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Clostridium Difficile Colonization in Hematopoietic Stem Cell Transplant Recipients: A Prospective Study of the Epidemiology and Outcomes Involving Toxigenic and Nontoxigenic Strains

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A B S T R A C T

Clostridium difficile is a leading cause of infectious diarrhea in hematopoietic stem cell transplant (HSCT) recipients. Asymptomatic colonization of the gastrointestinal tract occurs before development of C. difficile infection (CDI). This prospective study examines the rates, risk factors, and outcomes of colonization with toxigenic and nontoxigenic strains of C. difficile in HSCT patients. This 18-month study was conducted in the HSCT unit at the Karmanos Cancer Center and Wayne State University in Detroit. Stool samples from the patients who consented for the study were taken at admission and weekly until discharge. Anaerobic culture for C. difficile and identification of toxigenic strains by PCR were performed on the stool samples. Demographic information and clinical and laboratory data were collected. Of the 150 patients included in the study, 29% were colonized with C. difficile at admission; 12% with a toxigenic strain and 17% with a non-toxigenic strain. Over a 90-day follow-up, 12 of 44 (26%) patients colonized with any C. difficile strain at admission developed CDI compared with 13 of 106 (12%) of patients not colonized (odds ratio [OR], 2.70; 95% confidence interval [95% CI], 1.11 to 6.48; $P = .025$). Eleven of 18 (61%) patients colonized with the toxigenic strain and 1 of 26 (4%) of those colonized with nontoxigenic strain developed CDI (OR, 39.30; 95% CI, 4.30 to 359.0; $P < .001$) at a median of 12 days. On univariate and multivariate analyses, none of the traditional factors associated with high risk for C. difficile colonization or CDI were found to be significant. Recurrent CDI occurred in 28% of cases. Asymptomatic colonization with C. difficile at admission was high in our HSCT population. Colonization with toxigenic C. difficile was predictive of CDI, whereas colonization with a non-toxigenic C. difficile appeared protective. These findings may have implications for infection control strategies and for novel approaches for the prevention and preemptive treatment of CDI in the HSCT patient population.

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INTRODUCTION

Diarrhea is a major cause of morbidity in hematopoietic stem cell transplant (HSCT) recipients [1]. The etiology of diarrhea in this population is often multifactorial, including gastrointestinal graft-versus-host disease (GVHD), adverse effects of chemotherapy, and infections. Recent single-center retrospective studies suggest Clostridium difficile infection (CDI) is an important cause of infectious diarrhea in HSCT patients, with rates of 10% to 24% [1-4] and a 1-year incidence of 9.2% [5]. Furthermore, about 20% of the general

hospitalized patients [6] and HSCT patients [5] will develop recurrence of CDI despite therapy. However, the epidemiology of CDI has been evolving, with a significant increase in the incidence and severity of CDI in the last decade, in part due to the emergence of an easily transmissible and virulent North American pulsed-field gel electrophoresis type 1 (NAP1) strain of *C. difficile* [7,8]. The impact of the NAP-1 strains on the rates of CDI in the HSCT population is unknown.

Asymptomatic colonization with toxigenic *C. difficile* precedes symptomatic CDI. Colonization is common among hospitalized patients, with progression to CDI upon disruption of the enteric microbiome after antibiotic therapy [9]. Asymptomatic carriage of *C. difficile* may also contribute to nosocomial transmission [10-13]. A recent multicenter prospective study of general hospitalized patient population reported a *C. difficile* colonization rate of 4.4% at admission and 3% after hospitalization [14]. Colonization was associated with recent hospitalization, prolonged length of hospital stay, and exposure to chemotherapy and proton pump inhibitors (PPI) and H2 blockers—factors prevalent in the HSCT population [14]. Two recent studies in HSCT recipients using enzyme immunoassay and PCR assays have reported rates of colonization with toxigenic *C. difficile* of 10.7% and 39% [3,4]. Early studies suggest colonization with nontoxigenic strains of *C. difficile* might protect against colonization with toxigenic strains of *C. difficile* and subsequent CDI [15]. However, rates of colonization with nontoxigenic *C. difficile* and comparative outcomes associated with colonization with toxigenic and nontoxigenic strains have not been reported in the HSCT population.

