Metronidazole Exposure Prior to *Clostridiodes difficile Infection* (CDI) is a Risk Factor for Severe *C. difficile* Disease in Cancer Patients

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ABSTRACT

BACKGROUND

Antibiotic use is a risk factor for CDI. Few studies have correlated use of prior antibiotics with CDI severity in the oncologic population. We hypothesized that previous antibiotic exposure and microbiome composition at time of CDI presentation, are risk factors for severe disease in cancer patients.

METHODS

This non-interventional, prospective, single-center cohort study examined patients with cancer who had their first episode or first recurrence of CDI between Oct 27, 2016 and Jul 1, 2019.
C. difficile was identified using nucleic acid amplification testing (NAAT). Multivariate analysis was used to determine significant clinical risk factors for severe CDI as defined in the 2018 IDSA/SHEA guidelines. Alpha, and beta diversities were calculated to measure the average species diversity and the overall microbial composition. Differential abundance analysis and progressive permutation analysis were used to single out significant microbial features that differed across CDI severity levels.

RESULTS

This cohort (n=200) included patients with a mean age of 60 yrs., 53% female, majority White (76%) and non-Hispanic (85%). Prior 90-day metronidazole use (Odds Ratio OR 4.68 [1.47-14.91] p= 0.009) was a significant risk factor for severe CDI. Other factors included Horn's Index > 2 (OR 7.75 [1.05-57.35] p= 0.045), Leukocytosis (OR 1.29 [1.16-1.43] p< 0.001), Neutropenia (OR 6.01 [1.34-26.89] p= 0.019) and Serum Creatinine >0.95 mg/dL (OR 25.30 [8.08-79.17] p< 0.001). Overall, there were no significant differences in alpha and beta diversity between severity levels. However, when identifying individual microbial features, the high presence of *Bacteroides uniformis*, *Ruminococceae*, *Citrobacter koseri* and *Salmonella* were associated with protection from severe CDI (p< 0.05).

CONCLUSION

A number of risk factors for severe CDI were identified among this population, including the use of metronidazole for non CDI indications within 90 days of diagnosis. Also, an increased relative abundance of *Bacteroides uniformis*, *Ruminococceae*, *Citrobacter koseri* and *Salmonella* were linked to protection from severe CDI. Reducing metronidazole use in patients with cancer may help prevent subsequent severe CDI.

INTRODUCTION

- *C. difficile* infection (CDI) is a leading cause of health care associated diarrhea in the US. In the oncologic population, the incidence of CDI ranges from 6%-33% [1]. A study showed that severe infection (based on 2010 IDSA Guidelines) was seen in 32% of cases with an all-cause mortality of 16% [2].
- Because of its intrinsic anti-*C. difficile* activity, metronidazole use for non-CDI indications has been associated with decreased risk for subsequent CDI [3]. However, metronidazole efficacy has decreased due to emergence of resistance and reduced fecal excretion [4] [5] and is no longer a first line agent to treat CDI
- Antibiotic induced dysbiosis of the normal gut microbiome favors *C difficile* growth in the colon via a decrease in secondary bile acids leading to spore germination and an increase in the vegetative growth of the bacteria. This is most apparent with prior use of fluroquinolones, beta lactams, cephalosporins, carbapenems, and clindamycin
- Assessing the relative contribution of prior antibiotic exposure and microbiome composition on CDI severity in patients with cancer may provide insights that inform strategies to reduce risk of more severe disease in this immunocompromised population

METHODS

Objectives:
This study aimed to identify clinical and microbiological risk factors associated with severe CDI in patients with cancer. We hypothesized that previous antibiotic exposure and microbiome composition at time of CDI presentation, are risk factors for severe disease in patients with cancer

STUDY DESIGN

Non-interventional, prospective, single-center cohort study at a large academic medical center examining patients with cancer who had their first episode or first recurrence of CDI between Oct 27, 2016 and Jul 1, 2019. C. difficile was identified by nucleic acid amplification testing (NAAT) and enzyme immunoassay was used for C. difficile toxin A/B detection

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• Age > 18 • Diarrhea: > 3 unformed stools or >200 mL/day unformed stool/24 hours before therapy • CDI NAAT +, followed by EIA test • With a malignancy, active or in remission • Inpatient	• Use of anti-motility agents or oral contrast • Concurrent participation in a CDAD trial • Prior fecal microbial transplant • Severe underlying disease with an expected survival of < 4 days

