and then added either *S. boulardii* (1 g/day for 28 days) or placebo. A significant decrease in recurrences was observed only in patients treated with high-dose vancomycin (2 g/day) and *S. boulardii* (16.7%), compared with those who received high-dose vancomycin and placebo (50%; $P = .05$). No serious adverse reactions were observed in these patients. Comparison of data from this trial with data from previous studies indicates that recurrent CDD may respond to a short course of high-dose vancomycin or to longer courses of low-dose vancomycin when either is combined with *S. boulardii*.

Clostridium difficile–associated disease (CDD) is the most frequently identified cause of nosocomial diarrhea, colitis, and pseudomembranous colitis; however, the recurrent, intractable form of this disease has not been well studied [1–3]. Recurrent CDD has been traditionally treated with 1 of 2 antibiotics (metronidazole or vancomycin); this usually results in an initial resolution of the diarrhea [1, 4]. However, in up to 20% of patients, the symptoms recur within 4 weeks of the end of antibiotic therapy. Some patients may require repeated courses of antibiotics for months and even years in attempts to cure multiple episodes of CDD before *C. difficile* is finally eradicated [5]. The wide use of antibiotics and the risks (e.g., sepsis, surgery, toxic megacolon, and death) resulting from complications make a more effective treatment for recurrent CDD necessary.

Saccharomyces boulardii is a nonpathogenic yeast that has been used successfully as a biotherapeutic agent to prevent antibiotic-associated diarrhea and to treat other types of diarrhea; it is currently undergoing investigational clinical trials in the United States [6–12]. A previous randomized, double-blind trial in patients with either initial or recurrent CDD found that *S. boulardii* was effective in reducing the rate of recurrence from 44.8% in patients receiving placebo and a standard antibiotic to 26.3% in patients receiving *S. boulardii* and a standard antibiotic [13]. This effect was more pronounced in the 60 patients with the recurrent form of CDD (rate of recurrence was 64.7% in the patients receiving placebo, compared with 34.6% in those receiving *S. boulardii;* $P = .04$). However, in this study, neither the dose nor the duration of antibiotic was controlled for in the study design [13]. We report the effect of a short course (10 days) of treatment with high-dose vancomycin (2 g/day) in combination with *S. boulardii* (1 g/day for 28 days) on the prevention of subsequent recurrences of CDD.

Patients and Methods

This study presents data from part of a national double-blind, placebo-controlled trial of the treatment of adult patients with recurrent CDD. Both the epidemiology of the patient population and the enrollment have been described elsewhere [14]. The study was approved by the institutional review board administration of each participating hospital or clinic, and each patient gave written informed consent. The inclusion criteria for patients were as follows: patient age, 18–100 years; occurrence of active diarrhea before standard antibiotic treatment; positive *C. difficile* assay (either by

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Table 1. Comparison of patient characteristics and indicators of recurrent *Clostridium difficile* disease (CDD) among patients treated with 1 of 3 standard antibiotic regimens.

Variable	Patients with CDD treated with		
	High-dose vancomycin ($n = 32$)	Low-dose vancomycin ($n = 85$)	Metronidazole ($n = 53$)
Patient characteristics			
Age, mean y ± SD	61.4 ± 18.0	62.2 ± 19.6	66.0 ± 17.9
Sex, female	19 (59.4)	63 (75.9)	39 (73.6)
Outpatient	23 (72.0)	57 (68.7)	27 (51.0)
Study drug			
Saccharomyces boulardii	18 (56.3)	45 (54.2)	27 (50.9)
Placebo	14 (43.8)	38 (45.8)	26 (49.1)
Severity of CDD episode at enrollment			
Diarrhea	17 (53.1)	61 (73.5)	43 (81.1)
Colitis	14 (43.8)	19 (22.9)[a]	9 (17.0)[b]
Pseudomembranous colitis	1 (3.1)	3 (3.6)[a]	1 (1.9)[b]
Fecal WBCs	12 (48)	19 (26.8)[a]	9 (19.6)[b]
Stools per day, mean no. ± SD	6.8 ± 3.6	6.8 ± 3.6	6.5 ± 2.9
Fever (oral temperature >37.8°C)	5 (22.7)	16 (24.6)	11 (28.2)
C. difficile persistence at end of antibiotic treatment			
Culture positive	0 (0)	4 (5.7)	6 (12.5)
Toxin A positive	0 (0)	2 (2.8)	13 (26.5)[b]
Toxin B positive	0 (0)	3 (4.3)	14 (29.8)[b]
Recurrence			
With *S. boulardii*	3 (16.7)[c]	23 (51.1)	13 (48.1)
With placebo	7 (50.0)	17 (44.7)	13 (50.0)

