**Title:** *Clostridioides difficile* infection in pediatric patients with cancer and hematopoietic stem cell transplant recipients

**Running Head:** *C. difficile* infection in pediatric cancer

**Authors**: Gabrielle M Haeuslera-f; Thomas Lehrnbecherh; Phillip KA Agyemani,j; Robyn Lovesk; Elio Castagnolal; Andreas H Grollm; Marianne van de Weteringn; Catherine C Aftandiliano; Bob Phillipsp,q; Krishna Mohan Chirrak; Christine Schneiderj; L. Lee Dupuisk,r; Lillian Sungk,s

**Affiliations**:

aDepartment of Infectious Diseases, Royal Children’s Hospital, Melbourne, Australia

bDepartment of Infectious Diseases, Peter MacCallum Cancer Centre, Melbourne, Australia

cNHMRC National Centre for Infections in Cancer, Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia.

dMurdoch Children’s Research Institute, Parkville, Australia

eDepartment of Paediatrics, University of Melbourne, Parkville, Australia

fPaediatric Integrated Cancer Service, Victoria, Australia

hPediatric Hematology and Oncology, Hospital for Children and Adolescents, Johann Wolfgang Goethe University, Frankfurt, Germany

iDepartment of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

jDivision of Pediatric Hematology/Oncology, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

kChild Health Evaluative Sciences, The Hospital for Sick Children, 555 University Avenue, Toronto, Canada

lInfectious Diseases Unit, Department of Pediatrics, Istituto Giannina Gaslini, Genova, Italy

mInfectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Pediatric Hematology/Oncology, University Children’s Hospital, Muenster, Germany

nDepartment of Pediatric Oncology, Princess Maxima Centre, Utrecht, Netherlands

oDivision of Hematology/Oncology, Department of Pediatrics, Stanford University, Palo Alto, CA

pLeeds Children’s Hospital, Leeds General Infirmary, Leeds Teaching Hospitals, NHS Trust, Leeds, United Kingdom

qCentre for Reviews and Dissemination, University of York, Yorkshire, United Kingdom

rDepartment of Pharmacy, The Hospital for Sick Children, and Leslie Dan Faculty of Pharmacy, University of Toronto, The Hospital for Sick Children, Toronto, ON, Canada

sDivision of Haematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada

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**Corresponding Author:**

Lillian Sung MD, PhD

Division of Haematology/Oncology

The Hospital for Sick Children

555 University Avenue, Toronto, Ontario, M5G1X8

Telephone: 416-813-5287

Fax: 416-813-5979

Email: Lillian.sung@sickkids.ca

**Abstract**

**Background:** Epidemiology of*Clostridioides difficile* infection (CDI) in pediatric cancer patients is uncertain. The primary objective was to describe the prevalence of CDI outcomes among pediatric patients receiving cancer treatments. Secondary objectives were to describe clinical features of CDI, propose a definition of severe CDI and to determine risk factors for CDI clinical outcomes.

**Methods:** A multi-center retrospective cohort study that included pediatric patients (1-18 years of age) receiving cancer treatments with CDI. Severe CDI definition was achieved by consensus. Univariable and multivariable regression was conducted to evaluate risk factors for CDI outcomes.

**Results:** There were 627 eligible patients who experienced 721 CDI episodes. Prevalence of clinical cure was 82.9%, recurrence was 9.6%, global cure was 75.0% and repeated new CDI episode was 12.8%. The proposed definition of severe CDI was the presence of colitis, pneumatosis intestinalis, pseudomembranous colitis, ileus or surgery for CDI, occurring in 70 (9.7%) episodes. In univariable regression, initial oral metronidazole or initial oral vancomycin were not significantly associated with failure to achieve clinical cure or CDI recurrence. In multiple regression, oral metronidazole was significantly associated with higher odds (odds ratio (OR) 1.7, 95% confidence interval (CI) 1.0-2.7) and oral vancomycin was significantly associated with lower odds (OR 0.4, 95% CI 0.2-0.8) of repeated new episodes.

**Conclusion**: The prevalence of clinical cure was 82.9% and recurrence was 9.6% in pediatric patients receiving cancer treatments. Severe CDI, as per our proposed definition, occurred in 9.7% episodes. Initial oral vancomycin was significantly associated with a reduction in repeated new CDI episodes.