VARIABLES

- Demographics (age, gender, race, ethnicity)
- Clinical
  - Severity of CDI based on 2018 IDSA Guidelines
    - Severe and Fulminant disease cases were combined due to the low number of Fulminant cases
  - First episode or recurrence
  - Presenting symptoms
  - Previous Exposure to Medications in the past 90 days before diagnosis
  - CDI acquisition (healthcare facility onset, health care facility associated, community onset but healthcare associated, community onset)
  - Charlson comorbidity index
  - Other severity scoring systems (Horn's Index and Zar Score)
  - Laboratory parameters
  - Co-pathogen present in the gastrointestinal panel
  - Inflammatory markers (lactoferrin, calprotectin, IL-1B, IL-8)
  - Underlying malignancy (solid tumor, hematologic, SCT)
- Microbiology (alpha diversity, beta diversity)

ANALYSES:

- Multivariable logistic regression analysis was used to assess significant risk factors for severe CDI.
  - The dependent variable in the regression model was severe CDI
  - Independent variables included clinical and microbiology parameters. Only variables with p-value ≤0.25 in univariate analysis were included in multivariate analysis
- Alpha, and beta diversities were calculated to measure the average species diversity and the overall microbial composition.
- Differential abundance analysis and progressive permutation analysis were used to single out significant microbial features that differed across CDI severity levels.
- Analyses was carried out using *IDM SPSS Statistics Version 24.* An alpha level of 0.05 was used to test for significance with the final multivariate model

RESULTS

Table 2: Descriptive Characteristics at Baseline Included in the Multivariate Analysis

Variables	Overall Sample (N=200)	Non-Severe CDI (n=158)	Severe and Fulminant (n=42)	P-value
Episode				
First Episode	185 (93%)	144 (92%)	41 (98%)	0.202*
Presenting Symptoms				
Abdominal pain	73 (37%)	54 (34%)	19 (45%)	0.186
Bloating	17 (9%)	11 (7%)	6 (14%)	0.208*
Mucus in stools	4 (2%)	2 (1%)	2 (5%)	0.195*
Antibiotic Exposure (Past 90 days)				
Cephalosporin	94 (47%)	69 (44%)	25 (60%)	0.067
Metronidazole	26 (13%)	16 (10%)	10 (24%)	**0.019**
Use of GABA mimetics (Past 90 days)				
Other benzodiazepines (not zolpidem)	48 (24%)	41 (26%)	7 (17%)	0.211
Charlson Co-Morbidity Index (SD)	5.53 (2.68)	5.41 (2.52)	6.26 (2.96)	0.063
Horn's Index				**0.050***
1 – Medical management	193 (97%)	154 (97%)	39 (93%)	
2 – ICU stay, no invasive	2 (1%)	0 (0%)	2 (5%)	
3 – ICU stay, with invasive procedures	5 (3%)	4 (3%)	1 (2%)	
4 – Critically ill, shock	0 (0%)	0 (%)	0 (0%)	
Zar Score				**0.001**
Not severe (<2)	157 (79%)	132 (84%)	24 (57%)	
Severe (> 2)	43 (22%)	26 (16%)	18 (43%)	
MD Anderson Severity Scoring				**0.013**
Non-Severe	39 (20%)	38 (24%)	1 (2%)	
Severe	161 (81%)	120 (76%)	41 (98%)	
Laboratory Parameters				
WBC (SD, continuous)	6.16 (5.54)	5.24 (4.34)	9.6 (7.83)	**0.001****
Neutropenia (<500) (N = 194)	48 (25%)	42 (27%)	7 (17%)	0.174
Lymphopenia (<1000) (N = 192)	134 (70%)	111 (70%)	24 (57%)	0.063
Serum albumin (SD)	3.28 (0.68)	3.32 (0.66)	3.14 (0.75)	0.142**
Serum Cr (SD)	1.15 (1.20)	0.81 (0.24)	2.44 (2.14)	**0.001****
Diagnostic modality				
Toxin A/B positive	62 (31%)	43 (27%)	19 (45%)	**0.025**

