Annals of Gastroenterology (2022) **35,** 393-399

Clostridioides difficile infection in cancer patients receiving immune checkpoint inhibitors

Shaleen Vasavada[a], Kavea Panneerselvam[a], Rajan Amin[b], Krishnavathana Varatharajalu[c], Pablo C. Okhuysen[d], Isabella C. Glitza Oliva[e], Jianbo Wang[f], Petros Grivas[g], Anusha S. Thomas[c]*, Yinghong Wang[c]*

Baylor College of Medicine, Houston, Texas; The University of Texas Health Science Center, Houston, Texas; The University of Texas MD Anderson Cancer Center, Houston, Texas; University of Washington and Fred Hutchinson Cancer Center, Seattle, Washington, USA

Abstract

Background Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but are associated with immune-mediated diarrhea and colitis (IMDC). *Clostridioides difficile* infection (CDI) can cause infectious diarrhea with overlapping symptoms. Thus, we sought to elucidate the characteristics of CDI in patients treated with ICI, in the context of IMDC.

Methods We conducted a retrospective, single-center study of adult cancer patients (N=421) with ICI exposure from 2015-2020 and a positive stool nucleic acid amplification test and/or enzyme immunoassay for CDI. Baseline characteristics, treatments, and outcomes were compared between patients with and without concurrent IMDC.

Results Forty-one eligible patients were included, 27 with concurrent IMDC and 14 without. Twenty-seven patients were taking programmed death-1 or its ligand inhibitors and 14 were taking cytotoxic T-lymphocyte-associated antigen 4 inhibitors. Patients with concurrent CDI and IMDC had a longer symptom duration (20 vs. 5 days, P=0.003) and a higher rate of grade 3-4 diarrhea (41% vs. 7%, P=0.033). Among patients with concurrent IMDC, preceding antibiotics (P=0.050) and proton pump inhibitors (PPI) (P=0.038) were used more frequently among individuals who developed CDI after immunosuppressant exposure. Thirty-eight patients received antibiotics for CDI, while 5 required fecal microbiota transplantation for concurrent CDI & IMDC.

Conclusions CDI is common in ICI-treated cancer patients, especially those with IMDC requiring immunosuppressants. Antibiotics did not alter the need for immunosuppressants in those with concurrent IMDC. Use of PPI and antibiotics while receiving immunosuppressants for IMDC was associated with a greater risk of CDI. Further large-scale studies are warranted to clarify the role of CDI, antibiotics and immunosuppression treatment in IMDC patients.

Keywords Immune checkpoint inhibitors, immune-mediated diarrhea and colitis, *Clostridioides difficile* infection

Ann Gastroenterol 2022; 35 (4): 393-399

Introduction

Immune checkpoint inhibitors (ICIs) enhance antitumor activity by blocking negative regulators of T-cell function: e.g., cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 or its ligand (PD-[L]1) [1,2]. By upregulating the immune system, ICIs have revolutionized the treatment of various cancers in recent years; however, they are associated with immune-related adverse events (irAE). Immune-mediated diarrhea and colitis (IMDC) is the most common irAE affecting the gastrointestinal (GI) tract and has been well recognized in current clinical practice [3-5]. However, there are limited data related to *Clostridioides difficile* infection (CDI) in patients treated with ICIs [6]. In a single-center retrospective study,

*Equal contribution

Correspondence to: Yinghong Wang, MD, PhD, Department of Gastroenterology, Hepatology and Nutrition, Unit 1466, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA, e-mail: ywang59@mdanderson.org

Received 5 February 2022; accepted 1 April 2022; published online 2 June 2022

DOI: https://doi.org/10.20524/aog.2022.0722

Del Castillo *et al* reported that the incidence of infections requiring hospitalization in 740 melanoma patients on ICI therapy was 7.3%, with the majority of GI infections being secondary to CDI [7].