NOTE. Data are no. (%) of patients, unless otherwise indicated, and % is based on the no. of patients for whom data were available. High-dose vancomycin was given at a dosage of 2 g/day. Low-dose vancomycin was given at a dosage of 500 mg/day. Metronidazole was given at a dosage of 1 g/day.

[a] Trend at $.05 < P < .1$ compared with the group receiving high-dose vancomycin.

[b] $P < .05$ compared with the group receiving high-dose vancomycin.

[c] *Saccharomyces boulardii* compared with placebo.

culture or by toxin A or B) during episodes of diarrhea; and ⩾1 recent, prior episode of CDD within 1 year.

Patients enrolled in the national trial were given a 10-day orally administered course of either high-dose vancomycin (2 g/day), or low-dose vancomycin (500 mg/day) or metronidazole (1 g/day). This article focuses on the patients who were given high-dose vancomycin, because neither the 10-day course of the lower dose of vancomycin nor metronidazole given with either *S. boulardii* or placebo was significantly effective. Patients were randomized to receive *S. boulardii* or placebo, and administration of the blinded study drug was begun on day 7 of treatment with vancomycin and was continued for 28 days. The study drug (*S. boulardii* or placebo) was taken orally at a dosage of 1 g/day (two 250-mg capsules b.i.d.) for 4 weeks. The 1:1 (*S. boulardii* to placebo) randomization and packaging of the blinded study drug kits was performed at Laboratoires Biocodex (Montrouge, France), to ensure that the study centers did not have access to the identity of the study drug.

Because patients were enrolled at many sites, all patients and physicians were required to use standardized diagnostic criteria, protocols, case report forms, and *C. difficile* assay methods. The patients kept a standardized daily diary of stool frequency and consistency, other symptoms, medications, and adverse reactions during the 8 weeks of the study. To document delayed recurrences of CDD after the study, the patients were also contacted by telephone monthly for 5 months afterward.

"Diarrhea" was defined either as (a) a change in bowel habits with ⩾3 loose or watery bowel movements per day for ⩾2 consecutive days, or (b) >8 loose stools within 48 h [3, 14]. "CDD" was defined as diarrhea associated with ⩾1 positive *C. difficile* assay (either culture, or toxin A or toxin B) and with no other known etiology (other enteric pathogens, chronic gastrointestinal condition, or medications). Patients whose samples were found to be positive by culture only needed current endoscopic evidence of *C. difficile* or a history of toxin A or B positivity during ⩾1 of the previous episodes of CDD. "Recurrent CDD" was defined as ⩾1 previous episode of CDD that was positive for *C. difficile* and that had initially responded to antibiotic treatment. "Diarrhea cessation" was defined as a return to normal bowel frequency (<3 loose or watery stools per day) for at least 48 h. If patients developed a subsequent episode of *C. difficile*–positive diarrhea after the antibiotics were discontinued and before the end of the 2-month follow-up, we considered those patients to have study drug failure [14].

Stool samples were collected at enrollment, at the end of standard antibiotic therapy, at the end of the 4-week blinded study drug treatment, at the end of the 4-week follow-up period, and when there was any suspected recurrence of CDD. *C. difficile* was assayed with the use of standardized culture methods (prereduced taurocholate-enriched broth and cycloserine cefoxitin fructose agar plates) and with assays for toxin A and toxin B [15, 16]. Toxin A was detected by use of commercial ELISA kits (Premier Kit, Meridian Diagnostics, Cincinnati, OH). Toxin B was detected by means of the standard cytopathic cell culture assay [16]. In addition, the stool specimens from the enrollment episode and from any suspected recurrences were assayed for other enteric pathogens (*Salmonella* species, *Shigella* species, *Escherichia coli* O157:H7, or *Campylobacter* species) and for fecal WBCs with the use of standard assays [17].