**Key Words:** *Clostridioides difficile* infection; pediatric, oncology, hematopoietic stem cell transplantation; risk factors

**Background**

*Clostridioides difficile* is a gram-positive, spore-forming bacillus that can colonize the intestinal tract of healthy individuals.[1] Toxin-producing isolates may result in symptomatic infection with clinical presentation varying from mild diarrhea to life-threatening inflammation with toxic megacolon, bowel perforation and death.[2] *C. difficile* infection (CDI) is the most common cause of healthcare-associated infectious diarrhea.[3][4] Risk factors for CDI include antibiotic exposure, older age, hospitalization[5] as well as immunosuppression and chemotherapy.[6-8]

In pediatric patients, CDI has also been associated with prolonged hospitalization and an increased risk of death.[9] Children with cancer and hematopoietic cell transplant (HCT) recipients are commonly exposed to risk factors for CDI and not surprisingly, CDI has emerged as an important healthcare-associated infection in these patients.[10]

A multi-national and multi-disciplinary panel developed a clinical practice guideline (CPG) for the management of CDI in pediatric patients with cancer and HCT recipients.[11] This CPG highlighted the absence of precise estimates of CDI outcomes including recurrence among patients receiving cancer treatments. The CPG made a strong recommendation to administer either oral metronidazole or oral vancomycin for the initial treatment of non-severe CDI and oral vancomycin for the initial treatment of severe CDI. However the definitions of severe CDI, incorporating high white blood cell count (WBC) and age >60 years, used in the predominantly adult trials that informed this CPG, are not applicable to immunocompromised pediatric patients.[12, 13]

The primary objective was to describe the prevalence of clinical cure, recurrence, global cure and repeated new CDI episodes among pediatric patients receiving cancer treatments. Secondary objectives were to describe the clinical features of CDI, propose a definition of severe CDI and determine risk factors for CDI outcomes.

**Methods**

 This multi-center retrospective cohort study included pediatric patients with cancer and HCT recipients at nine institutions: Bern University Hospital, Bern, Switzerland; Hospital for Children and Adolescents, Frankfurt, Germany; The Hospital for Sick Children, Toronto, Canada; Istituto Giannina Gaslini, Genova, Italy; Leeds Children’s Hospital, York, United Kingdom; Lucile Packard Children’s Hospital, Palo Alto, United States; Princess Maxima Centre for Pediatric Oncology, Utrecht, Netherlands; Royal Children’s Hospital, Melbourne, Australia; and University Children’s Hospital, Muenster, Germany. These institutions belong to the Umbrella Consortium, which focuses on clinical research on infectious complications in pediatric cancer and HCT patients.[14] The Research Ethics Boards of The Hospital for Sick Children and all participating institutions (where required) approved this study.

**Subjects:** Potential cases were identified from hospital microbiology databases. We included pediatric patients 1 to 18 years of age with cancer or HCT procedure with CDI as defined as diarrhea (change in stool patterns with at least three unformed bowel movements in 24 hours before screening) and detection of toxigenic *C. difficile* using ≥1 local tests (presence of toxin A or B on toxigenic stool culture, enzyme immunoassay (EIA) or polymerase chain reaction (PCR)) from stool within 72 hours of diarrhea onset. The CDI episode start date was the date the positive stool sample was collected. Episodes between January 2010 and April 2020 were included.

**Outcomes:** The primary outcomes were clinical cure, recurrence, global cure and repeated new CDI episodes. Clinical cure was defined as resolution of diarrhea (two or fewer unformed stools for two consecutive days), with maintenance of resolution by day 12 of start of CDI-directed therapy.[15, 16] Recurrence was defined as three diarrheal stools within 24 hours, a positive stool toxin test and need for retreatment by day 40 of start of CDI-directed therapy after achievement of clinical cure.[15, 16] Global cure was defined as clinical cure without recurrence.[15, 16] Repeated new CDI episodes were defined as episodes meeting CDI criteria after day 40 of start of the previous CDI-directed therapy with resolution of diarrhea after the initial episode.

**Data Collection:** Demographic data were sex, age, diagnosis, metastatic disease, relapse status, time from diagnosis, inpatient status, active treatment and HCT. Pre-episode data included the following prior to the CDI episode: neutropenia (absolute neutrophil count <500/uL) for at least 7 days, systemic chemotherapy within 28 days, gastroprotectants within 28 days and systemic, non-CDI directed antibiotics within one day prior to the episode start.