A prior study done at our institution reported a 9-fold higher rate of CDI in hospitalized HSCT patients compared with the general patient population (24 of 10,000 patient days versus 2.6 of 10,000 patient days) [16]. Given the high rates of CDI and limited data on the epidemiology of *C. difficile* colonization and infection in HSCT patients, this prospective study aims to do the following: (1) report rates of colonization and infection with toxigenic and nontoxigenic *C. difficile* in hospitalized HSCT patients using culture and PCR testing, (2) evaluate relevant risk factors associated with colonization and infection, and (3) examine outcomes associated with colonization and infection.

PATIENTS AND METHODS

Study Population

The study was conducted at the Bone Marrow Transplant (BMT) inpatient unit at the Karmanos Cancer Institute and Wayne State University in Detroit, Michigan. All HSCT patients admitted to the BMT unit between December 1, 2010 and June 31, 2012 were invited to participate in the study. We included all HSCT recipients admitted during the study period regardless of the time from HSCT. Patients who signed informed consent for the study were requested to provide a stool sample within 72 hours of admission and weekly thereafter until discharge from the hospital. Patients were excluded if they were diagnosed with CDI within 72 hours of hospital admission or if they were unable to provide a stool sample within 72 hours of admission. The study was approved by the institutional review board at Wayne State University, Detroit, Michigan.

Study methodology

In order to detect colonization with *C. difficile* the study was designed to collect a stool specimen at admission and then weekly thereafter as long as the patient remained in hospital. No samples were collected for the study after hospital discharge. The research assistant obtained weekly stool samples for the study as per study schedule. The study stool samples were tested in the research laboratory using stool cultures followed by *C. difficile* toxin PCR. This was done in order to detect colonization with toxigenic and non-toxigenic strains of *C. difficile*. The results were not provided to the clinicians.

The treating physician and other providers ordered stool testing as clinically indicated if *C. difficile* infection was suspected. These samples were tested in the hospital clinical microbiology laboratory using *C. difficile* toxin PCR.

Microbiology Methodology

The stool samples were stored at −70°C and batched for testing. The testing of study samples was performed in the research laboratory. Stool samples were inoculated on cycloserine cefoxitin fructose agar enriched with horse blood and incubated at 35°C under anaerobic conditions. The presumptive identification of *C. difficile* was made after colonies with the characteristic yellow/off-white, ground-glass morphology grown of cycloserine cefoxitin fructose agar enriched with horse blood showed gram positive/gram variable bacilli in Gram stain.

The samples that were culture positive for *C. difficile* were further tested by PCR to identify toxigenic strains. EasyMag instrument (BioMerieux, Durham, NC, USA) was used for DNA extraction from stool samples before PCR. Briefly, 500-μL lysis buffer was added to 500 μL of liquid stool sample and then allowed to sit for 10 minutes. The sample was centrifuged at 16,000 rpm for 1 minute. Then, 200 μL of cleared lysate supernatant was extracted and the DNA was eluted into 50 μL of elution buffer. Detection of toxigenic strains of *C. difficile* was performed by using the LightMix Kit Clostridium difficile (TIB Mol Biol, Adelphia, NJ, USA) and LightCycler Fast-Start DNA Master HybProbe (Roche Diagnostics, Indianapolis, IN, USA) on the LightCycler 1.2 instrument as recommended by the manufacturer. A 176-bp fragment of the *C. difficile* tcdC gene and a 158-bp fragment of the 18bp deletion found in mutant *C. difficile* del. strains BI/NAP1/027 (ribotype 027) were amplified. The resulting PCR fragments were analyzed with hybridization probes labeled with Roche LightCycler Red 640 (channel 640). The distinction between *C. difficile* del and wild-type *C. difficile* was made by melting temperature analysis of the PCR products. The *C. difficile* del DNA exhibits a melting temperature of 65°C in channel 640. *C. difficile* shows a broader melting profile, between 55°C and 65°C. The PCR reaction is monitored by an additional PCR product of 349bp formed from the internal control. The supplied vials of *C. difficile* DNA with known concentrations permitted estimation of the quantity of the *C. difficile* DNA in the samples (linear measuring range of the assay is 100 to 1,000,000 copies *C. difficile* DNA). The study results were not utilized for patient care.

