Clostridium difficile Infection Incidence and Risk Factors within Pediatric and Adult Hospitalization Sites after Allogeneic Hematopoietic Cell Transplantation

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Abstract

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BACKGROUND: Although *Clostridium difficile* infection (CDI) is the most common cause of nosocomial infectious diarrhea, the relationship between hematopoietic cell transplantation (HCT) and CDI is not well understood. Furthermore, although the experiences of adult and pediatric HCT recipients differ, studies of CDI in HCT have primarily focused on adults; the potential CDI risk imposed by many unique experiences in pediatric HCT warrants separate study.

METHODS: To explore these questions, we retrospectively reviewed CDI incidence and risk factors 100-days post-transplant among allogeneic recipients of the Fred Hutchinson Cancer Research Center from 2008-2012. All analyses were conducted separately per hospital (pediatric or adult). Patients under 1-year of age and those with evidence of preexisting CDI were excluded. Cumulative incidence curves were estimated from subdistribution hazards, and potential CDI risk factors (age, year, graft type, myeloablative transplant, graft-versus-host disease [GVHD] prophylaxis, and acute GVHD severity) were analyzed using Cox proportional hazard models.

RESULTS: CDI was diagnosed in 33/192 (17%) pediatric recipients at a median of 51 days (Interquartile Range [IQR]: 5, 72); and 107/990 (11%) adults at a median of 16 days (IQR: 5, 49). Overall, testing for CDI appeared less widespread among pediatric patients (47% tested at least once) than adults (76%). In risk factor analyses, year of transplant was associated with CDI among pediatric patients ($p<.05$). Among adults, univariate analyses found myeloablative transplant (Hazard Ratio [HR]: 1.81, $p=.0047$) associated with increased CDI risk and ages 60+ (HR: 0.61, $p=.037$) protective against CDI; these associations did not persist in multivariable models.

DISCUSSION: Our novel examination of CDI risk among allogeneic HCT recipients shows that children and adults are at high risk of CDI during the first 100-days post-transplant. The possible increased risk found among pediatric recipients in our study supports the differentiation of children from adults in future studies of CDI after HCT.

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Chapter 1

INTRODUCTION

Clostridium difficile infection (CDI) is the most common cause of nosocomial infectious diarrhea in the US, resulting in substantial morbidity and mortality among hospitalized patients [1–5]. Costs associated with this healthcare-associated pathogen, the emergence of a new hypervirulent strain of *C. difficile* (NAP-1), and reports of increasing incidence and severity of CDI in both hospitals and in the community have led to major efforts to control and prevent this infection [1–5].

Patients undergoing hematopoietic cell transplantation (HCT) have been shown to be at increased risk of CDI when compared to both general and cancer patient populations [6]. However, CDI among HCT patients remains largely understudied, and incidence estimates among HCT recipients have varied from 0% to 27% [7–15]. While these studies validate many common CDI risk factors within the HCT patient population, such as antibiotic use, the risk imposed by factors exclusive to HCT recipients, such as acute graft-versus-host disease (GVHD) and conditioning regimens, differs across studies [16]. It is unclear if differences in center-based transplant care, patient populations or testing methods and strategies contribute to some of this variance.

To date, studies on CDI among HCT recipients have focused exclusively on adults or adult-dominated cohorts, with little study of this infection among pediatric HCT recipients. However, the pediatric HCT experience is unique, as there are potential risk factors for CDI that occur more frequently during the pediatric HCT experience, such as lack of prior hospitalization and the presence of G/J tubes [17]; similarly, risk factors described within adult HCT populations may not be applicable to the pediatric HCT experience.

Given the unique experiences and pathophysiology of HCT recipients, variance in results across prior studies, and the need for pediatric data, we estimated the incidence of and risk factors for CDI among allogeneic HCT recipients at the Fred Hutchinson Cancer Research Center (FHCRC). We also addressed possible changes in incidence associated with a center-wide shift in testing methods to better understand how such changes can affect CDI at large comprehensive transplant centers.

Chapter 2

METHODS

2.1 Study Design

Study activities were approved by the FHCRC Institutional Review Board, and participants provided written informed consent according the principles of the Declaration of Helsinki.

2.2 Study Population

All patients who received an allogeneic HCT at the FHCRC between January 1, 2008–December 31, 2012 were eligible for inclusion in this study. We excluded patients with preexisting CDI, defined as a positive test for *C. difficile* toxin A or B $\geq$8 weeks prior to transplantation [18], from primary analysis to avoid possible misclassification of recurrence as an incident event; these preexisting cases were separately analyzed as a supplement to the primary analysis. In addition, infants under one-year of age were excluded due to evidence of higher rates of asymptomatic carriage of toxigenic *C. difficile* within this age group [19].

2.3 Data Collection

We retrieved information from a prospectively collected database of demographic and medical information on the first 100-days after HCT. Diagnosis of CDI and other post-transplant outcome data were identified through a center-based electronic database and confirmed with subsequent chart review.