The significance of the differences between continuous variables was assessed by the Student *t* test; if the variances were significantly different, a separate variance estimate was calculated. Nonparametric data were analyzed by means of the Mann-Whitney ranked sum test. Significant differences between nominal variables were assessed by χ^2 analysis or Fisher exact test, by use of EPISTAT software (Gustafson, Round Rock, TX). Two-tailed tests of significance were used for all tests at a level of $P \leqslant .05$.

Results

Patient characteristics. From August 1993 through December 1996, 209 patients were initially enrolled in the study; 41 did not meet the definition of recurrent CDD or failed to fulfill inclusion criteria. There were no statistical differences in eligible and ineligible groups with regard to study center, sex, blinded study drug assignment, outcome, or severity of CDD. Of the 168 eligible patients, 32 patients were prescribed high-dose vancomycin, 83 were given low-dose vancomycin, and 53 were given metronidazole. The patient characteristics, according to the type of antibiotic treatment used, are shown in table 1.

There were no significant differences between the 3 antibiotic groups with regard to patient characteristics or assignment to

Table 2. Study center comparison of characteristics of and outcomes for patients with recurrent *Clostridium difficile* disease (CDD) at 4 study centers.

Variable	Patients with CDD at centers in			
	Seattle (n= 86)	Lexington (n = 41)	Ann Arbor (n = 8)	New York City (n = 33)
Antibiotic group				
High-dose vancomycin	19 (22)	10 (24)	1 (13)	2 (6)
Low-dose vancomycin	44 (51)	18 (44)	7 (87)	14 (42)
Metronidazole	23 (27)	13 (32)	0 (0)	17 (51)[a]
Blinded study drug				
Saccharomyces boulardii	45 (52)	24 (58)	5 (62)	16 (48)
Placebo, no. patients	41	17	3	17
Outcome				
CDD recurrence	36 (42)	16 (39)	6 (75)	18 (54)
No CDD recurrence, no. patients	50	25	2	15
Age in years, mean ± SD	60 ± 20	66 ± 17	45 ± 15[a]	72 ± 13[a]
Sex, female	69 (80)	24 (58)	6 (75)	22 (67)
Severity of CDD episode				
Diarrhea	63 (73)	25 (61)	5 (62)	28 (85)
Colitis, no. patients	20	15	3	4
Pseudomembranous colitis, no. patients	3	1	0	1
Total days in study, mean ± SD	60.9 ± 6.9	60.1 ± 8.7	62.6 ± 1.8	58.1 ± 11.5

NOTE. Data are no. (%) of patients, unless otherwise noted.
[a] $P < .05$ compared with Seattle center.

the blinded study drug. Patients who were given high-dose vancomycin were found to have more-severe CDD (defined either by the presence of colitis or pseudomembranous colitis or by the presence of fecal leukocytes). There were no significant differences between antibiotic groups when CDD severity was measured by stool frequency or by the presence of significant fever. Because blood samples were not obtained for this study, the value of other risk factors reported in the literature, including leukocytosis and hypoalbuminemia, could not be determined [18–20]. Patients who were given metronidazole had significantly higher rates of persistence of *C. difficile* at the end of antibiotic therapy than did patients who were given high-dose vancomycin. There were no other significant differences between the 3 antibiotic groups, with the exception of the efficacy of *S. boulardii.* When study center populations were compared at study entry, 1 site (New York City) prescribed more metronidazole and 2 sites had different mean age distributions; however, these 2 factors had no significant impact on the rate of recurrence of CDD (table 2). No other significant differences were found, by study site, with regard to recurrence rates, assignment to blinded study drug, severity of CDD, or frequency of enrolled female patients.

Of the 168 patients with recurrent CDD, 6 (3.6%) were culture positive but toxin negative for the stool sample collected at enrollment. All 6 patients had previous episodes of CDD with positive toxin A or B assays and did not have other etiologies of diarrhea (i.e., enteric pathogens or medications) present at enrollment. One patient with a stool specimen positive for culture but negative for toxins had endoscopic evidence characteristic of CDD colitis. The remaining 162 patients (96.4%) had detectable toxin A or B present in their enrollment stool samples.