 Episode data were the number of stools per day (2 or less, 3-5, 6-9 or ≥10), macroscopic blood in stool, abdominal pain (none, mild – no opioids, moderate – opioid bolus or severe - opioid infusion), temperature, WBC, absolute neutrophil count, serum albumin and C-reactive protein. Measurements at the start of the CDI episode and the worst value during the CDI episode were recorded.

 CDI treatment data included antibiotic and non-antibiotic treatments. We recorded the CDI-directed antibiotic agent at the start of treatment, whether other CDI-directed antibiotic agents were added on or after day 1 of treatment, and the duration of CDI antibiotic treatment.

 Potential indicators of severe CDI were hypotension requiring intervention (bolus or ionotropic support), ventilator support (invasive and non-invasive ventilation), total parenteral nutrition, nil per os due to gastrointestinal signs or symptoms, abdominal imaging (no imaging, imaging with no intestinal abnormalities or imaging with intestinal abnormalities including colitis, typhlitis, enteritis or pneumatosis intestinalis), clinical diagnosis of ileus, pseudomembranous colitis upon endoscopy, intensive care unit admission, surgery for CDI, death within 30 days and death due to CDI.

**Procedures:** All data were de-identified and entered into a REDCap database. To develop a definition of severe CDI, potential indicators of severe CDI were tabulated. Using the distribution of these variables and clinical impression of factors more likely to be specific for severe CDI in a pediatric population receiving myelosuppressive treatments, a proposed definition of CDI was developed by the co-investigators during a teleconference.

**Statistics:** The primary objective was descriptive. The prevalence of CDI recurrence was calculated among those who achieved clinical cure. Determining factors associated with CDI outcomes was accomplished using univariate logistic regression analysis and the association was expressed using the odds ratio (OR) and 95% confidence interval (CI). As global cure was a composite outcome consisting of clinical cure and absence of recurrence, regression was not conducted for this outcome. We also did not conduct regression for severe CDI as some of the factors constituting severe CDI (such as ileus) may have been present prior to episode onset, thus making any clinical actions confounded by indication. In the event of multiple factors associated with an outcome, multiple logistic regression was conducted in the absence of collinearity. A P value <0.05 was considered statistically significant. Analyses were performed using R studio version 4.1.2.

**Results**

There were 721 eligible CDI episodes occurring in 627 patients during the study period (Figure 1). 94 multiple episodes occurred within 80 patients (70 patients had two episodes, 7 had three, 2 had four and 1 had five). In patients who experienced multiple episodes, the median time between episodes was 105 days (interquartile range (IQR) 75.5 – 171.8 days).

 Demographic and pre-episode data are shown in Table 1 and Supplementary Table 1. The most common diagnosis group was leukemia in 359 (49.8%) and 248 (34.4%) were HCT recipients.

|  |  |
| --- | --- |
| **Characteristics** | **Value** |
| Male, n (%) | 426 (59.1%) |
| Age, n (%) |  |
| 1 to 9 yearsa | 493 (68.4%) |
| 10 to 18 years | 228 (31.6%) |
| Diagnosis, n (%) |  |
| Leukemia | 359 (49.8%) |
| Lymphoma | 58 (8.0%) |
| Solid tumor | 194 (26.9%) |
| Brain tumor | 75 (10.4%) |
| Other | 35 (4.9%) |
| Metastatic Disease, n (%) | 221 (30.7%) |
| Relapse, n (%) | 122 (16.9%) |
| Median Months from Diagnosis (IQR) | 6.0 (3.0, 11.0) |
| Inpatient at Episode Onset, n (%) | 590 (81.8%) |
| Active Cancer Treatment, n (%0 | 657 (91.1%) |
| HCT, n (%) | 248 (34.4%) |
| Autologous | 156 (21.6%) |
| Allogeneic | 92 (12.8%) |
| Neutropenia Previous 7 Days, n (%) | 114 (15.8%) |
| Systemic Chemotherapy within 28 Days, n (%) | 640 (88.8%) |
| Vincristine | 226 (31.3%) |
| Methotrexate | 213 (29.5%) |
| Cytarabine | 118 (16.4%) |
| Cyclophosphamide | 171 (23.7%) |
| Corticosteroids | 329 (45.6%) |
| Melphalan | 34 (4.7%) |
| Etoposide | 89 (12.3%) |
| Other | 452 (62.7%) |
| Gastroprotectant within 28 Days, n (%) | 334 (46.3%) |
| Histamine-2 receptor antagonist | 113 (15.7%) |
| Proton pump inhibitor | 250 (34.7%) |
| Systemic antibiotics one day prior, n (%) | 356 (49.4%) |
| Sites |  |
| Bern University Hospital, Bern  | 39 (5.4%) |
| Hospital for Children and Adolescents, Frankfurt, | 108 (15.0%) |
| The Hospital for Sick Children, Toronto | 300 (41.6%) |
| Istituto Giannina Gaslini, Genova | 44 (6.1%) |
| Leeds Children’s Hospital, York | 5 (0.7%) |
| Lucile Packard Children’s Hospital, Palo Alto | 22 (3.1%) |
| Princess Maxima Centre for Pediatric Oncology, Utrecht | 26 (3.6%) |
| Royal Children’s Hospital, Melbourne | 148 (20.5%) |
| University Children’s Hospital, Muenster | 29 (4.0%) |