Study Design

A prospective cohort study was performed. All HSCT patients colonized with *C. difficile* at admission were considered cases and patients not colonized with *C. difficile* were the controls. Demographic, clinical, and laboratory data and outcomes were collected from review of the electronic medical records. Patient data were reviewed for the 30 days preceding admission to the BMT unit to identify potential risk factors for *C. difficile* colonization and infection. Similarly, patient data were reviewed for 90 days after study enrollment to evaluate outcomes. The risk factors evaluated included prior CDI, recent hospitalization or clinic visit, and use of antibiotics and PPI. We also reviewed variables related to the HSCT, including indication, type of transplantation, and presence of GVHD. The Charlson comorbidity index was used to grade the severity of comorbid illnesses and the Karnofsky score was used to assess performance status. In patients who developed CDI, the severity of CDI, treatment used for CDI, relapse and recurrence rates, number of recurrences, and time to recurrence were evaluated. The outcomes evaluated included occurrence of CDI in patients colonized with *C. difficile*; in those with CDI, outcomes examined included intensive care unit admission, need for colectomy, or death related to CDI.

Definitions

C. difficile colonization was defined as isolation of *C. difficile* from stool specimen on culture in a patient without diarrhea. *CDI* was defined as presence of diarrhea confirmed with positive stool PCR for *C. difficile* done in the clinical laboratory. Testing for *C. difficile* was performed at the discretion of the treating physician. Per laboratory protocol, only diarrheal stools were accepted for *C. difficile* testing. *Recurrent CDI* was defined as a new onset of diarrhea and a *C. difficile*−positive stool PCR assay within 90 days of previous CDI. There is no standardized definition of severe CDI; however, several parameters associated with severe disease are white count > 15,000 cells/mm^3, elevation of serum creatinine >1.5 times the baseline, abdominal distension, and low albumin [17,18]. Because several of these parameters may be abnormal in patients with HSCT, for the purposes of this study, *severe CDI* was defined as the presence of the following: CDI necessitating admission to an intensive care unit or resulting in colectomy or death within 30 days after disease onset [19]. *Neutropenia* was defined as absolute neutrophil count less than 500 cells/mm^3. *Lymphopenia* was defined as absolute lymphocyte count less than 300 cells/mm^3. Only biopsy-proven cases of GVHD of the gastrointestinal tract were included.

Statistical Methodology

Data analyses were performed using SPSS version 21 (IBM, Armonk, NY). Chi-squared and Fisher exact tests were used to compare categorical variables among patients colonized with *C. difficile* versus patients not colonized with *C. difficile* and among patients colonized with toxigenic *C. difficile* strains versus patients colonized with nontoxigenic strains. *T*-test and Wilcoxon rank-sum test were used to analyze continuous variables among the different groups. Variables with a *P* value of less than .10 in bivariate analyses were included as candidates for multivariate analyses. Logistic regression with backwards selection was performed to select for variables in the final model. Variables excluded in the backwards selection model that changed the β-coefficients of selected variables by >10% were considered confounders and were added back to the model. All *P* values were 2 sided and used a 5% level of significance.

RESULTS

Study Patients

During the 18-month study period, a total of 533 HSCT patients were admitted to the BMT unit. Of these, 21 patients were not eligible as they had diarrhea, were diagnosed with community-onset CDI, or had CDI diagnosed within 72 hours

of admission to the unit. Of the remaining eligible 512 patients, 157 agreed to participate in the study. Seven patients were excluded, as they could not provide a stool sample for testing within 72 hours of admission. The 150 evaluable patients provided a total of 430 stool samples for testing. The percentage of patients who provided sequential weekly stool samples for testing were as follows: at admission, 100%; week +1, 67%; week +2, 48%; week +3, 32%; week +4, 16%; week +5, 13%; and week +6, 7%. No samples were collected for the study after hospital discharge. Hence the drop off rate reflects patients being discharged from the hospital. 100% of enrolled patients provided a sample at admission and all enrolled patients provided weekly stool samples as long as they remained in hospital.