2.4 Transplant Standards, Infection Prevention and Surveillance

Nearly all patients received acyclovir or valacyclovir prophylaxis for prevention of herpes simplex virus 1 and 2 and varicella-zoster virus; cord blood transplant recipients after June 2008 instead received high-dose valacyclovir for cytomegalovirus (CMV) prevention [20]. Daily fluconazole as administered as antifungal prophylaxis; those with known or presumptive fungal infections instead

received mold specific therapy with voriconazole, posaconazole or liposomal amphotericin B. All patients received standard *Pneumocystis jirovercii* prophylaxis with trimethoprim-sulfamethaxazole, dapsone, or atovaquone following engraftment. Screening and preemptive therapy for CMV depended on the donor–recipient serostatus match and the type of donor graft [20, 21]. Conditioning for HCT, as well as prophylaxis and treatment of GVHD, were performed using current protocols standardized within the center [22].

2.4.1 Antibacterial Prophylaxis and Treatment

All adult patients who developed neutropenia (absolute neutrophil count $<500/\text{mm}^3$) received levofloxacin prophylaxis until neutrophil recovery, while adult patients with allergy/intolerance to levofloxacin received intravenous (IV) ceftazidime. All pediatric patients under the age of 18 received ceftazidime. Patients who developed neutropenic fever underwent routine blood cultures and generally received ceftazidime as first line therapy; the addition of gram-positive coverage with vancomycin was recommended only for patients with known mucositis. Decisions regarding continuation of therapy, changes in antibiotic coverage, and clinical and laboratory assessment of fever were made by the primary team.

2.5 Definitions

2.5.1 CDI Case

C. difficile testing was ordered at the discretion of the primary team. Per guidelines [23], *C. difficile* testing was performed only on liquid stool. Repeat testing following a positive test ("test-of-cure") was discouraged.

Various testing methods are employed throughout the FHCRC network (Appendix C), and include the enzyme immunoassay (EIA) test, cell culture cytotoxin neutralization assay (CTA), and polymerase chain reaction (PCR). Due to alterations in testing protocols and differences in individualized physician-dictated patient care, various testing combinations were administered. As such, streamlined criteria were applied to determine CDI positivity/negativity (Figure A.1).

2.5.2 Risk Factors

All covariates were chosen *a priori* based on clinical relevance and previously published risk factors.

Acute GVHD severity was graded according to standard criteria [24] and was modeled in two versions: grades III-IV versus grades 0-II to capture the immunosuppressive effects of high dose steroid treatment; and grades II-IV versus grades 0-I to distinguish those with clinically significant GVHD and to capture the immunosuppressive effects of any steroids. Dates of GVHD diagnosis were modeled as time-dependent covariates.

GVHD prophylactic regimens were also included to differentiate their immunosuppressive effects and were categorized as follows: cyclosporine (CSP) or tacrolimus (FK506) with methotrexate (MTX); other regimens with mycophenolate mofetil (MMF); or all other regimens (including no prophylaxis).

In 2010, center-wide CDI testing protocols changed from assay-based testing to PCR testing. Because the adherence to the guidelines was gradual, year of transplantation was included in analyses.

For the pediatric patients, age was categorized in intervals of 1-5 (reference), 6-10, 11-15, and 16+ years to capture CDI incidence and risk factors from toddlerhood to adulthood. For the adults, age was dichotomized at 60 to assess the risk imposed by older age [13].

Other factors studied were graft type (bone marrow, peripheral blood stem cells [PBSC], and cord blood) and myeloablative transplantation [25].

2.5.3 Site of Hospitalization

Adults received outpatient care at the Seattle Cancer Care Alliance (SCCA) and were hospitalized at the University of Washington Medical Center (UWMC); children and some young adults received care at Seattle Children's Hospital (SCH). Because patient age and site of hospitalization were correlated, all analyses were conducted separately by the site of care. For brevity, patients of the pediatric site will be referred to as "pediatric" or "children," and those of the adult site as "adult."

2.6 Statistical Analyses

Cumulative incidence functions for CDI in the presence of the competing risk (death) were derived from the subdistribution hazard functions and plotted for each site. Time at second transplantation, loss to follow-up, and observation time beyond 100-days post-transplantation were censored.

Univariate and multivariable Cox proportional hazard models were fit to assess the contributions of age, year of transplant, stem-cell source, myeloablative transplant, GVHD prophylactic regimen, and GVHD severity (modeled as time-dependent) to the risk of CDI. Since GVHD severity was modeled in two versions, separate models were fit to estimate each version's contribution to CDI risk and model fit. To test the assumption of proportional hazards, the Therneau and Grambsch tests for non-zero slope of the Schoenfeld residuals were conducted; additionally, the scaled Schoenfeld residuals and their lowess smooths were plotted over time for visual assessment of this assumption.

Proportions of those "ever-tested" for *C. difficile*, defined as tested $\geq$ once while at risk, were examined to assess the breadth of testing. Testing frequencies required to achieve a positive *C. difficile* test were also examined.

Statistical significance was defined at an α of .05. Bonferroni corrected 95% confidence intervals (CI) were provided for variables including multiple comparisons. Data analyses were conducted using Stata version 12.1 (StataCorp; College Station, TX).

Chapter 3

RESULTS

Analyses included 192 children and 990 adults (Figure A.2). Demographic and clinical descriptions are detailed per site in Table B.1. Bone marrow was the most common graft type transplanted among children (53%) while PBSCs were most commonly transplanted among adults (75%). Myeloablative transplant was received by nearly all children (91%) and by about half of adults (57%). Most children (67%) and about half of adults (56%) were diagnosed with grade II acute GVHD during the first 100 days after transplantation.