Abbreviation: IQR – interquartile range, HCT – hematopoietic cell transplantation

a 57 episodes occurred in patients between 1 and 2 years of age

**Table 1: Demographic and pre-episode characteristics of pediatric cancer and hematopoietic stem cell transplant recipients with *Clostridioides difficile* infection (N=721)**

Table 2 illustrates episode characteristics at the start of the CDI episode and the worst value during the episode. When considering the worst values, the number of episodes with 6 or greater stools per day was 269 (37.3%), macroscopic blood in stool was 86 (11.9%) and requiring opioid analgesia for abdominal pain was 180 (25.0%). In 543 (75.3%) episodes, non-CDI directed systemic antibiotics were administered during the CDI episode (Supplementary table 1).

|  |  |  |
| --- | --- | --- |
|  | **Start of CDI Episode** | **Worst During CDI Episode** |
| **Clinical Variables** |  |  |
| Number of Stools per Day, n (%) |  |  |
| Normal (2 or less per day) | 184 (25.5%) | 75 (10.4%) |
| Mild (3-5 per day) | 337 (46.7%) | 333 (46.2%) |
| Moderate (6-9 per day) | 114 (15.8%) | 194 (26.9%) |
| Severe (≥ 10 per day) | 23 (3.2%) | 75 (10.4%) |
| UTD | 63 (8.7%) | 44 (6.1%) |
| Median Days to Worst Stool Frequency (IQR) | NA | 1.0 (0.0, 3.0) |
| Macroscopic Blood in Stool, n (%) | 56 (7.8%) | 86 (11.9%) |
| Abdominal Pain, n (%) |  |  |
| None | 453 (62.8%) | 336 (46.6%) |
| Mild – no opioids | 165 (22.9%) | 200 (27.7%) |
| Moderate – opioid bolus | 69 (9.6%) | 111 (15.4%) |
| Severe – opioid infusion | 31 (4.3%) | 69 (9.6%) |
| UTD | 3 (0.4%) | 5 (0.7%) |
| Median Days to Worst Abdominal Pain (IQR) | NA | 1.0 (0.0, 4.0) |
| Median Maximum Temperature (IQR) | 37.8 (37.1, 38.7) | 38.5 (37.6, 39.3) |
| Median Days to Maximum Temperature (IQR) | NA | 1.00 (0.0, 5.0) |
|  |  |  |
| **Laboratory Variables** |  |  |
| Median WBC x 109/L (IQR) | 1.9 (0.3, 5.2) | 0.8 (0.1, 2.7) |
| Median Days to Lowest WBC (IQR) | NA | 2.0 (0.0, 6.0) |
| Median ANC x 109/L (IQR) | 0.9 (0.1, 3.4) | 0.1 (0.0, 1.3) |
| Median Days to Lowest ANC (IQR) | NA | 3.0 (0.0, 7.0) |
| Median Serum Albumin in g/L (IQR) | 32.0 (29.0, 36.0) | 29.0 (25.0, 33.0) |
| Median Days to Lowest Albumin (IQR) | NA | 3.0 (1.0, 7.0) |
| Median CRP  | 24.7 (4.0, 87.9) | 50.0 (16.7, 137.8) |
| Median Days to Highest CRP | NA | 2.0 (0.0, 5.0) |

Abbreviations: IQR – interquartile range; CDI - *Clostridioides difficile* infection; WBC – white blood cell count; ANC – absolute neutrophil count; CRP – C-reactive protein; NA – not applicable; UTD – unable to determine