Testing for CDI consists of a nucleic acid amplification test (NAAT) via polymerase chain reaction (PCR) and an initial enzyme immunoassay (EIA) screening for glutamate dehydrogenase antigen and/or toxins A and B. The most recent Infectious Disease Society of America (IDSA) guideline recommends a multistep algorithm combining the use of both NAAT and toxin detection methods to diagnose CDI [8]. In conjunction, a positive result for both tests is diagnostic for CDI; however, the clinical value of discordant results, such as NAAT-positive and EIA-negative (NAAT+/EIA-) remains unclear. In a cohort study by Polage *et al*, NAAT+/EIA- patients had a significantly lower bacterial load and a lower incidence of fecal inflammation, diarrhea, CDI-related complications and death than the NAAT+/EIA+ cohort [9]. The same study found similar outcomes between those NAAT+/EIA- patients with and without antibiotic treatment; however, it is unclear whether this applies to patients with underlying advanced malignancy on ICI therapy. Because of overlapping symptoms, it is often difficult to interpret positive stool studies in the context of IMDC. In a study by Ma *et al*, antimicrobial treatment did not avert the need for immunosuppressive therapy for IMDC or improve clinical outcomes among cancer patients with common *Escherichia coli* and viral infections of the GI tract [10]. In the present study, we compared the clinical characteristics and outcomes of cancer patients with concurrent IMDC and CDI with those of a matched control group of patients with CDI but no IMDC.

[a]Department of Internal Medicine, Baylor College of Medicine, Houston, Texas (Shaleen Vasavada, Kavea Panneerselvam); [b]Department of Internal Medicine, The University of Texas Health Science Center, Houston, Texas (Rajan Amin); [c]Department of Gastroenterology, Hepatology, and Nutrition, The University of Texas MD Anderson Cancer Center, Houston, Texas (Krishnavathana Varatharajalu, Anusha S. Thomas, Yinghong Wang); [d]Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, Houston, Texas (Pablo C. Okhuysen); [e]Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas (Isabella C. Glitza Oliva); [f]Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas (Jianbo Wang); [g]Department of Medicine, University of Washington, Fred Hutchinson Cancer Research Center/Seattle Cancer Care Alliance, Seattle, Washington (Petros Grivas), USA

Conflict of Interest: P. Grivas provided consulting to AstraZeneca, Astellas Pharma, Bayer, Bristol Myers Squibb, Dyania Health, EMD Serono, Exelixis, Foundation Medicine, Genentech/Roche, Genzyme, GlaxoSmithKline, Guardant Health, Gilead Sciences, Infinity Pharmaceuticals, Janssen, Lucence Health, Merck, Mirati Therapeutics, Pfizer, QED Therapeutics, Regeneron Pharmaceuticals, Seattle Genetics, UroGen, SilverBack Therapeutics, 4D Pharma PLC. His institution has received grants from Bavarian Nordic, Bristol Myers Squibb, Clovis Oncology, Debiopharm, EMD Serono, G1 Therapeutics, Gilead Sciences, GlaxoSmithKline, Merck, Mirati Therapeutics, Pfizer, QED Therapeutics. Dr. Wang has disclosed serving as a consultant for Tillotts Pharma, and AzurRx Pharma.

The remaining authors have disclosed that they have not received any financial consideration from any person or organization to support the preparation, analysis, results, or discussion of this article

Patients and methods

Patient selection and data collection

We performed a retrospective, descriptive, single-center study by screening 421 adult cancer patients with ICI exposure who were tested for CDI at The University of Texas MD Anderson Cancer Center between November 1, 2015, and April 30, 2020. The ICIs (including ipilimumab, tremelimumab, nivolumab, pembrolizumab, atezolizumab, durvalumab and avelumab) were used as monotherapy or as part of a multi-agent regimen. Patients with positive stool CDI NAAT PCR and/or EIA test during ICI exposure up to 1 year after the last dose were eligible for this study. CDI was defined as positivity of either NAAT or EIA with associated GI symptoms. CDI recurrence was defined as GI symptom recurrence together with a positive NAAT PCR or EIA within the study window in a patient who had achieved symptom resolution after treatment for a prior episode of CDI. The diagnosis of IMDC was based on clinical symptoms, colonoscopy, inflammatory stool markers, and the assessment of the treating oncologist or gastroenterologist, after other etiologies had been excluded. Patients who had a preexisting inflammatory bowel condition, CDI outside the study window, or positive non-CDI GI infections were excluded from the study.