Note: Only variables with p-value ≤0.25 in univariate analysis were included in multivariate analysis
* Fisher's Exact Test Used (For categorical data)
** Binary Logistic Regression Used (For continuous data)

- The mean age of patients in the study was 60 years, with a slight female majority (53%)
- Most patients were non-Hispanic (71%) and White (76%)

Table 3: Results of multivariate logistic regression analysis of factors associated with severe CDI

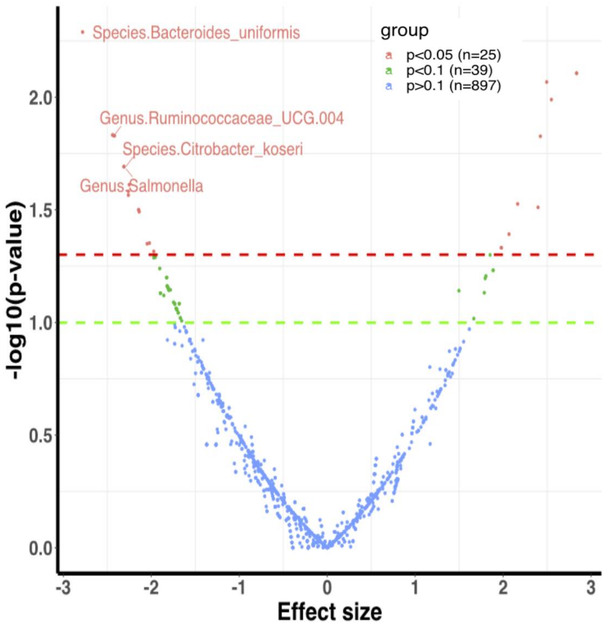
Variable	Multivariate analysis	
	Adjusted OR (95% CI)	p-value
Antimicrobial Exposure		
Metronidazole	4.68 (1.47, 14.91)	0.009
Horn's Index		
1 - Medical management	Reference	
2,3,4 - ICU stay or critically ill	7.75 (1.05, 57.35)	0.045
Laboratory Parameters		
WBC	1.29 (1.16, 1.43)	< .001
Neutropenia	6.01 (1.34, 26.89)	0.019
Serum Creatinine > 0.95 mg/dL	25.30 (8.08, 79.17)	< .001

Abbreviations: OR = Odds ratio; 95% CI = 95% confidence interval
Elimination procedure (p> 0.05 in multivariate analysis).
Backward regression analysis was preferred to forward regression analysis due to small sample size and our study not having enough events per variable. Multivariate model with all factors was not used due to overfitting.

- Prior 90-day metronidazole use (Odds Ratio OR 4.68 [1.47-14.91] p= 0.009) was a significant risk factor for severe CDI.
- Other significant factors included Horn's Index > 2 (OR 7.75 [1.05-57.35] p= 0.045), Leukocytosis (OR 1.29 [1.16-1.43] p< 0.001), Neutropenia (OR 6.01 [1.34-26.89] p= 0.019) and Serum Creatinine >0.95 mg/dL (OR 25.30 [8.08-79.17] p< 0.001).

MICROBIOME ANALYSIS

- There was no significant difference between alpha and beta diversity when comparing severe and fulminant vs non-severe disease



- When identifying individual microbial features, the high presence of *Bacteroides uniformis*, *Citrobacter koseri* at the species level and *Salmonella* and *Ruminococceae* at the genus level were associated with protection from severe CDI (p< 0.05). This was confirmed by differential analysis when compared to non-severe CDI cases.

LIMITATION & CONCLUSIONS

LIMITATIONS

- This study evaluated a relatively small sample size done across a variety of malignancies from a single health care system that can limit its generalizability
- No susceptibility testing to Metronidazole was done

CONCLUSIONS

- Risk factors for severe CDI in patients with cancer include prior 90-day metronidazole use, increase in WBC count, increased creatinine, and presence of neutropenia.
- Based on microbiome analyses, there was no significant difference in alpha and beta diversity between patients with severe versus non-severe CDI, however, an increase in the relative abundance of *Bacteroides uniformis, Ruminococceae, Citrobacter koseri* and *Salmonella* were linked to protection from severe CDI.
- Findings provide valuable insights on risk factors for severe CDI in an underserved population with cancer that warrants further exploration

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