**Table 2: Details of *Clostridioides difficile* infection episode (N=721)**

Table 3 describes CDI treatments. The initial antibiotic treatment on day 1 consisted of one antibiotic in 646 episodes and two antibiotics in 28 episodes, with 47 not receiving any CDI-directed antibiotic therapy.

|  |  |
| --- | --- |
|  | **n (%)** |
| **Antibiotic Treatment** |  |
| CDI-Directed Antibiotic Agent Start of Treatment |  |
| Oral Metronidazole | 388 (53.8%) |
| Oral Vancomycin | 151 (20.9%) |
| Intravenous Metronidazole | 162 (22.5%) |
| Fidaxomycin | 0 (0.0%) |
| Othera | 1 (0.1%) |
| None | 47 (6.5%) |
| CDI-Directed Antibiotic Agent Added after Day 1 of Treatment |  |
| Oral Metronidazole | 8 (1.1%) |
| Oral Vancomycin | 54 (7.5%) |
| Intravenous Metronidazole | 14 (1.9%) |
| Fidaxomycin | 3 (0.4%) |
| Otherb | 2 (0.3%) |
| Single Antibiotic Throughout | 598 (82.9%) |
| Combination Antibiotics at Start of Treatment |  |
| Oral metronidazole and oral vancomycin | 0 |
| Intravenous metronidazole and oral vancomycin | 10 (1.4%) |
| Combination Antibiotics at Start or During Treatment |  |
| Oral metronidazole and oral vancomycin | 40 (5.5%) |
| Intravenous metronidazole and oral vancomycin | 45 (6.2%) |
| Median Duration of CDI Treatment (IQR) | 10.0 (10.0, 14.0) |
|  |  |
| **Other CDI Treatment** |  |
| Probiotic Administration | 1 (0.1%) |
| Fecal Microbial Transplantation | 1 (0.1%) |
| Bezlotoxumab | 2 (0.3%) |

Abbreviations: IQR – interquartile range; CDI - *Clostridioides difficile* infection

aOthers were intravenous vancomycin (n=1)

bOthers were intravenous vancomycin (n=1), and nitazoxanide (n=1)

**Table 3: *Clostridioides difficile* infection treatment (N=721)**

Table 4 describes potential indicators of severe CDI. Eight patients died within 30 days of the CDI episode; CDI was not reported as the cause of death in any of these patients. Based on data distribution and considering factors that might be more specific for severe CDI, the proposed definition of severe CDI was any of the following: radiological diagnosis of colitis or pneumatosis intestinalis, pseudomembranous colitis, clinical diagnosis of ileus or surgery for CDI. There were 70 (9.7%) episodes classified as severe using this definition. Table 4 also shows the distribution of potential indicators of severe CDI.

|  |  |  |
| --- | --- | --- |
|  | **All Episodes (N=721)****n (%)** | **Severe Episodes**a **(N=70)****n (%)** |
| Hypotension Requiring Intervention | 49 (6.8%) | 14 (20.0%) |
| Bolus only | 36 (5.0%) | 7 (10.0%) |
| Ionotropic support | 14 (1.9%) | 8 (11.4%) |
| Ventilator Support | 15 (2.1%) | 6 (8.6%) |
| Total Parenteral Nutrition | 252 (35.0%) | 53 (75.7%) |
| NPO due to CDI | 65 (9.0%) | 29 (41.4%) |
| Abdominal Imaging Abnormalities |  |  |
| No imaging | 474 (65.7%) | 5 (7.1%) |
| Imaging – no intestinal abnormalities | 170 (23.6%) | 4 (5.7%) |
| Imaging – intestinal abnormalities | 74 (10.3%) | 61 (87.1%) |
| Not available | 3 (0.4%) | 0  |
| Small or Large Bowel Abnormalities on Imagingb |  |  |
| Colitisa | 52 (7.2%) | 52 (74.3%) |
| Typhlitis | 26 (3.6%) | 20 (28.6%) |
| Enteritis | 13 (1.8%) | 6 (8.6%) |
| Pneumatosis intestinalisa | 4 (0.6%) | 4 (5.7%) |
| Clinical Diagnosis of Ileusa | 16 (2.2%) | 16 (22.9%) |
| Pseudomembranous Colitisa | 8 (1.1%) | 8 (11.4%) |
| Intensive Care Unit Admission | 31 (4.3%) | 12 (17.1%) |
| Surgery for CDIa | 2 (0.3%) | 2 (2.9%) |
| At least Ten Stools per Day | 23 (3.2%) | 1 (1.4%) |
| Abdominal Pain Requiring Opioids | 100 (13.9%) | 22 (31.4%) |