Clinical characteristics and outcomes

Patients' clinical characteristics were collected, including demographics (age, sex, and race), oncological histories, Charlson Comorbidity Index, performance status, GI irAE-related data, GI infection-related data, treatment of IMDC, antecedent immunosuppressive and antimicrobial therapy, duration of symptoms and overall survival. Oncologic variables included cancer type and status of cancer progression at the time of CDI and ICI treatment. Clinical symptoms related to CDI included diarrhea, abdominal pain, blood or mucus in the stool, nausea and vomiting, abdominal distension and fecal incontinence. Peak grades of diarrhea and colitis (according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 5.0) were also collected.

Statistical analysis

Descriptive statistical analyses were performed in our study. Distributions of continuous variables were summarized as medians and interquartile ranges. Distributions of categorical variables were defined using frequencies and percentages. The Fisher exact test or the chi-square test was used to assess associations between categorical variables. Continuous variables were compared using non-parametric statistics. All tests were 2-sided, with a statistical significance level of 0.05. All statistical analyses were conducted using SPSS software (version 24.0; IBM Corporation, Armonk, NY).

Results

Baseline characteristics

Among 421 patients receiving ICI therapy who were tested for CDI within the study period, 41 met the inclusion criteria (Fig. 1). Patients' baseline clinical information is summarized in Table 1 while detailed characteristics are listed in Supplementary Table 1. Notably, 34 patients (83%) had positive NAAT and negative EIA for CDI. Immunosuppressant and proton pump inhibitor (PPI) use before CDI diagnosis was noted in 22 (54%) and 24 (59%) patients, respectively, and 56% of patients had taken antibiotics within the 3 months before the CDI diagnosis for various reasons, including infections and prophylaxis. All but 4 patients received antibiotics for CDI.

Comparing CDI with and without concurrent IMDC

Twenty-seven patients had concurrent CDI and IMDC, while 14 had CDI without IMDC (Table 2). Compared to those without IMDC, patients with concurrent IMDC had a higher frequency of systemic steroid use before CDI diagnosis (70% vs. 21%, P=0.007), a longer duration of diarrhea and colitis symptoms (20 vs. 5 days, P=0.003), and higher proportion of grade 3-4 diarrhea (41% vs 7%, P=0.033). The rest of the parameters were similar between the 2 groups.

Comparing CDI before and after immunosuppression among patients with concurrent IMDC

The 24 patients diagnosed with IMDC received immunosuppressive therapies such as steroids, vedolizumab and infliximab (Table 3). Among the 27 patients with concurrent IMDC, 67% had a positive NAAT test after steroid treatment for IMDC, while the rest had confirmed CDI before the initiation of immunosuppressive therapy. Those who developed CDI after the initiation of immunosuppression had significantly higher rates of preceding antibiotic (P=0.050) and PPI use (P=0.038) than those with CDI before immunosuppression administration. Over 90% of these patients received antibiotics for CDI regardless of their EIA status. Between patients who developed CDI before versus after the initiation of immunosuppressants, no differences were observed in rates of grade 3-4 diarrhea and colitis, duration of symptoms, need for hospitalization, overall symptom response rate or CDI recurrence.

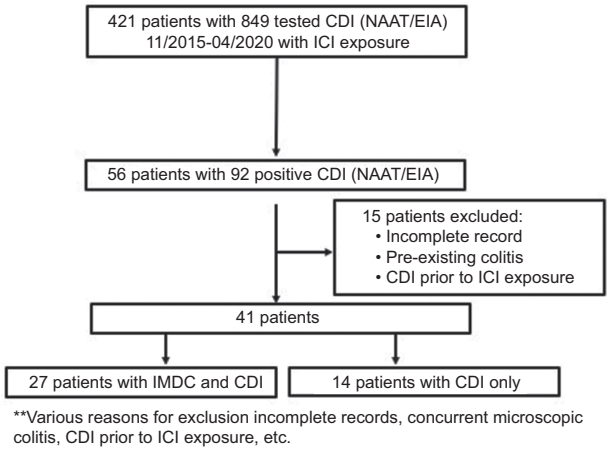

Figure 1 Flow chart showing patient selection process
CDI, Clostridioides difficile infection; NAAT, nucleic acid amplification test; EIA, enzyme immunoassay; ICI, immune checkpoint inhibitor; IMDC, immune-mediated diarrhea or colitis

Recurrent CDI

The overall rate of CDI recurrence was 24%. No significant associations were found between recurrent CDI and ICI type, preceding PPI or antibiotic use, cancer status at the time of CDI, severity of symptoms, CDI antibiotic treatment or concurrent IMDC (Supplementary Table 2).