Rates and Outcomes

Rates and potential risk factors

Of the 150 patients included in the study, 44 (29%) were found to be *C. difficile* culture positive at the time of
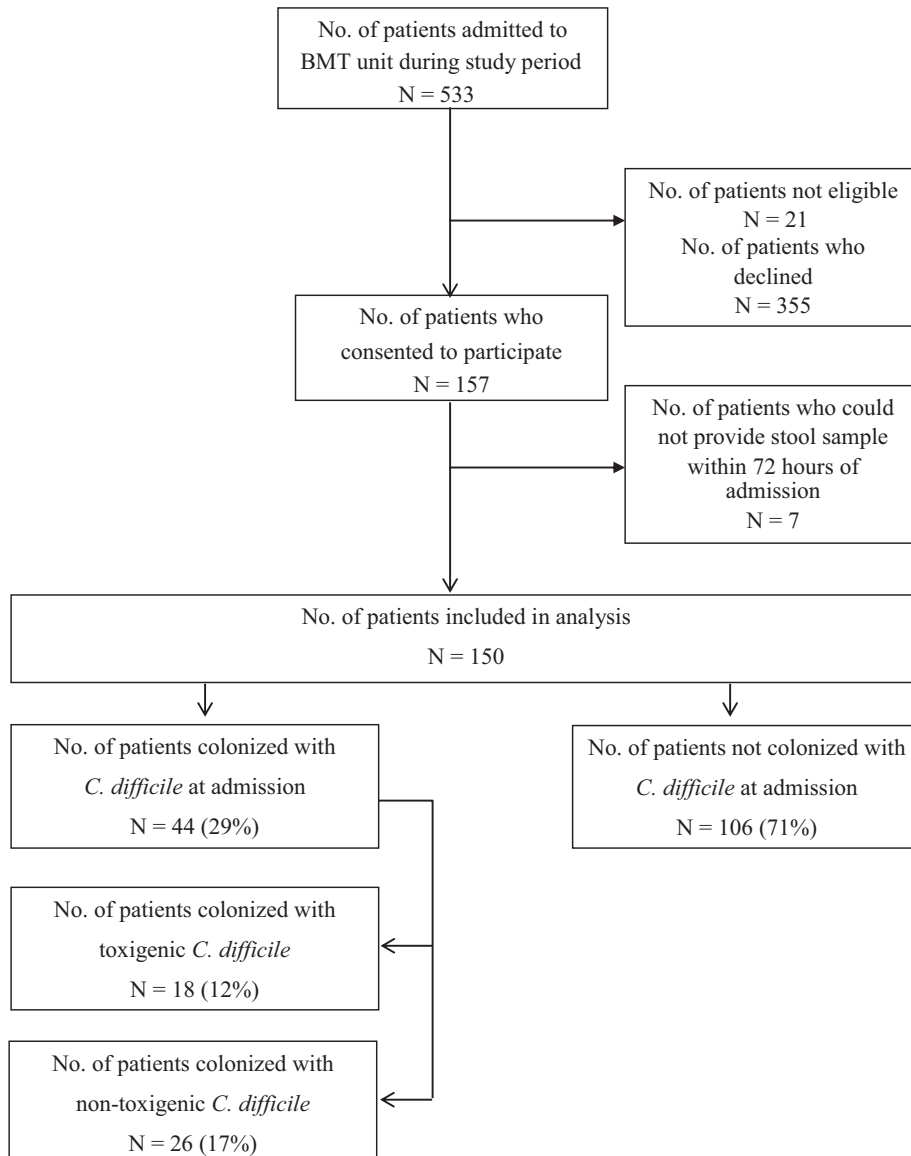

Figure 1. Study enrollment and colonization status at admission.

Table 1
Characteristics of HSCT Patients with and without *C. difficile* Colonization at Admission