Thirty-three pediatric patients (17%) developed post-transplant CDI at a median of 51 days (Interquartile Range [IQR]: 5, 72) for an incidence of 20 per 10,000 patient-days, compared with 107 adults (11%) at a median of 16 days (IQR: 5, 49) at an incidence of 12 per 10,000 patient-days (Table B.2). Figure A.3 displays cumulative incidence plots depicting the progression of CDI incidence over time per site.

We briefly analyzed CDI incidence among those patients originally excluded from primary analysis due to preexisting CDI (CDI within 8 weeks of transplant). Among preexisting cases, 6/47 (13%) adults and 3/11 (27%) children were diagnosed with CDI within 100 days after transplant.

Annual proportions of those ever-tested were compared to CDI positivity per each site (Table B.2, Figure A.4). Although children overall appeared to have higher incidence of CDI, less children were tested for *C. difficile* compared to adults; as such, *C. difficile* testing appeared to be more predictive of CDI among pediatric patients. While at risk for CDI, median testing frequencies prior to the first positive test appeared similar between pediatric and adult patients (Table B.2).

Among pediatric patients, year of transplant was significantly associated with CDI in univariate and multivariable analyses, and results suggest that the increased risk of CDI in 2012 drove this association. No significant associations between CDI and GVHD prophylaxis regimen, age group, graft type, myeloablative transplantation, or acute GVHD severity were detected in either univariate or multivariable analyses (Table B.3).

Among adults, older age appeared significantly protective and myeloablative transplantation was significantly associated with increased risk of CDI in univariate analyses; these associations did not persist in the multivariable analyses. When comparing the relationship between myeloablative transplantation and older age, chi-squared testing yielded a strong association between adults aged <60 and myeloablative transplantation (myeloablative transplantation: 69% aged <60 versus 25% aged 60+, $p<0.0001$). No significant associations were observed between CDI and year of transplant, graft type, GVHD prophylaxis, or GVHD severity in either univariate or multivariable analyses (Table B.4).

Chapter 4

DISCUSSION

Our retrospective cohort study of allogeneic HCT recipients demonstrates that adult and pediatric allogeneic recipients are at high risk for CDI during the early post-transplant period. Within the first 100-days post-transplant, 11% of adults and 17% of children developed incident CDI. Diarrhea leading to *C. difficile* testing was common within the entire cohort, but testing appeared more widespread among patients at the adult site. We found that later year of transplant among pediatric patients was significantly associated with higher CDI incidence, with no similar association detected among adult patients; since center-wide conversion to PCR testing for *C. difficile* was dependent on year, these results suggest possible differences in the diagnostic value of PCR testing between children and adults. Unlike other studies [10–13], no additional pre- or post-transplant factors were found to significantly influence CDI risk.

Not surprisingly, CDI incidence estimates observed among allogeneic HCT recipients are much higher than those reported in other hospitalized populations [14], as frequent use of antibiotics for prophylaxis, empiric coverage, and treatment of documented bacterial infections results in heavy antibiotic exposure in this population. Additionally, HCT recipients require repeated and extensive healthcare interactions both pre- and post-transplant, thereby increasing opportunities for nosocomial transmission. Other exposures in HCT, including immunosuppressive agents, gastric acid suppression, and damage to the gastrointestinal mucosa, may also contribute to the increased risk [6].

Reported CDI incidence estimates for allogeneic recipients vary between 0-27% [7–15]. Most recent studies report incidence estimates similar to those detected in our study: Alonso et al. [10] reported 12.5% among 510 allogeneic recipients, Chakrabarti et al. [11] and Willems et al. [12] reported 13% among 75 and 407 (respectively), Trifilio et al. [13] reported 14.5% among 207, and Chopra et al. [14] reported 18% among 216. Differences in incidence seen in some studies [10, 12] could be due to longer follow-up periods post-HCT. Additionally, some studies present

overall incidence estimates undifferentiated by transplant type (autologous or allogeneic), without accounting for the particularly high CDI risk conferred by allogeneic transplantation [14].

Incidence differences across studies [10, 11, 13, 14] may also be related to the inclusion of pre-transplant CDI cases. Since HCT patients have demonstrated a considerable affinity for CDI recurrence [12, 13], including preexisting cases may therefore have increased incidence estimates. Indeed, in our data, patients with prior CDI appeared to be at higher risk for recurrence than were patients at risk of *de novo* infection.

Incidence variations may also reflect institutional *C. difficile* testing strategies. *C. difficile* testing methods have been suggested to contribute to differences in reported incidences, as PCR results in a higher rate of detection than other assay-based methods [26]. Although our results suggest that the center-wide switch to PCR testing for C. difficile detection in 2010 did not significantly increase the incidence of CDI in our adult population, it is possible that this resulted from the early incorporation of PCR in the EIA protocol. As most recent studies of CDI among allogeneic patients report little to no use of PCR in testing strategies, results of these studies may have underreported their CDI incidence estimates due to use of a less sensitive test. Additionally, since the criteria for stool sample testing for C. difficile can dramatically vary across studies, with some requiring one diarrheal sample [11, 14] whereas others require three consecutive days of diarrhea [9], differences in testing requirements may limit or expand the opportunities for *C. difficile* detection from study to study.