Clinical characteristics related to CDI tests

Among the 41 patients in our cohort, 34 were NAAT+/EIA- and only 7 were NAAT+/EIA+ (Supplementary Table 3). EIA positivity was associated neither with more severe CDI in terms of severity and duration of symptoms, nor with a need for more aggressive CDI treatment. Rates of concurrent IMDC, and concurrent antibiotic and immunosuppressive treatment were also comparable between EIA- and EIA+ patients.

Clinical characteristics of patients with CDI and concurrent IMDC undergoing different treatments

Twenty-four patients with concurrent CDI and IMDC received immunosuppressants. Of these patients, 19 were treated with CDI antibiotics, while the remaining 5 patients, who had refractory colitis symptoms and severe CDI, underwent additional fecal microbiota transplantation (FMT) (Supplementary Table 4). Overall, patients who received FMT were the individuals with significantly higher grade 3-4 diarrhea (100% vs. 32%, P=0.011) and more frequent presentations of ulcers and non-ulcer inflammation on endoscopy at the time of IMDC diagnosis. Rates of symptom improvement were similar between the 2 groups, as were rates of recurrent diarrhea related to colitis and overall mortality. FMT-treated patients had no recurrent CDI, while the antibiotic/immunosuppressant-treated group had a 26% recurrence rate. Characteristics of the 5 patients who received FMT are listed in Supplementary Table 5.

Discussion

Cancer patients with CDI have significantly higher mortality and longer hospital stays than those without CDI [11]. IMDC

Table 1 Baseline clinical characteristics of cancer patients on ICI therapy and having CDI (N=41) identified by NAAT

Characteristic	Value
Age, median (SD) years	61 (19)
Male sex, no. (%)	21 (51)
Race, no. (%)	
Caucasian	36 (88)
Other	5 (12)
Concomitant comorbidities, no. (%)[a]	26 (63)
ECOG status, no. (%)	
0-2	29 (71)
3-4	12 (29)
Concurrent ICI-related colitis, no. (%)	27 (66)
Cancer type, no. (%)	
Melanoma	11 (27)
Genitourinary	15 (37)
Other[b]	15 (37)
Cancer status at time of CDI, no. (%)	
Remission	1 (2)
Stable disease	4 (10)
Progression	31 (76)
ICI type, no. (%)	
CTLA-4	3 (7)
PD-(L)1	27 (66)
Combination	11 (27)
Immunosuppressant use 3 months before CDI, no. (%)	22 (54)
PPI use 6 months before CDI, no. (%)	24 (59)
Antibiotic use 3 months before CDI, no. (%)	23 (56)
Antibiotic treatment for CDI, no. (%)	38 (93)
Median duration from CDI to last encounter, months (IQR), N=41	8 (23)
Overall mortality (%)[c]	17 (41)

[a]Comorbidities included diabetes mellitus, hypertension, cirrhosis, autoimmune disorder, coronary artery disease, chronic kidney disease, HIV, gastrointestinal graft vs. host disease

[b]Other cancers were primary lung, hematologic and gynecologic origin

[c]All deaths were due to underlying malignancy

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; FMT, fecal microbiota transplantation; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; ICI, immune checkpoint inhibitor; IQR, interquartile range; PD-(L)-1, programmed death-1/programmed death-1 ligand; CDI, Clostridioides difficile infection; NAAT, nucleic acid amplification test

from ICI therapy can mirror the diarrheal symptoms of CDI, and treatment of IMDC has been shown to predispose patients to GI infections [12]. Limited data exist concerning the clinical course and outcomes of CDI in patients with IMDC. In our study, we found that patients with concurrent CDI and IMDC were more likely to have a higher grade of diarrhea and a longer duration of symptoms than those with CDI alone. Despite antibiotic treatment for CDI, a majority of patients with IMDC still required immunosuppressant treatment in their disease course.