High-dose vancomycin group. Of the 46 patients who had been prescribed high-dose vancomycin by their physician, 14 were ineligible for the following reasons: 3 had competing etiology of diarrhea, 3 were *C. difficile* negative, 2 had taken exclusion drugs, 2 had an initial episode of diarrhea, 1 had received no inciting antibiotic, and 1 had no diarrhea. We were unable to evaluate the other 2 ineligible patients because one died and because the other had unresolved diarrhea throughout the study. There were no significant differences for any variables examined among the patients comprising the eligible and ineligible groups. Various factors were examined for potential confounding of the association between treatment and outcome, but no significant differences were noted for patients randomized to receive *S. boulardii,* compared with those randomized to receive placebo, with regard to mean age, sex, medical history variables, number of previous CDD episodes, severity of illness, type or number of inciting antibiotics, or severity of CDD (table 3).

Efficacy. *S. boulardii* significantly reduced the frequency of recurrence of CDD when combined with high-dose vancomycin, but it had no effect when combined with either a 10-day course of low-dose vancomycin or metronidazole (table 1). As shown in figure 1, 3 (16.7%) of the 18 patients receiving high-dose vancomycin and *S. boulardii* had a recurrence of CDD, compared with 7 (50%) of 14 patients receiving high-dose vancomycin and placebo ($P = .05$, by the Fisher exact test). The percentage of CDD that was prevented by *S. boulardii* in combination with high-dose vancomycin was 66.6%. The time of recurrence was usually shortly after the antibiotic treatment was discontinued (median time to recurrence after discontinuation of the antibiotic, 12 days [for patients receiving *S. boulardii*] and 7 days [for patients receiving placebo]).

Table 3. Characteristics of patients with recurrent *Clostridium difficile* disease (CDD) receiving high-dose vancomycin (10 g/day) and randomized to receive either *Saccharomyces boulardii* or placebo.

Parameter	Patients with recurrent CDD receiving: High-dose vancomycin and *S. boulardii* ($n = 18$)	Patients with recurrent CDD receiving: High-dose vancomycin and placebo ($n = 14$)	Statistical significance
Enrollment CDD episode			
Stools per day, mean no. ± SD	7.5 ± 4.1	5.9 ± 2.8	$t = 1.23$, $P = .19$
Abdominal pain	12 (71)	10 (77)	$P = .51$
Fever (oral temperature >37.8°C)	2 (11)	3 (21)	$P = .37$
Uncomplicated diarrhea	10 (56)	7 (50)	$\chi^2_1 = 1.33$
Colitis or pseudomembranous colitis	8 (44)	7 (50)	$P = .51$
At enrollment			
Age, mean y ± SD	61.8 ± 20.0	60.9 ± 15.7	$t = 0.14$, $P = .89$
Sex, female	10 (55.6)	9 (64.3)	$\chi^2_1 = 0.01$, $P = .89$
Prior surgeries, mean no. ± SD	0.4 ± 0.8	0.6 ± 0.6	$t = 0.93$, $P = .36$
Prior CDD episodes, mean no. ± SD	2.5 ± 1.3	2.4 ± 1.2	$t = 0.28$, $P = .78$
Medical conditions, mean no. ± SD	2.5 ± 3.0	1.7 ± 1.7	$t = 0.88$, $P = .39$
Outpatient	14 (77.8)	9 (64.3)	—
Inpatient	4 (22.2)	5 (35.7)	$P = .33$
During study			
Intervening antibiotics,[a] mean no. ± SD	0.4 ± 0.7	0.2 ± 5.8	$t = 0.99$, $P = .33$
Medications, mean no. ± SD	3.9 ± 3.2	4.3 ± 2.4	$t = 0.45$, $P = .65$

NOTE. Data are no. (%) of patients, unless otherwise noted, and % is based on the number of patients for whom data were available.

[a] Intervening antibiotics were those given after enrollment and before the end of the follow-up period for infections other than *C. difficile*.