Variable	Colonized Patients n = 44	Noncolonized Patients n = 106	OR	95% CI	P Value
Age, mean (SD), yr	50.95 (±12)	51.07 (±13)			.96
Female gender	26 (59)	35 (33)	2.93	1.42-6.05	.003
Ethnicity					
White race	29 (66)	73 (69)			
Black race	4 (9)	11 (10)	.92	.27-3.11	.89
Other	11 (25)	22 (21)	1.26	.54-2.92	.59
Charlson score ≥ 3	17 (39)	53 (50)	.63	.31-1.29	.20
Karnofsky score ≥ 70	39 (88.6)	94 (88.7)	.99	.33-3.02	.99
Prior hospitalization[*]	15 (34)	28 (26)	1.44	.68-3.08	.34
Prior clinic visit[*]	42 (95.5)	98 (92.5)	1.71	.35-8.42	.50
Prior CDI[*]	3 (7)	15 (14)	.44	.12-1.62	.21
Antibiotic use[*]	33 (75)	76 (72)	1.18	.53-2.64	.68
PPI use[*]	19 (43)	59 (56)	.61	.30-1.23	.16
Steroid use[*]	18 (41)	43 (41)	.99	.49-2.04	.99
Immunosuppressant use[*]	31 (70.5)	80 (76)	.75	.34-1.64	.46
Indication for HSCT					
Leukemia	31 (70.5)	76 (72)	.94	.43-2.04	.88
Lymphoma	10 (22.7)	24 (22.6)	1.01	.43-2.33	.99
Solid tumor	6 (13.6)	14 (14)	.96	.35-2.65	.93
Allogeneic transplantation	43 (98)	100 (94)	2.58	.30-22.10	.37
Unrelated donor source	34 (79)	61 (62)	2.35	1.02-5.45	.042
GVHD[*]	10 (23)	24 (23)	1.01	.43-2.33	.99
Gastrointestinal GVHD[*]	8 (18)	22 (21)	1.46	.56-3.77	.43
Neutropenia[*]	10 (23)	24 (23)	1.01	.43-2.33	.99
Lymphopenia[*]	29 (66)	62 (58.5)	1.37	.66-2.86	.40
Low albumin (<2.5 g/dL)[*]	6 (14)	12 (11)	1.24	.43-3.53	.69

Data presented are n (%), unless otherwise indicated.
  [*] Assessed within 30 days before hospital admission.

admission and the remaining 106 (71%) were not colonized. Of the 44 colonized patients, 18 (41%) were colonized with toxigenic strain of *C. difficile* and 26 (59%) were colonized with nontoxigenic strain. Figure 1 summarizes the enrollment and colonization status.

Table 1 shows the baseline characteristics of the study population. Compared with the noncolonized patients, colonized patients were more commonly women (odds ratio [OR], 2.92; 95% confidence interval [CI], 1.42 to 6.05; P = .003). Unrelated donor status was the only other variable associated with *C. difficile* colonization (OR, 2.35; 95% CI, 1.02 to 5.45; P = .042). The other characteristics related to demographics, potential risk factors for *C. difficile* acquisition, and transplantation-related variables were comparable in the 2 groups. In a regression model adjusted for confounding effects of relation to donor, the only independent predictor of colonization with *C. difficile* was female gender (P = .007). Likewise, as summarized in Table 2, the demographics and other characteristics were similar in study patients colonized with toxigenic and nontoxigenic strains of *C. difficile*.

Outcomes

In the follow up-period of 90 days from study enrollment, 12 of 44 (26%) patients colonized with any *C. difficile* strain developed CDI compared with 13 of 106 (12%) of patients not colonized with *C. difficile* (OR, 2.70; 95% CI, 1.11 to 6.48; P = .025). Of the colonized patients, 11 of 18 (61%) colonized with a toxigenic strain of *C. difficile* and 1 of 26 patients (4%) colonized with a nontoxigenic strain of *C. difficile* developed CDI (OR, 39.30; 95% CI, 4.30 to 359.0; P < .001) (Table 3). In the HSCT patients colonized with toxigenic *C. difficile*, the presence of potential risk factors for CDI, including exposure to antibiotics, PPIs, and chemotherapy, were similar in those who developed CDI and those who did not.

CDI was primarily characterized by diarrhea (100%), fever (56%), and abdominal pain (32%). Leukocytosis of ≥15,000/mm³ was present in 16% patients at time of CDI (Table 4). Most patients (76%) with CDI were treated with oral vancomycin. Recurrent CDI occurred in 28% of patients within the 90-day follow-up. No cases of severe CDI occurred as defined by the study criteria, and no patients died as a result of CDI.

Of the 150 patients in the study cohort, 127 patients were enrolled in the study within 100 days of HSCT. The rate of gastrointestinal GVHD within 100 days of the transplantation in these patients was 40% (10 of 25) in those who developed CDI compared with 36% (36 of 102) in patients who did not develop CDI.

DISCUSSION

This prospective study shows that colonization with toxigenic and nontoxigenic *C. difficile* is common and present in about one quarter of our HSCT patients at the time of hospitalization. The study also demonstrates that the majority of patients colonized with toxigenic *C. difficile* will develop symptomatic CDI within 2 weeks of hospital admission. In contrast, colonization with nontoxigenic *C. difficile* may be protective against CDI.