The IDSA states that, although more cases of CDI are detected from PCR NAAT, not all result in clinically significant CDI that requires treatment, citing insufficient evidence regarding CDI carriers [8]. The majority of cancer patients tested for CDI are EIA negative, so it is important for clinicians to understand what the best practice is in these cases [13]. In 2 separate studies, NAAT followed by a multistep toxin test showed greater detection of clinically significant CDI cases associated with a greater risk of developing CDI-related complications, compared with only NAAT positive cases [14,15]. It is thought that the failure to detect toxins may be due to a lack of toxin production, host defense successfully binding toxins, or a low bacterial burden of CDI at the time of testing [9,16]. In contrast, Kaltsas *et al* conducted a retrospective study among cancer patients with PCR+/EIA+ and PCR+/EIA- test results, and found similar symptom severity and 30-day mortality, with testing carried out mainly in patients presenting with diarrhea [17]. In all these studies, few conclusions were drawn in relation to treatment. This selective testing, when applied to patients receiving ICI therapy, can be more challenging because of the symptom overlap between CDI and IMDC. In our study, positive CDI NAAT testing was found in less than 10% of all ICI treated patients being tested for CDI. While the vast majority of these patients were EIA toxin negative, more than 90% received antibiotics at the discretion of the treatment team. Given the worse prognosis and mortality in this vulnerable cancer population with CDI [18], and the need for prompt treatment of IMDC with potent immunosuppressive agents, an aggressive approach is favored over conventional practice in real-world settings. The benefit of antibiotic treatment for CDI among patients with IMDC cannot be fully elucidated, given the lack of adequate control cases in our study; however, the need for immunosuppressive therapy to control IMDC, regardless of antibiotic treatment, argues against its beneficial role.

There are few publications in the literature on the overlap of IMDC and CDI. In a case series of 5 patients diagnosed with IMDC, only 1 was exposed to antibiotics before CDI, and 3 were administered immunosuppressants before the CDI episode, suggesting that IMDC was a risk factor for developing CDI [6]. Among our cohort of 41 patients, which is the largest study to date, 66% had concurrent IMDC. Compared to those without IMDC, patients with concurrent CDI and IMDC had more preceding systemic steroid use, a longer duration of diarrhea and colitis symptoms, and higher grades of diarrhea. On one hand, CDI contributes to inflammation by recruiting additional T-cells, leading to the development of autoimmunity to colonic mucosa and disruption of the gut microbiome [19,20]. On the other hand, more severe IMDC itself could result in greater susceptibility to CDI. Recognizing the challenge in differentiating between *Clostridioides difficile* colonization and real infection in the context of IMDC, treating physicians are more reluctant to initiate systemic immunosuppressant monotherapy for these patients, e.g. steroids without antibiotics, as steroids are associated with worse outcomes—including death—in patients with CDI [21]. Hypothetically, treating IMDC alone with immunosuppressants among our cohort might be sufficient to achieve symptom resolution and non-inferior to a combination of antibiotics and immunosuppression, acknowledging IMDC to be the main driver of symptoms. This is especially crucial considering that further disruption of the intestinal microbiome by multiple courses of antibiotics for various indications may

Table 2 Clinical characteristics of patients with CDI and IMDC compared with those who had CDI without IMDC