Abbreviations: IQR – interquartile range; CDI - *Clostridioides difficile* infection; NPO – nil per os

aSevere defined as presence of colitis, pseudomembranous colitis, pneumatosis intestinalis, ileus or surgery for CDI

bMultiple abnormalities were possible

**Table 4: Potential indicators of severe *Clostridioides difficile* infection**

When considering the 627 first episodes of CDI, the number of patients with clinical cure was 520/627 (82.9%, 95% CI 79.8 to 85.8%), recurrence was 50/520 (9.6%, 95% CI 7.2 to 12.5%), global cure was 470/627 (75.0%, 95% CI 71.4 to 78.3%) and repeated new CDI episodes was 80/627 (12.8%, 95% CI 10.2 to 15.6%) (Supplementary table 2). Table 5 illustrates results of univariate logistic regression for the outcomes of failure to achieve clinical cure, recurrence and repeated new CDI episodes. Patients undergoing HCT had significantly greater odds of failing to achieve clinical cure (OR 2.4, 95% CI 1.6 to 3.7). None of the evaluated factors were significantly associated with CDI recurrence, including the initial use of oral vancomycin or metronidazole. For repeated new CDI episodes, HCT was associated with significantly lower odds (OR 0.5, 95% CI 0.3 to 0.8), initial therapy with oral metronidazole was associated with significantly higher odds (OR 1.8, 95% CI 1.1 to 2.9) and initial therapy with oral vancomycin was associated with significantly lower odds (OR 0.4, 95% CI 0.2 to 0.9) of the outcome. For multiple regression, there was a substantial correlation between initial oral metronidazole and initial oral vancomycin (Spearman rho = -0.54) and thus, they were not incorporated into the same model. When multiple regression including HCT and initial antibiotic was conducted, the association between initial oral metronidazole with repeated new CDI episode remained significant (adjusted OR 1.7, 95% CI 1.0 to 2.7; P=0.042). Similarly, the association between initial oral vancomycin with repeated new CDI episode also remained significant (adjusted OR 0.4, 95% CI 0.2 to 0.8; P=0.013). In both models, HCT was independently associated with lower odds of repeated new episodes (data not shown).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Failure to Achieve Clinical Cure (n=107)** | **CDI Recurrence** **(n=50)** | **Repeated New CDI Episodes** **(n=80)** |
|  | **OR** | **95% CI** | **P Value** | **OR** | **95% CI** | **P Value** | **OR** | **95% CI** | **P Value** |
| Age 10-18 vs. 1-9 Years | 0.8 | 0.5 to 1.3 | 0.431 | 1.5 | 0.9 to 2.8 | 0.151 | 0.7 | 0.4 to 1.2 | 0.223 |
| Diagnosis |  |  | 0.625 |  |  | 0.652 |  |  | 0.362 |
| Leukemia | 0.7 | 0.4 to 1.4 | 0.321 | 0.7 | 0.3 to 2.0 | 0.532 | 0.6 | 0.3 to 1.2 | 0.154 |
| Lymphoma | 0.6 | 0.2 to 1.5 | 0.251 | 1.4 | 0.4 to 4.9 | 0.606 | 0.9 | 0.3 to 2.4 | 0.807 |
| Solid tumors | 0.6 | 0.3 to 1.2 | 0.164 | 1.1 | 0.4 to 3.1 | 0.894 | 0.8 | 0.4 to 1.7 | 0.507 |
| Brain | REF |  |  | REF |  |  | REF |  |  |
| Not oncology | 0.9 | 0.3 to 2.6 | 0.878 | 1.1 | 0.3 to 5.1 | 0.882 | 0.3 | 0.1 to 1.4 | 0.132 |
| Active Treatment | 0.8 | 0.4 to 1.6 | 0.471 | 0.9 | 0.3 to 2.6 | 0.843 | 2.3 | 0.7 to 7.6 | 0.171 |
| HCT | ***2.4*** | ***1.6 to 3.7*** | ***<0.001*** | 0.5 | 0.3 to 1.1 | 0.092 | ***0.5*** | ***0.3 to 0.8*** | ***0.011*** |
| Neutropenia Previous 7 Days | 0.6 | 0.3 to 1.2 | 0.163 | 0.8 | 0.3 to 1.8 | 0.549 | 1.5 | 0.9 to 2.7 | 0.142 |
| Inpatient | 0.9 | 0.5 to 1.6 | 0.727 | 1.5 | 0.6 to 3.6 | 0.382 | 1.2 | 0.6 to 2.2 | 0.678 |
| Any Non-CDI Antibiotic One Day Prior | 0.8 | 0.5 to 1.2 | 0.322 | 1.6 | 0.9 to 2.9 | 0.137 | 1.0 | 0.6 to 1.6 | 0.976 |
| Any Non-CDI Antibiotic During Episode | 1.5 | 0.9 to 2.5 | 0.130 | 1.4 | 0.7 to 2.9 | 0.340 | 1.1 | 0.7 to 2.0 | 0.665 |
| Chemotherapy within 28 Days | 0.8 | 0.4 to 1.6 | 0.591 | 1.9 | 0.6 to 6.3 | 0.293 | 1.1 | 0.5 to 2.4 | 0.832 |
| Gastroprotectant within 28 Days | 1.3 | 0.8 to 1.9 | 0.259 | 1.0 | 0.6 to 1.8 | 0.959 | 1.2 | 0.8 to 1.9 | 0.457 |
| Initial Oral Metronidazole | 0.8 | 0.6 to 1.3 | 0.416 | 1.0 | 0.6 to 1.8 | 0.982 | ***1.8*** | ***1.1 to 2.9*** | ***0.024*** |
| Initial Intravenous Metronidazole | 1.5 | 1.0 to 2.4 | 0.067 | 1.0 | 0.5 to 2.0 | 0.929 | 0.8 | 0.5 to 1.5 | 0.549 |
| Initial Oral Vancomycin | 0.7 | 0.4 to 1.2 | 0.136 | 1.2 | 0.6 to 2.4 | 0.630 | ***0.4*** | ***0.2 to 0.9*** | ***0.018*** |