Apart from the known risk factors for CDI, allogeneic HCT recipients may have unique exposures with the potential to increase CDI risk. Unique to allogeneic recipients post-transplant is GVHD, and the interplay of GVHD with CDI has been of growing interest. Unlike other studies [10–12], GVHD did not confer an increased risk of CDI in our study. In contrast, analyses conducted by Alonso et al. [10] and Chakrabarti et al. [11] detected GVHD as a risk factor for CDI. However, both studies were of case-control design and did not account for the temporal relationship between GVHD and CDI. Willems et al. [12] did account for the time-dependent nature of GVHD and found a significant association between GVHD and CDI. Interestingly, GVHD only predicted CDI incidence among those with CDI occurring between two months to one year post-transplant in their study, while no association between GVHD and CDI occurring less than two months after transplant was detected. Since we only observed patients for 100 days (approximately three months)

post-transplant, it is possible that the association between GVHD and CDI detected by Willems et al. [12] may have been detected in our study if patients were followed for a longer period.

Advanced age has been suggested as a risk factor for CDI among general patients [23]. A notable advancement in HCT enabling more allogeneic recipients of advanced age was the advent of nonmyeloablative transplantation, which poses lower risk for bacterial infection during the early post-transplant period than myeloablative transplantation [25] and thus may be protective against early post-transplant CDI. During our univariate analyses among adults, older age was associated with decreased risk of CDI while myeloablative transplantation was associated with increased risk of CDI. Since the significance of these associations did not persist once in the multivariable model, it is likely that the strong relationship between nonmyeloablative transplantation and older age detected in our population contributed these null associations. Our results differ with those of Trifilio et al. [13], who found an increased risk for CDI among older patients and no association between myeloablative transplantation and CDI.

Our study is one of the first examine CDI incidence and risk factors specifically among pediatric HCT recipients. Most published research assesses CDI risk either for adult HCT recipients or for a mixed population of children and adults. However, studies suggest that CDI incidence may be higher among younger children [27], perhaps reflecting the high risk of asymptomatic carriage of *C. difficile* among infants [28]. Among pediatric cancer patients, 1-4 year olds had a greater risk of CDI than those aged 15-18 [29]. Despite the evidence supporting separate analyses of pediatrics from adults when studying CDI, many published studies do not discern between these populations.

One previous study evaluated C. difficile among a 1990s cohort of pediatric allogeneic recipients and reported 9% with *C. difficile* positive diarrhea [30]. Pediatric allogeneic recipients in our study had a higher incidence of CDI, perhaps owing to the recent rise in *C. difficile* and differences in testing sensitivities.

Results of our study suggest a possible difference in post-transplant CDI risk between pediatric and adult allogeneic recipients. Since no HCT-associated risk factors evaluated in our study proved significantly associated with CDI in multivariable analyses, it is likely that other unmeasured factors may be responsible for the possible increased the risk among children. For instance, the experiences of pediatric allogeneic HCT recipients often differ from those of adults in ways potentially meaningful to CDI risk. Pediatric patients are more likely to remain inpatient than adults at our

center, potentially increasing opportunities for nosocomial infection. In addition, differences between pediatrics and adults in administration of G/J tubes, proton-pump inhibitors, myeloablative transplant, and prophylactic antibiotic regimens may further alter risk for CDI [17, 27].

Evidence suggests that the very young may act as *C. difficile* reservoirs due to asymptomatic toxigenic *C. difficile* colonization [19,28,31], and transmission from asymptomatic pediatric carriers has been demonstrated in several studies [32, 33]. Furthermore, exposure to other children directly (e.g., during play activities) or indirectly (e.g., shared toys) may facilitate the transmission of *C. difficile* within pediatric facilities. Considering the environmental persistence of this organism and the rigorous hand hygiene necessary to limit its transmission, aggressive infection control practices may help limit but not overcome many of these opportunities for transmission.

As with all retrospective studies, there are limitations imposed by the availability and accuracy of data. We did not assess length of inpatient stay, frequency of outpatient visits, or the duration and type of antibiotic use; accurate evaluation of these universal exposures would require complex modeling of multiple time-dependent variables using data more detailed than is currently available. Therefore, possible risk differences between pediatric and adult patients seen in this study were not tested for statistical significance due to the presence of these potential confounders. Although this is the largest study to address CDI in pediatric transplant patients, the limited size of our pediatric population may have restricted our ability to detect associations. As with many studies of HCT patients, many aspects of the HCT experience are not independent of one another, allowing for opportunities for possible collinearity during multivariable risk factor analyses.

In conclusion, our study provides a novel examination of CDI incidence and risk factors among pediatric and adult patients after allogeneic HCT and shows that both pediatric and adult recipients are at high risk of CDI during the first 100 days post-transplant. The possible increased risk among children compared to adults supports the differentiation of these groups in future evaluations of CDI incidence and risk factors after HCT.