Patients receiving high-dose vancomycin and *S. boulardii* also experienced fewer recurrences during the trial (3 patients given *S. boulardii* had only 1 recurrence, whereas of those receiving placebo, 3 patients had 1 recurrence and 4 had 2 recurrences). The number of recurrences in these patients was 0.17 ± 0.38 (mean ± SD), compared with 0.78 ± 0.89 among patients receiving high-dose vancomycin and placebo (t test = 2.4; $P = .03$).

C. difficile *clearance.* Treatment with high-dose vancomycin completely cleared *C. difficile* by the end of the antibiotic treatment (as measured by stool culture and by toxin A and toxin B). Treatment with high-dose vancomycin did not prevent the regrowth of *C. difficile* in patients at the end of 1 month of administration of *S. boulardii* (27% of patients were positive for toxin B) or placebo (18% were positive for toxin B) ($P = .49$). Of the 21 patients providing stool samples at the end of the 8-week trial, there was no significant difference in *C. difficile* positivity for patients who had been receiving *S. boulardii* (9.1%) or placebo (33.3%; $P = .2$).

Of 29 patients who were successfully followed for the 5 months after the 8-week study, 3 of 13 (23%) patients who had been receiving placebo had a recurrence of CDD, whereas none of the 16 patients who had been receiving *S. boulardii* experienced recurrences of infection ($P = .08$).

Adverse reactions. While patients were taking the study drug, no significant differences were found in the number of adverse reactions reported by the 18 patients taking *S. boulardii* (number of reactions [mean ± SD], 1.9 ± 1.8) compared with those reported by the 14 patients taking placebo (number of reactions [mean ± SD], 2.0 ± 1.7; $t = 0.09$; $P = .93$). No specific type of adverse reaction was more common in patients taking *S. boulardii* than in those receiving placebo, nor were any significant adverse reactions found during the 4-week follow-up period.

Discussion

The results of this exploratory study showed that, in patients with a prior history of CDD, combination treatment with *S. boulardii* and high-dose vancomycin was 67% more effective in preventing further CDD recurrences than was treatment with high-dose vancomycin alone. Treatment for recurrent CDD has been a dilemma for clinicians since its first description in the medical literature. Patients with recurrent CDD represent a distinct subgroup of patients who acquire *C. difficile* [5, 14]. The initial episode of CDD is usually nosocomially acquired, and 80% of patients are successfully cured with either vancomycin or metronidazole therapy [4]. However, in ~20% of patients, the initial episode is followed by a recurrence, and these patients tended to have recurrences repeatedly [1, 13]. This pattern of repeating episodes has been previously reported to persist for up to 4 years, regardless of subsequent antibiotic regimens [13, 21, 22]. These repeated episodes of disease are debilitating and expensive, and the affected individuals often become frustrated and depressed by the refractory illness [5, 14].

Previous trials have reported that neither the choice of vancomycin or metronidazole nor the specific doses given have any proven efficacy in the treatment of patients with the recurrent form of CDD [1, 4, 20, 23]. Most recurrences occur shortly

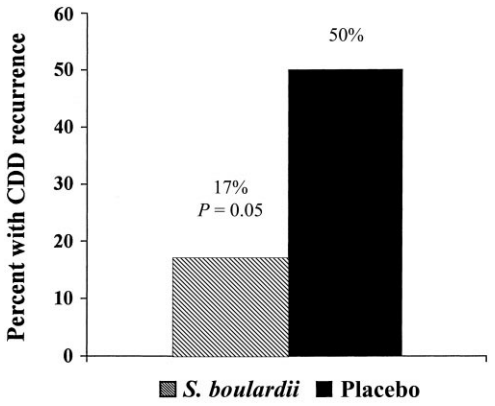

Figure 1. Frequency of a subsequent recurrence of *Clostridium difficile* disease (CDD) after treatment with high-dose vancomycin (2 g/day for 10 days) and either *Saccharomyces boulardii* or placebo (1 g/day for 28 days) in adult patients with active CDD.

after antibiotics have been discontinued. One hypothesis is that residual *C. difficile* spores, which are not susceptible to antibiotics, may then germinate, resulting in *C. difficile* overgrowth and subsequent recurrence of diarrhea. Even if all vegetative *C. difficile* cells are cleared from the colon by the end of the antibiotic regimen (as was seen in the group receiving high-dose vancomycin), the remaining spores may be sufficient to cause a recurrence of disease.