Characteristic	CDI w/ IMDC (N=27)	CDI w/o IMDC (N=14)	P-value[a]
Immune checkpoint inhibitors, no. (%)	2 (7)	1 (7)	>0.99
CTLA-4	15 (56)	12 (86)	0.084
PD-(L)1	10 (37)	1 (7)	0.064
Combination			
PPI use <3 months before CDI, no. (%)	17 (71)	7 (50)	0.512
Antibiotic use <3 months before CDI, no. (%)	15 (63)	8 (57)	0.623
Cancer progression at time of CDI, no. (%)	20 (74)	12 (86)	0.462
Steroid use <3 months before CDI, no. (%)	19 (70)	3 (21)	0.007
CTCAE grade of diarrhea			
1-2	16 (59)	13 (93)	0.033
3-4	11 (41)	1 (7)	
CTCAE grade of colitis			
1-2	24 (89)	12 (86)	>0.99
3-4	3 (11)	2 (14)	
Median duration of symptoms, days (IQR), N=41	20 (10-30)	5 (1-10)	0.003
ANC<1.5 K/μL within 7 days of CDI, no. (%)	4 (15)	2 (14)	>0.99
Peak Cr>1.5 within 7 days of CDI, no. (%)	0 (0)	2 (14)	0.111
Hospitalization/emergency room requirement related to CDI, no. (%)	17 (63)	7 (50)	0.424
Antibiotic treatment for CDI, no. (%)	23 (85)	14 (100)	0.280
Metronidazole monotherapy	4 (15)	4 (29)	0.411
Vancomycin monotherapy	17 (63)	9 (64)	0.675
Fidaxomicin monotherapy	0 (0)	0 (0)	N/a
Vancomycin–fidaxomicin combination therapy	2 (7)	1 (7)	>0.99
Colitis treatment[b], no. (%)	26 (96)	0 (0)	<0.001
Mesalamine	2 (7)	0 (0)	>0.99
Steroid/infliximab/vedolizumab	24 (89)	1 (7)[c]	<0.001
Fecal microbiota transplantation, no. (%)	5 (19)	0 (0)	0.147
Concurrent use of CDI antibiotic and immunosuppressants, no. (%)	22 (81)	1 (7)	<0.001
Symptom improvement after treatment, no. (%)	21 (78)	13 (93)	0.389
Recurrence of CDI after initial episode until last follow up, no. (%)	8 (30)	1 (7)	0.131

[a]P values were calculated via Pearson's chi-square test. Fisher's exact text was used when there were less than 5 subjects in a group

[b]One patient with mild non-diarrheal symptoms was treated symptomatically. This patient received corticosteroids empirically for initial suspicion of IMDC, but these were quickly discontinued after a CDI-positive result and no further investigation was done

IMDC, immune-mediated diarrhea or colitis; CDI, Clostridioides difficile infection; ANC, absolute neutrophil count; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-(L)-1, programmed death-1/programmed death-1 ligand; IQR, interquartile range; PPI, proton pump inhibitor; CTCAE, common terminology criteria for adverse events

have a deleterious effect on tumor response to immunotherapy [22]. Furthermore, antibiotic treatment has also been associated with an increased risk of severe IMDC, especially when given after immunotherapy [23]. With the higher recurrence rate of CDI among patients with concurrent IMDC, likely due to the multiple rounds of immunosuppression administered, future studies are urgently required to explore a safer and more effective treatment strategy for patients with coexisting IMDC and CDI.

Until those studies are performed, we can look to an existing disease, such as inflammatory bowel disease (IBD), to provide a plausible biological basis linking inflammation and CDI. Many studies have already shown the link between CDI and IBD [24]. It is thought that IBD results from an overactive inflammatory response to commensal intestinal microbiota; this dysbiosis creates an environment susceptible to CDI [21]. In a similar fashion, immunotherapy upregulates the immune system and could disrupt the gut microbiome to provide the ideal environment for *Clostridioides difficile* colonization and infection. Given the high rates of colonization with PCR-only positive stool studies, it is recommended to only test and treat IBD patients with significant diarrhea and both PCR/EIA positive [22]. After being treated with antibiotics initially for CDI, patients generally receive escalating immunosuppressive therapy. In one recent study, PCR+/EIA- IBD patients had a poor response to antibiotics, and a majority of these patients required escalation of IBD therapy, thus suggesting that many of these patients were colonized rather than having true CDI [20]. However, complication rates in PCR+ and EIA+ patients were similar. This is in contrast to other studies

Table 3 Clinical characteristics of patients with CDI and immune-mediated diarrhea and colitis, before and after immunosuppressant use (N=24)