In our HSCT population, the overall rates of colonization with *C. difficile* at hospital admission was high at 29.3%, of which 17% were due to a nontoxigenic strain and 12% due to a toxigenic strain of *C. difficile*. Rates of asymptomatic carriage of toxigenic *C. difficile* in recent studies has ranged from 0.6% in the geriatric population [20] to up to 15% in a general patient population [14,21]. Our rate of asymptomatic carriage of toxigenic *C. difficile* of 12% is comparable to rates recently reported from other HSCT centers. Alasmari et al. reported an overall *C. difficile* colonization rate of 21%, with 6% due to nontoxigenic and 15% due to toxigenic strains in HSCT patients at hospitalization [22]. Using a 2-tier assay of glutamate

Table 2
Characteristics of HSCT Patients Colonized with Toxigenic and Nontoxigenic *C. difficile*

Variable	Colonized with Toxigenic *C.difficile* n = 18	Colonized with Nontoxigenic *C. difficile* n = 26	OR	95% CI	*P* Value
Age, mean (SD), yr	51 (±13)	50.77 (±12)			.91
Female gender	8 (44)	10 (56)	.78	.23-2.65	.69
Ethnicity					
White race	13 (72)	16 (62)			Reference
Black race	0	4 (15)			.99
Other	5 (28)	6 (23)	1.03	.25-4.14	.97
Charlson score ≥ 3	7 (39)	10 (38.5)	1.02	.30-3.50	.97
Karnofsky score ≥ 70	17 (94)	22 (85)	3.10	.32-30.25	.63
Prior hospitalization*	3 (17)	12 (46)	.23	.05-1.01	.057
Prior clinic visit*	17 (94)	25 (96)	.68	.04-11.63	1.00
Prior CDI*	2 (11)	1 (4)	3.13	.26-37.36	.56
Antibiotic use*	15 (83)	18 (69)	2.22	.50-10.00	.48
PPI use*	6 (33)	3 (50)	.50	.14-1.74	.27
Steroid use*	7 (39)	11 (42)	.87	.25-2.96	.82
Immunosuppressant use*	11 (61)	20 (77)	.47	.13-1.76	.26
Indication for HSCT					
Leukemia	12 (67)	19 (73)	.74	.20-2.73	.65
Lymphoma	4 (22)	6 (23)	.95	.23-4.01	1.00
Solid tumor	2 (11)	4 (15)	.69	.11-4.22	1.00
Allogeneic HSCT	18 (100)	25 (96)	.96	.89-1.04	1.00
Unrelated donor source	14 (78)	20 (80)	.88	.20-3.85	1.00
GVHD*	2 (11)	8 (31)	.28	.05-1.52	.16
Gastrointestinal GVHD*	2 (11)	6 (23)	.16	.02-1.43	.12
Neutropenia	2 (11)	8 (31)	.28	.05-1.52	.16
Lymphopenia	10 (56)	19 (73)	.46	.13-1.64	.23
Low albumin	3 (17)	3 (11.5)	1.53	.27-8.63	.67

Data presented are n (%), unless otherwise indicated.

  * Assessed within 30 days before hospital admission.

dehydrogenase followed by enzyme immunoassay, Bruminhent et al. found that 10.7% of patients undergoing HSCT were colonized with toxigenic *C. difficile* at admission [3]. Kinnebrew et al., using a PCR assay, noted rates of colonization with toxigenic *C. difficile* of 39% during the pretransplantation conditioning and up to day 35 after HSCT, with most cases detected in the pretransplantation period [4]. As expected, rates of detection are higher at HSCT centers utilizing the more sensitive PCR assays. The sensitive PCR assays do not distinguish between colonization and CDI and may potentially overestimate cases of CDI in the HSCT population, in whom diarrhea is a common symptom. The high rates of colonization with *C. difficile* in the HSCT population, compared with the general population, may reflect the acquisition through frequent exposures to the health care environment, antibiotics, antacids, and chemotherapy. Furthermore, the underlying immune dysfunction in HSCT patients may contribute to the persistent carriage of *C. difficile*.