Abbreviations: CDI - *Clostridioides difficile* infection; OR - odds ratio; HCT – Hematopoietic cell transplant

**Table 5: Univariate Regression for Failure to Achieve Clinical Cure, CDI Relapse, and Multiple CDI Episodes (N=627 Initial CDI Episodes)**

Supplementary table 3 shows the prevalence of CDI outcomes among the non-severe and severe first CDI episodes. Initial oral metronidazole, intravenous metronidazole or oral vancomycin were not significantly associated with failure to achieve clinical cure or CDI recurrence when stratified by severe CDI. However, initial oral vancomycin was significantly associated with lower odds of repeated new CDI episodes among the non-severe group while among the severe group, the odds ratio was not calculatable as none of the patients who received initial oral vancomycin had a repeated new CDI event.

**Discussion**

 In this large multi-center and multi-national epidemiological study of CDI in pediatric patients receiving cancer treatments or HCT, we found the prevalence of clinical cure was 82.9%, recurrence was 9.6%, global cure was 75.0% and repeated new CDI episode was 12.8%. Macroscopic blood in stool was uncommon. Most common initial treatment was oral metronidazole followed by intravenous metronidazole and oral vancomycin. Using our proposed definition, severe CDI occurred in just below 10% of episodes. Initial therapy with oral vancomycin was associated with significantly lower odds of experiencing a repeated new CDI episode.

 In order to compare the prevalence of CDI outcomes in our study of pediatric cancer and HCT patients, we used the data from the five randomized trials comparing vancomycin to metronidazole that informed the CDI CPG.[12, 13, 17, 18] None included children and only 65 patients with cancer were included across all studies. The prevalence of clinical cure (82.9%) and recurrence (9.6%) in our study was similar to the reported prevalence of cure (range 74.8% to 94.4%)[12, 13, 17, 18] and recurrence in these studies (range 5.1% to 27.1%).[12, 13, 17, 18]

 We found that initial oral metronidazole or initial oral vancomycin were not significantly associated with failure to achieve clinical cure or CDI recurrence, either among the entire group or when stratified by severe CDI. The finding in the overall cohort is consistent with the randomized trials that also showed that oral vancomycin was not significantly associated with higher cure rates or lower recurrence.[11] This is in contrast to three of the randomized trials that stratified analysis by severe CDI and found vancomycin was associated with higher cure rates at the end of the antibiotic treatment.[12, 13] There are several potential explanations for the difference in findings including confounding, use of adult-based severe CDI definitions that include age and rising WBC or a different effect among pediatric or immunocompromised patients. It is also possible that our study was under powered to show such an association, as we only had 58 patients with severe CDI.