Characteristic	CDI before immunosuppression (N=6)	CDI after immunosuppression (N=18)	P-value
Immune checkpoint inhibitors (%)			
CTLA-4	0 (0)	2 (11)	>0.99
PD-1/L1	4 (67)	9 (50)	0.694
Combination	2 (33)	7 (39)	>0.99
PPI use <3 months before CDI, no. (%)	2 (33)	15 (83)	0.038
Antibiotic use <3 months before CDI, no. (%)	1 (17)	13 (72)	0.050
Cancer progression at time of CDI, no. (%)	4 (67)	12 (67)	>0.99
CTCAE grade of diarrhea, no. (%)			
1-2	2 (33)	12 (67)	0.192
3-4	4 (67)	6 (33)	
CTCAE grade of colitis, no. (%)			
1-2	5 (83)	16 (89)	>0.99
3-4	1 (17)	2 (11)	
Median duration of symptoms, days (IQR), N=24	20 (10-30)	16 (9-28)	0.680
ANC<1.5 K/μL within 7 days of CDI, no. (%)	1 (17)	3 (17)	>0.99
Hospitalization/emergency room requirement related to CDI, no. (%)	4 (67)	12 (67)	>0.99
Antibiotic treatment for CDI, no. (%)	6 (100)	16 (89)	>0.99
Treatments for the initial CDI episode, no. (%)			
Metronidazole monotherapy	1 (13)	3 (17)	>0.99
Vancomycin monotherapy	5 (83)	11 (61)	0.621
Fidaxomicin monotherapy	0 (0)	0 (0)	N/A
Combination	0 (0)	2 (11)	>0.99
Symptom improvement after treatment, no. (%)	5 (83)	13 (72)	>0.99
Recurrence of CDI by the last follow up, no. (%)	1 (17)	6 (33)	0.629

Fisher's exact test was used given the small number of categories

IQR, interquartile range; CTCAE, common terminology criteria for adverse events, CDI, Clostridioides difficile infection; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-(L)-1, programmed death-1/programmed death-1 ligand

examining CDI in the general population, which have shown that patients with only EIA+ had significantly higher CDI-related complication rates compared to patients with only PCR+ [15]. As shown in our study, most patients at MD Anderson receive antibiotics, considering the potential implications of untreated infections in immunocompromised patients. While similarities exist between IBD and IMDC, cancer patients receiving immunotherapy deserve unique considerations pertaining to CDI diagnosis and treatment.

Gut dysbiosis has been shown to be associated with CDI, carcinogenesis, and IMDC [25]. FMT is the standard of care for recurrent CDI and has demonstrated a benefit, not only for refractory IMDC [8,26], but even for malignancy that is refractory to immunotherapy to recapture response [27,28]. Regarding the restoration of healthy gut microbiota, in our study, FMT eradicated CDI test positivity in all 5 treated patients and significantly improved IMDC symptoms in 4 of them. This avoided the need to interrupt IMDC management and cancer treatment because of CDI later on. Thus, FMT appeared to be the more favorable alternative, with higher efficacy and a better safety profile in eradicating CDI and treating IMDC than the standard immunosuppressive and antibiotic regimens.

Our study was limited by its small sample size and retrospective nature. Inconsistent documentation in conjunction with the subjectivity of symptom duration and severity in certain patients' electronic health records made data collection more challenging. Most of our patients received antibiotic treatment and only a small group did not, so our study did not have sufficient power to assess the benefit from antibiotic treatment. In addition, we had a very small proportion of patients with EIA+/NAAT+ results, which limited our subgroup analysis to measure the predictive value of EIA among this population.

In conclusion, CDI can occur in nearly 10% of ICI-treated cancer patients with GI symptoms, and may coexist with IMDC at the onset of and after immunosuppressant treatment. Antibiotics did not alter the need for immunosuppressant treatment for those with concurrent IMDC in our cohort; hence, FMT could be an optimal alternative option in treating CDI and concurrent IMDC with favorable outcomes, while avoiding unnecessary exposure to antibiotics and immunosuppression. CDI with IMDC may lead to recurrent symptoms, a prolonged disease course and ICI discontinuation with potentially worse cancer outcomes. This finding emphasizes the need for a multidisciplinary approach to evaluate and treat these patients

appropriately. Further large-scale prospective studies are warranted to clarify the role of CDI in patients with IMDC and the efficacy and safety of antibiotics, immunosuppressants and FMT in this population.