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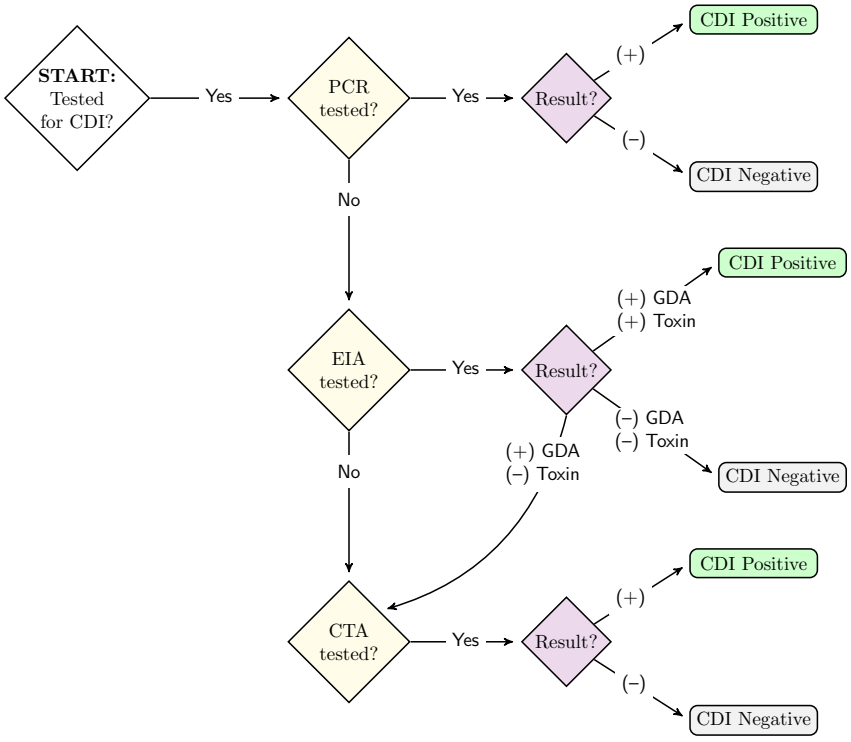
Appendix A

FIGURES

Figure A.1: Algorithm for *C. difficile* infection diagnosis via various testing methods



Note: In the event that the testing method was unclear in the database, a thorough chart review was executed to determine the actual testing method used.

Definitions: CDI: *C. difficile* infection; PCR: polymerase chain reaction for detection of *tcdB* gene or *tcdC* gene; EIA: enzyme immunoassay for simultaneous detection of both GDA and toxin presence–either Toxin A or both Toxins A and B, depending on testing clinic; GDA: glutamate dehydrogenase antigen; CTA: cell culture cytotoxin neutralization assay

Figure A.2: Study population exclusion, per site of hospitalization

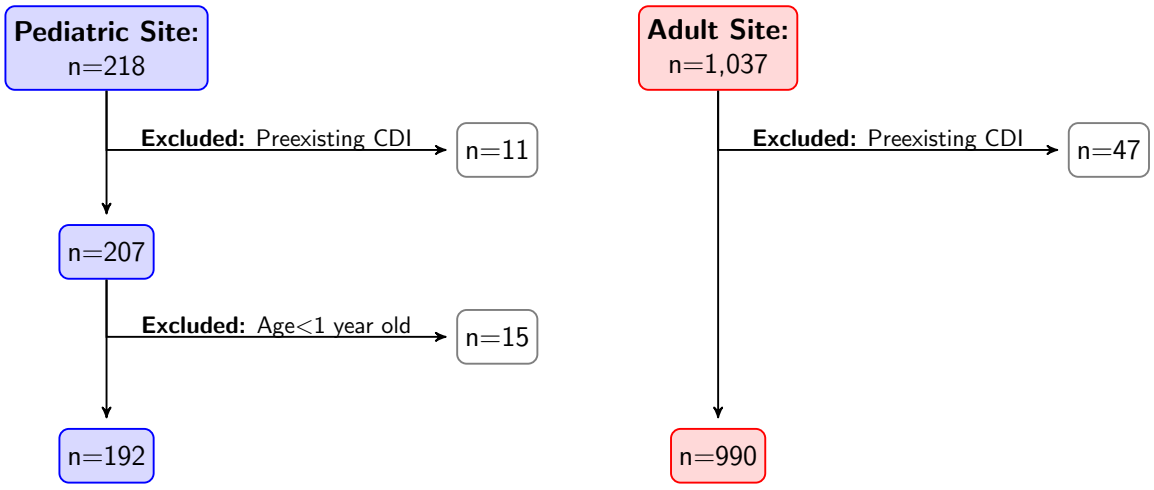


Figure A.3: Cumulative incidence curves for post-allogeneic HCT *C. difficile* infection, per site of hospitalization