 We also found that initial oral vancomycin was significantly associated with lower odds of experiencing a new CDI episode. It is possible that this affect is confounded by unmeasured variables, including compliance with oral metronidazole, or is a spurious finding. It is also possible that oral vancomycin is better at eradicating *C. difficile*, although it is difficult to understand why oral vancomycin was not then associated with a reduction in CDI recurrence. With fidaxomicin only used in three CDI episodes the impact of this agent on outcome in our cohort is unknown. In a randomized trial of pediatric patients, fidaxomicin was associated with higher rates of global cure as compared to oral vancomycin.[19] Of note the rates of global cure in the fidaxomicin arm were 68.4% and below the overall rate of 75.0% in our study.

 Interestingly, we found that HCT was associated with higher odds of failing to achieve clinical cure but also associated with lower odds of repeated new episodes. A study of adult patients receiving solid organ transplant or HCT found a high percentage of clinical cure and low percentage of recurrence.[20] It is possible that our association between HCT and lower odds of clinical cure relate to the multiple factors associated with ongoing diarrhea in this population, including but not limited to intestinal graft-vs.-host disease (GvHD).[21] The lower odds of recurrent new episodes may be explained because many HCT patients no longer continue to have CDI risk factors after the engraftment period.

 We have proposed a definition of severe CDI for pediatric patients receiving cancer treatments in which we prioritized factors likely to be specific rather than sensitive for severe disease. This effort is challenging as we do not have a gold standard for severe CDI in pediatric patients. We did not collect whether hypotension or invasive ventilation were directly attributable to CDI. However, we noted that these events were often temporally distant from CDI onset, suggesting they were not directly attributable to CDI in these cases. While a definition of severe CDI has been proposed among a general pediatric population[22], it cannot be applied to our patients since it included rising WBC, which is frequently absent in patients receiving cancer therapy, and fever, which is commonly present in patients receiving cancer therapy and thus, is not discriminative for CDI. It is also important to note that this definition is not specific to pediatric patients and was derived from a UK-based CDI prevention and control guidance document. Our proposed definition of severe CDI, composed of microbiological, clinical, and radiographic criteria is pediatric specific and takes into account the unique characteristics of cancer and HCT patients. We believe this is a reasonable starting point, and that users should categorize severe CDI only if the components cannot be attributable to other factors.

 The strengths of our study include its large sample size and conduct in multiple centers in multiple countries. Another strength is the use of the same terms (including clinical cure, recurrence and global cure) and definitions for CDI outcomes as contemporary major CDI clinical trials.[15,16] However, our study is limited by its observational design that may be susceptible to confounding. To define CDI, abstractors needed to evaluate stool frequency and consistency and thus, standard operating procedures were developed and followed at each institution. There were likely to be differences between centers in CDI testing and treatment algorithms that may have impacted case ascertainment and treatment response. While we could not adjust for heterogeneity in testing indication, it was the heterogeneity in treatment that allowed evaluation of how initial treatment choice impacted on CDI outcomes. Potential indicators of severe CDI focused on more objective features to minimize classification error. A final limitation was that a small number of patients were between 1 and 2 years of age and it is possible that some of these cases could have represented colonization

 In conclusion, we found that among pediatric patients receiving cancer treatments, the prevalence of clinical cure was 82.9.0%, recurrence was 9.6%, global cure was 75.0% and repeated new CDI episode was 12.8%. We propose a definition of severe CDI. Initial oral vancomycin was significantly associated with a reduction in repeated new CDI episodes.

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**Figure 1: Flow Diagram of Participant Identification and Study Participation**

Episodes Screened for Eligibility (n=933)

Did not Meet Eligibility Criteria (n=212)

* Date episode start outside eligibility window (n=10)
* Did not have cancer or receive HCT (n=16)
* Did not meet criteria for distinct episode (n=118)
* Did not meet microbiological criteria for CDI (n=20)
* Data incomplete or unavailable (n=48)

Patients with Multiple Episodes (n=80)

* Two episodes (n=70)
* Three episodes (n=7)
* Four episodes (n=2)
* Five episodes (n=1)

Abbreviations: CDI: *Clostridioides difficile* infection; HCT – hematopoietic stem cell transplantation

Patients with at Least One Episode

(n=627)

Patients with One Episode

(n=547)

Episodes Analyzed (n=721)

Excluded from analysis (n = 0)

Episodes Included (n=721)