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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

Tania Jain [1,5], Christopher Croswell [1], Varinia Urday-Cornejo [1,6], Reda Awali [1,6], Jessica Cutright [1,6], Hossein Salimnia [1,7], Harsha Vardhan Reddy Banavasi [1,6], Alyssa Liubakka [1], Paul Lephart [2], Teena Chopra [1,3,6], Sanjay G. Revankar [1,3,6], Pranatharthi Chandrasekar [1,3,6], George Alangaden [1,3,4,6,*]

[1] *Wayne State University School of Medicine, Detroit, Michigan*
[2] *Detroit Medical center University Laboratories, Detroit, Michigan*
[3] *Karmanos Cancer Center, Detroit, Michigan*
[4] *Henry Ford Hospital, Detroit, Michigan*
[5] *Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan*
[6] *Department of Internal Medicine, Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, Michigan*
[7] *Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan*

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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 nontoxigenic 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 nontoxigenic *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

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* Correspondence and reprint requests: George Alangaden, MD, 2799 West Grand Boulevard, Clara Ford Place Suite 316, Detroit, MI 48202.
E-mail address: galanga1@hfhs.org (G. Alangaden).

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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 FastStart 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.

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