Studies since the 1980s have reported a significant association between colonization with *C. difficile* and older age, recent hospitalization, exposure to antibiotics, PPI, H2 blockers, chemotherapy, corticosteroids, and chronic dialysis [10,14,19,21,23,24]. None of these factors were independently associated with *C. difficile* colonization in our HSCT patients.

A similar lack of association with the traditional risk factors and colonization with *C. difficile* in HSCT recipients has been reported [22]. Furthermore, in the HSCT population, use of chemotherapy before conditioning, intensity of condition regimen, and total body irradiation have been associated with increased risk of CDI [4,5,25]. Studies have also reported GVHD as a risk for the development of CDI in HSCT recipients [5,25]. None of these factors were associated with colonization or infection with *C. difficile* in our study. It is likely that these potential risk factors, such as exposure to the health care environment, antibiotics, PPI, and chemotherapy, were so prevalent in our HSCT population they could not serve as independent predictors for *C. difficile* colonization or infection. This observation suggests that, in the HSCT population, screening based solely on clinical and epidemiological factors will not be useful in guiding targeted surveillance for *C. difficile*. In our study, women were more commonly colonized at admission. Higher rates of colonization and CDI have been noted in women, although postulated explanations include greater contact with children, more clinic visits, and more exposure to antibiotics; however, the exact reasons remain unclear [7].

Notably, over a 90-day follow-up period, 27% of asymptomatic patients colonized with *C. difficile* developed CDI

Table 3
Odds Ratios of Occurrence of *C. Difficile* Infection Based on Colonization Status at Time of Hospital Admission

	Colonized with *C. difficile* n = 44	Not Colonized with *C. difficile* n = 106	OR (95% CI)	*P* Value	Colonized with Toxigenic *C. difficile* n = 18	Colonized with Nontoxigenic *C. difficile* n = 26	OR (95% CI)	*P* Value
Occurrence of CDI, n (%)	12 (27%)	13 (12%)	2.70 (1.11-6.48)	.025	11 (61%)	1 (4%)	39.30 (4.30-359.0)	<.001
Time from admission to CDI, median [IQR], d	12 [7-25.25]	12 [7.5-19.5]		.89	11 [7-23]	26 [26-26]		.50

IQR indicates interquartile ratio.

Table 4
Characteristics and Outcomes of Patients with *C. difficile* Infection (n = 25)

Characteristic(s)	Value
Clinical features	
Diarrhea	25 (100)
Fever	14 (56)
Abdominal pain	8 (32)
Treatment	
Metronidazole	4 (16)
Vancomycin	19 (76)
Both	2 (8)
Recurrent CDI	7 (28)
<30 Days	4 (57% of recurrence)
30-90 Days	3 (43% of recurrence)
Complications of CDI	
CDI-related colectomy	0 (0)
ICU admission for CDI	0 (0)
30-Day mortality	0 (0)

ICU indicates intensive care unit.

(OR, 2.7; 95% CI, 1.11 to 6.48; $P = .025$) and 61% of patients colonized with toxigenic *C. difficile* at admission developed CDI (OR, 39.30; 95% CI, 4.30 to 359.0; $P < .001$), at a median of 12 days. Although an early study reported asymptomatic carriage of *C. difficile* was associated with a low risk (1%) of CDI [26], more recent studies in the general population have reported rates of CDI ranging from 14% to 51% over 5 to 18 months of follow-up in patients colonized with toxigenic *C. difficile* [23,27-29]. The high OR signifying increased risk of CDI in our patients colonized with toxigenic *C. difficile* is comparable to ORs of 68.5% [3] and 17.6% [4] reported in the 2 recent studies. The high rate of CDI in HSCT patients colonized with toxigenic *C. difficile* might reflect the underlying immunocompromised state of these patients, as well as exposure to several other risk factors, such as antibiotics, PPIs, and chemotherapy. The attenuated immune response and prevalence of hypogammaglobulinemia in HSCT patients may contribute to development of CDI. Higher concentrations of IgA and IgM were reported in asymptomatic carriers of *C. difficile* compared with patients with symptomatic CDI [30]. Furthermore patients who developed higher levels of IgG to *C. difficile* toxin A after colonization with *C. difficile* were more likely to remain asymptomatic carriers rather than develop CDI [27,31,32]. A phase 2 trial examining the clinical effectiveness of a human monoclonal antibody to *C. difficile* toxin A and B, to prevent recurrent CDI, has just been completed [33].