S. boulardii has been shown to produce a protease that specifically inactivates *C. difficile* toxin receptors [24, 25]. Once these toxin receptor sites have been acted upon by the *S. boulardii* protease, even the regrowth of *C. difficile* is not able to produce disease, because the toxins cannot attach to the inactivated receptor sites [25]. It may be that *S. boulardii* cannot inactivate receptor sites if the bound toxin is already present. This may explain why *S. boulardii* did not significantly reduce rates of recurrence in patients following 10-day treatment regimens with low-dose vancomycin or metronidazole, because *C. difficile* was not completely cleared in these patients. The ability of *S. boulardii* to prevent toxin binding may allow sufficient time for the restoration of normal intestinal microflora and the reestablishment of colonization resistance.

This present study also confirms results from a previous trial testing the use of *S. boulardii,* even though the design of the previous study did not specify the dosage of the antibiotic and the duration of antibiotic treatment and even though its blinded study drug overlapped by more days than did that of the current study [13]. This article analyzed the antibiotics, according to mean dose, for all patients and found no significant differences [13]. When the data for the patients with recurrent CDD in the previous study were stratified according to the dosage of antibiotics, high-dose vancomycin (2 g/day) was found to have completely eradicated *C. difficile* by the end of antibiotic therapy (mean number of days of antibiotic treatment, 16 ± 5 days), and when combined with *S. boulardii,* high-dose vancomycin reduced the rate of recurrence to 0%, compared with its rate of recurrence when used in combination with placebo (50%). *S. boulardii* also was found to reduce the frequency of CDD recurrences when it was given in combination with lower doses of vancomycin; however, the duration of antibiotic treatment was longer.

Lower doses of vancomycin (500 mg/day) were found to significantly reduce recurrences of CDD if used in combination with *S. boulardii* (21%), rather than with placebo (62%; $P = .01$), but the mean duration of low-dose vancomycin was longer (23 ± 24 days) than that observed the present study (10 days). At the end of a mean of 23 days of a low-dose vancomycin regimen, 12% of patients remained positive for *C. difficile.* Metronidazole treatment (mean, 1 g/day) was not found to be effective when combined with either *S. boulardii* (recurrences in 45% of patients) or placebo (recurrences in 33%), despite a longer duration of treatment (mean duration ± SD, 20 ± 11 days), and it failed to clear *C. difficile* at the end of therapy (31% of patients remained positive for *C. difficile*).

Another significant difference between the 2 studies is the time of overlapping treatment with the antibiotic and *S. boulardii.* In the previous study, treatment with the antibiotic and *S. boulardii* overlapped for a median of 8 days, whereas in the present study, the median time of overlapping treatment was only 4 days. Thus, it appears that both components of the investigational combination treatment—clearance of *C. difficile* at the end of the antibiotic therapy and inactivation of the toxin receptor site by *S. boulardii* before residual spores can germinate and overgrow in the colon—may be required for effectiveness.

Limitations of the present study include an inability to randomize the choice of standard antibiotic, which resulted in a low number of patients who received high-dose vancomycin. The choice of antibiotic was made by the enrolling physician, on the basis of the patient's previous history of exposure, tolerance to metronidazole, and severity of CDD. In addition, the referral nature of the design meant that patients had already started antibiotic therapy before study coordinators were notified. High-dose vancomycin is usually reserved for patients with severe CDD, because of recent recommendations to limit the use of vancomycin as a result of the emergence of vancomycin-resistant enterococci as well as of the high cost of vancomycin itself [1, 26]. In the present study, physicians reserved the use of high-dose vancomycin for the more severely ill patients with colitis or pseudomembranous colitis.

The results of this study may not apply to all patients with CDD, because the patient population in this study may represent cases of the most intractable and difficult recurrent CDD. The effect of persistence of *C. difficile* at the end of antibiotic treatment should also be further investigated as a predictor of efficacy of combination treatments (antibiotics and biotherapeutic agents), as should other factors that may predict treat-

ment failures (immune status and presence of spores). Biotherapeutic agents are emerging as a valuable adjunctive therapy for the difficult syndrome of recurrent CDD.

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