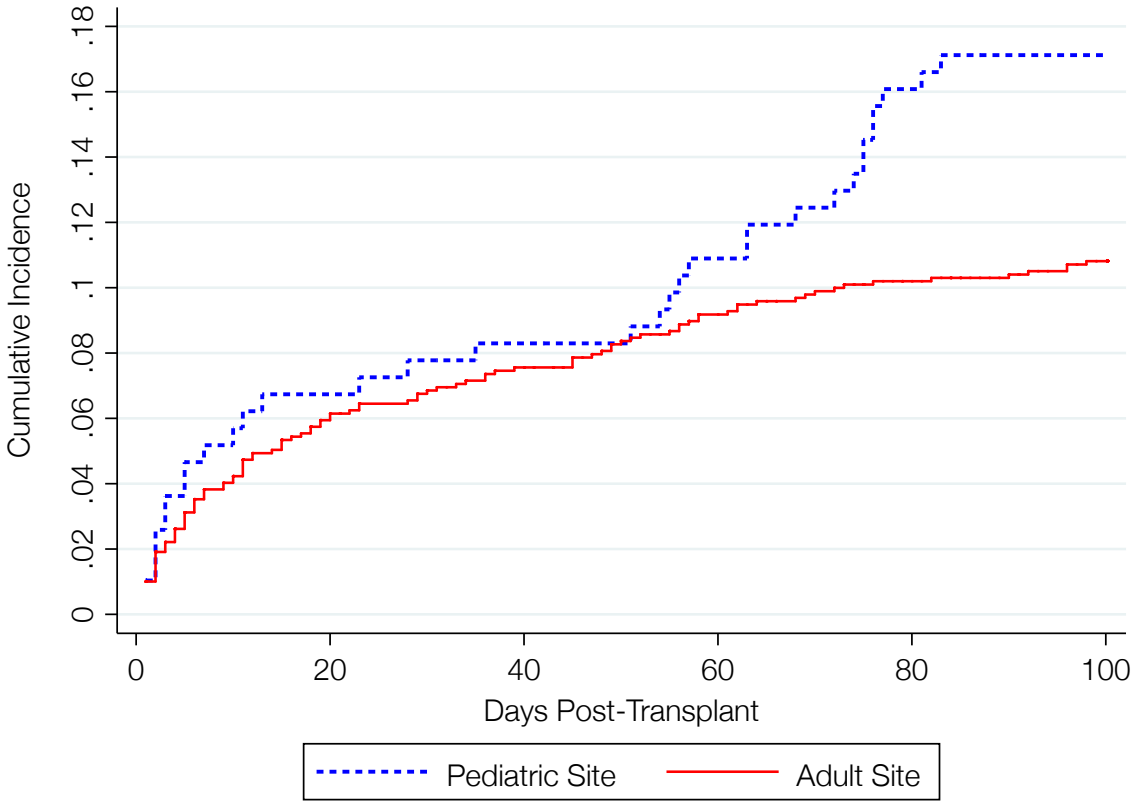
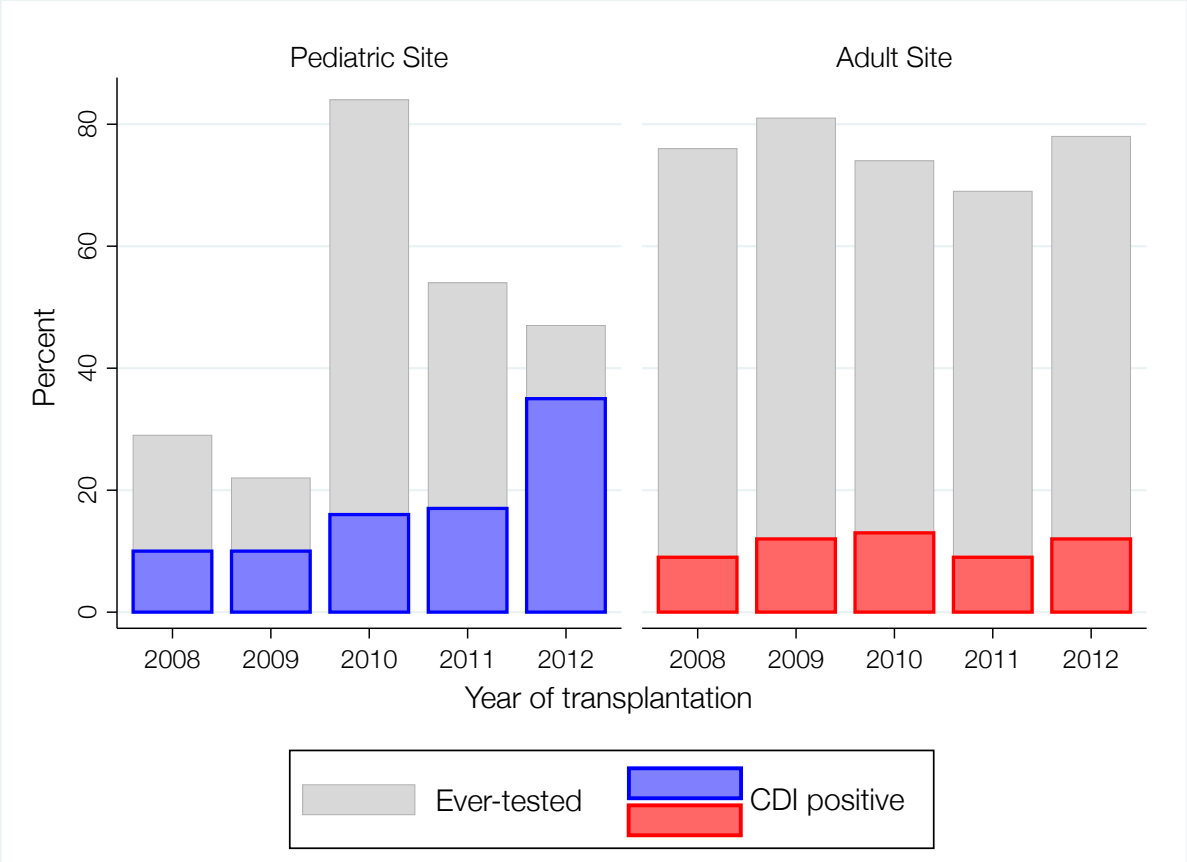