Strikingly, only 1 (4%) patient colonized with nontoxigenic strain developed CDI days during the 90-day follow-up period, compared with 61% patients colonized with toxigenic *C. difficile*. A similar observation was noted in a recent study by Hung et al. with rates of CDI over an 18-month follow-up of 14%, 0%, and .9% in general patients colonized with toxigenic *C. difficile*, nontoxigenic *C. difficile*, and no colonization, respectively [29]. The mechanism of the protective effect is unknown, but a plausible explanation is that nontoxigenic *C. difficile* competes with toxigenic strains and inhibits their colonization. This has important clinical implications, as colonization with nontoxigenic *C. difficile* may be protective towards development of CDI in HSCT population. This protective effect has been described in hamster models [34,35]. In healthy adult human subjects, Villano et al. reported good tolerance to non-toxigenic *C. difficile* VP20621/NTCD-M3 spores in a recent phase 1 study [15]. A follow-up phase 2 randomized controlled study reported that oral administration of spores of NTCD-M3 strain of non-toxigenic *C. difficile*, successfully colonized the gastrointestinal tract of patients treated for CDI and significantly reduced CDI recurrence [36].

Clinical CDI features in HSCT population were similar to generally reported symptoms of diarrhea, fever, and abdominal pain. Severe CDI and mortality rates of up to 9% have been reported in general population from community-acquired CDI [37,38], with higher death rates in the older population. However, severe CDI, as defined by McDonald et al. [39] was not found in any patient in our study. This is similar to previously reported low occurrence of severe CDI in the HSCT population, both allogeneic and autologous [1,4,40]. In our study population, 22 out of the 25 CDI patients were treated with vancomycin or both vancomycin and metronidazole as first-line therapy for the first episode of CDI. It is unclear if the use of vancomycin as initial therapy might have resulted in a more benign course of disease.

Recurrence within 90 days was noted in 6 of 25 (24%) patients in our study. Of these, 4 (16%) recurred within 30 days of first episode and 2 (8%) had symptoms after 30 days. The recurrence rate of CDI varies from about 8% to 22% in studies of CDI in HSCT patients [1,3,5] and is comparable to rate of 25% reported in the general population [6]. The high rates of recurrence in our study may also be due to the longer follow-up period of 90 days rather than the usual 4 to 8 weeks of follow-up used in other studies. Newer agents, such as fidaxomicin, that are associated with fewer recurrences of CDI [6] may be preferred in the HSCT population.

Retrospective studies have reported increased risk of the development of GVHD and gastrointestinal GVHD in patients with CDI [2,5,41]. In our study, the rates of development of GVHD within 100 days of HSCT were comparable in patients with CDI and those without. However, our study had too few patients to draw any firm conclusions. Two recent prospective studies reported no association with GVHD in patients with CDI [3,4]. Given the limited data, it is yet unclear if any association exists between GVHD and *C. difficile* colonization and infection.

Our study has several limitations. The study was conducted at a single center and, hence, the findings might not be applicable to other transplantation centers. The 157 patients who consented to participate in the study comprised about 30% of the 533 eligible patients admitted to the unit during the study period. Hence, the finding might not be representative of all of our HSCT patients. The culture used for the detection of *C. difficile* in our study is not as sensitive as PCR and may not have identified all patients who were colonized with toxigenic *C. difficile* [42]. However, we used cultures as we wished to identify and assess the outcomes associated with colonization with both toxigenic and nontoxigenic *C. difficile*. Since strain typing was not performed it was not possible to confirm if the colonizing toxigenic strain was the same strain that caused CDI.

C. difficile colonization at admission is common in our HSCT population. Colonization with toxigenic strains of *C. difficile* seems predictive of subsequent CDI. Notably, colonization with nontoxigenic strains appears to be protective against CDI in HSCT patients. Severe disease CDI is uncommon in this population and no association with GVHD was evident. Larger multicenter studies are necessary to confirm these findings. High rates of asymptomatic colonization at admission, with potential for unidentified nosocomial transmission, have implications for infection control. Novel strategies for prevention of CDI such as the "preemptive" therapy of patients colonized with toxigenic

C. difficile, use of monoclonal antibodies to *C. difficile* toxin A and B, or the therapeutic use of nontoxigenic strains of *C. difficile* need to be explored in this population.

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