Figure A.4: Yearly *C. difficile* infection incidence and testing among allogeneic HCT patients, per site of hospitalization



Appendix B

TABLES

Table B.1: Descriptive statistics, by site of hospitalization

	Pediatric Site	Adult Site
Variable	Statistic (N=192)	Statistic (N=990)
Age (yr) – median (IQR)	11 (7, 16)	53 (42, 61)
Sex – n (%)		
Male	104 (54)	583 (59)
Female	88 (46)	407 (41)
Race/Ethnicity – n (%)		
Caucasian	101 (53)	761 (77)
Hispanic	31 (16)	27 (3)
Asian Pacific-Islander	19 (10)	59 (6)
African (American or otherwise)	6 (3)	17 (2)
Native American	2 (1)	8 (1)
Other	22 (12)	53 (5)
Unknown	11 (6)	65 (7)
Stem-cell source – n (%)		
Bone marrow	102 (53)	155 (16)
PBSC	36 (19)	743 (75)
Cord blood	54 (28)	92 (9)
Donor – n (%)		
Sibling	52 (27)	300 (30)

Continued on next page

Table B.1 – continued from previous page

Variable	Pediatric Site Statistic (N=192)	Adult Site Statistic (N=990)
Sibling: Matched	*51 (27)*	*293 (30)*
Sibling: Mismatched	*1 (1)*	*7 (1)*
Unrelated	80 (42)	523 (53)
Cord	54 (28)	92 (9)
Haploidentical	6 (3)	68 (7)
Myeloablative transplant – n (%)		
Myeloablative	175 (91)	559 (57)
Nonmyeloablative	17 (9)	431 (44)
Acute GVHD grade– n (%)		
0	23 (13)	256 (26)
I	6 (3)	49 (5)
II	117 (67)	551 (56)
III	27 (15)	101 (10)
IV	2 (1)	29 (3)
GVHD prophylaxis – n (%)		
CSP or FK506 plus MTX	106 (55)	360 (36)
Other regimen with MMF	74 (39)	539 (54)
Other (including none)	12 (6)	91 (9)

Definitions: Interquartile range (IQR); graft-versus-host disease (GVHD); cyclosporine (CSP); tacrolimus (FK506); methotrexate (MTX); mycophenolate mofetil (MMF)

Note: Percentages may not add up to 100 due to rounding.

Table B.2: Summary statistics for *Clostridium difficile* infection incidence and testing, by site of hospitalization

Variable	Pediatric Site Statistic (N=192)	Adult Site Statistic (N=990)
Outcomes–n (%)		
C. difficile infection	33 (17)	107 (11)
Death	8 (4)	93 (9)
Lost to follow-up	0 (0)	1 (0)
Second transplant	0 (0)	11 (1)
Administrative (100 days)	151 (79)	778 (79)
CDI incidence (per yr[a])–n (%)		
2008	4 (10)	17 (9)
2009	4 (10)	25 (12)
2010	5 (16)	23 (13)
2011	7 (17)	19 (9)
2012	13 (35)	23 (12)
Days to incidence–median (IQR)	51 (5, 72)	16 (5, 49)
Ever-tested[b]–n (%)	91 (47)	748 (76)
Ever-tested[b] (per yr[a])–n (%)		
2008	12 (29)	153 (76)
2009	9 (22)	166 (81)
2010	27 (84)	130 (74)
2011	23 (56)	149 (69)
2012	20 (54)	150 (78)
Num. tests to incidence–median (IQR)	1 (1, 1)	1 (1, 2)

[a]Year of reference transplantation

[b]Tested for *C. difficile* at least once during observation

Note: Percentages may not add up to 100 due to rounding.

Table B.3: Pediatric site: Univariate and multivariable hazard ratios for *C. difficile* infection after allogeneic HCT

	Univariate		Multivariable			
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95%CI)	P
Age group (years)[a]		.50		.18		.18
1-5	Ref.		Ref.		Ref.	
6-10	0.76 (0.19, 3.01)		0.94 (0.19, 4.56)		0.98 (0.20, 4.70)	
11-15	1.51 (0.46, 5.02)		2.11 (0.51, 8.67)		2.07 (0.50, 8.55)	
16+	0.93 (0.26, 3.29)		0.58 (0.12, 2.74)		0.57 (0.12, 2.66)	
Year of transplant[a]		.028		.020		.017
2008	Ref.		Ref.		Ref.	
2009	0.94 (0.16, 5.47)		0.98 (0.17, 5.83)		0.95 (0.16, 5.64)	
2010	1.56 (0.29, 8.31)		2.30 (0.41, 12.97)		2.07 (0.37, 11.43)	
2011	1.71 (0.36, 8.21)		1.18 (0.19, 7.16)		1.26 (0.21, 7.62)	
2012	3.99 (0.96, 16.63)		5.48 (1.13, 26.64)		5.87 (1.19, 29.08)	
Graft type[a]		.32		.37		.42
Bone marrow	Ref.		Ref.		Ref.	
PBSC	1.87 (0.73, 4.81)		2.02 (0.61, 6.66)		1.94 (0.60, 6.30)	
Cord blood	1.16 (0.45, 2.97)		2.03 (0.29, 14.26)		1.81 (0.26, 12.65)	
Myeloablative transplant	1.52 (0.36, 6.34)	.57	1.51 (0.13, 17.35)	.74	1.46 (0.13, 16.81)	.76
GVHD prophylaxis[a]		.061		.19		.18
CSP/FK506 with MTX	Ref.		Ref.		Ref.	
Other with MMF	0.93 (0.39, 2.21)		0.49 (0.07, 3.65)		0.49 (0.07, 3.63)	
Other/none	3.05 (0.97, 9.55)		2.23 (0.43, 11.45)		2.28 (0.45, 11.67)	
aGVHD: Grade III-IV	0.30 (0.04, 2.24)	.24	0.33 (0.04, 2.67)	.30	–	–
aGVHD: Grade II-IV	1.13 (0.37, 3.46)	.83	–	–	0.89 (0.28, 2.84)	.84

[a] Bonferroni corrected 95% CIs; p-values represent multiple degrees of freedom test for overall variable.

[b] aGVHD severity dependent upon time of diagnosis.

Table B.4: Adult site: Univariate and multivariable hazard ratios for *C. difficile* infection after allogeneic HCT

	Univariate		Multivariable			
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95%CI)	P
Older age (60+ yrs)	0.61 (0.38, 0.97)	.037	0.78 (0.47, 1.31)	.35	0.78 (0.47, 1.31)	.35
Year of transplant[a]		.42		.34		.34
2008	Ref.		Ref.		Ref.	
2009	1.49 (0.68, 3.26)		1.50 (0.68, 3.32)		1.51 (0.68, 3.34)	
2010	1.58 (0.71, 3.51)		1.42 (0.63, 3.23)		1.42 (0.63, 3.22)	
2011	1.02 (0.44, 2.34)		0.92 (0.39, 2.17)		0.92 (0.39, 2.18)	
2012	1.45 (0.65, 3.21)		1.51 (0.66, 3.43)		1.51 (0.67, 3.44)	
Graft type[a]		.13		.40		.40
Bone marrow	Ref.		Ref.		Ref.	
PBSC	0.62 (0.37, 1.07)		0.71 (0.40, 1.26)		0.71 (0.40, 1.26)	
Cord blood	0.83 (0.36, 1.88)		0.83 (0.30, 2.27)		0.83 (0.30, 2.27)	
Myeloablative transplant	1.81 (1.20, 2.72)	.0047	1.57 (0.76, 3.24)	.23	1.54 (0.74, 3.19)	.25
GVHD prophylaxis[a]		.10		.96		.97
CSP/FK506 with MTX	Ref.		Ref.		Ref.	
Other with MMF	0.68 (0.43, 1.09)		1.01 (0.43, 2.37)		1.01 (0.43, 2.35)	
Other/none	1.13 (0.56, 2.29)		1.09 (0.52, 2.29)		1.08 (0.52, 2.28)	
aGVHD[b]: Grade III-IV	1.06 (0.45, 2.46)	.90	0.99 (0.42, 2.32)	.98	–	–
aGVHD[b]: Grade II-IV	1.31 (0.75, 2.26)	.34	–	–	1.20 (0.69, 2.08)	.52

[a] Bonferroni corrected 95% CIs; p-values represent multiple degrees of freedom test for overall variable.

[b] aGVHD severity dependent upon time of diagnosis.

Appendix C

CDI TESTING PROTOCOLS

Various testing methods are employed throughout the FHCRC network, and include combinations of the enzyme immunoassay (EIA) test, cell culture cytotoxin neutralization assay (CTA), and polymerase chain reaction (PCR). With respect to those years studied, the EIA (with possible PCR) and CTA were the primary methods of *C. difficile* detection from 2008 through 2009. In 2010, the FHCRC began transitioning to PCR as the sole testing method of *C. difficile*.

EIA and PCR

The EIA test simultaneously tests for both the presence of glutamate dehydrogenase antigen (GDA) and, depending on the site of hospitalization, toxin A (administered at the UWMC/SCCA) or both toxins A and B (administered at the SCH). Concordantly negative results (GDA–, toxin–) indicate the absence of any *C. difficile* (toxigenic or non-toxigenic), while concordantly positive results (GDA+, toxin+) indicate the presence of toxigenic *C. difficile*. In the event of discordant results (GDA+, toxin–), a follow-up PCR test for the toxin B gene (administered at the UWMC/SCCA) or the toxin A and B genes (administered at SCH) was conducted. If conducted, this follow-up PCR determines the overall toxigenic positivity or negativity of the sample.

CTA

The CTA tests for the presence of toxin B, and functions as a standalone test.

PCR (standalone)

PCR, as a standalone diagnostic test, detects the presence of genes responsible for the production of toxins A and B.