Summary Box

What is already known:

- Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but are associated with immune mediated diarrhea and colitis (IMDC)
- *Clostridioides difficile* infection (CDI) can cause infectious diarrhea with overlapping symptoms

What the new findings are:

- In our study, we found CDI to be relatively common in ICI-treated cancer patients, especially those with IMDC requiring immunosuppressants
- Antibiotics did not alter the need for immunosuppressants for those with concurrent IMDC
- The use of proton pump inhibitors and antibiotics while receiving immunosuppressant therapy for IMDC was associated with an increased risk of CDI

References

1. Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol* 2012;**30**:2691-2697.
2. Puzanov I, Diab A, Abdallah K, et al; Society for Immunotherapy of Cancer Toxicity Management Working Group. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society For Immunotherapy of Cancer (SITC) toxicity management working group. *J Immunother Cancer* 2017;**5**:95.
3. Gong Z, Wang Y. Immune checkpoint inhibitor-mediated diarrhea and colitis: a clinical review. *JCO Oncol Pract* 2020;**16**:453-461.
4. Wang Y, Abu-Sbeih H, Mao E, et al. Immune-checkpoint inhibitor-induced diarrhea and colitis in patients with advanced malignancies: retrospective review at MD Anderson. *J Immunother Cancer* 2018;**6**:37.
5. Dougan M, Wang Y, Rubio-Tapia A, Lim JK. AGA clinical practice update on diagnosis and management of immune checkpoint inhibitor colitis and hepatitis: expert review. *Gastroenterology* 2021;**160**:1384-1393.
6. Babacan NA, Tanvetyanon T. Superimposed *Clostridium difficile* infection during checkpoint inhibitor immunotherapy-induced colitis. *J Immunother* 2019;**42**:350-353.
7. Del Castillo M, Romero FA, Argüello E, Kyi C, Postow MA, Redelman-Sidi G. The spectrum of serious infections among patients receiving immune checkpoint blockade for the treatment of melanoma. *Clin Infect Dis* 2016;**63**:1490-1493.
8. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis* 2018;**66**:e1-e48.
9. Polage CR, Chin DL, Leslie JL, Tang J, Cohen SH, Solnick JV. Outcomes in patients tested for *Clostridium difficile* toxins. *Diagn Microbiol Infect Dis* 2012;**74**:369-373.
10. Ma W, Gong Z, Abu-Sbeih H, et al. Outcomes of immune checkpoint inhibitor-related diarrhea or colitis in cancer patients with superimposed gastrointestinal infections. *Am J Clin Oncol* 2021;**44**:402-408.
11. Delgado A, Reveles IA, Cabello FT, Reveles KR. Poorer outcomes among cancer patients diagnosed with *Clostridium difficile* infections in United States community hospitals. *BMC Infect Dis* 2017;**17**:448.
12. Ni J, Zhang X, Zhang L. Opportunistic bowel infection after corticosteroid dosage tapering in a stage IV lung cancer patient with tislelizumab-related colitis. *Thorac Cancer* 2020;**11**:1699-1702.
13. Yepez Guevara EA, Aitken SL, Olvera AV, et al. *Clostridioides difficile* infection in cancer and immunocompromised patients: relevance of a two-step diagnostic algorithm and infecting ribotypes on clinical outcomes. *Clin Infect Dis* 2021;**72**:e460-e465.
14. Longtin Y, Trottier S, Brochu G, et al. Impact of the type of diagnostic assay on *Clostridium difficile* infection and complication rates in a mandatory reporting program. *Clin Infect Dis* 2013;**56**:67-73.
15. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. *JAMA Intern Med* 2015;**175**:1792-1801.
16. Delmée M, Van Broeck J, Simon A, Janssens M, Avesani V. Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea: a plea for culture. *J Med Microbiol* 2005;**54**:187-191.
17. Kaltsas A, Simon M, Unruh LH, et al. Clinical and laboratory characteristics of *Clostridium difficile* infection in patients with discordant diagnostic test results. *J Clin Microbiol* 2012;**50**:1303-1307.
18. Gupta A, Tariq R, Frank RD, et al. Trends in the incidence and outcomes of hospitalized cancer patients with *Clostridium difficile* infection: a nationwide analysis. *J Natl Compr Canc Netw* 2017;**15**:466-472.
19. Becattini S, Taur Y, Pamer EG. Antibiotic-induced changes in the intestinal microbiota and disease. *Trends Mol Med* 2016;**22**:458-478.
20. Gupta A, Wash C, Wu Y, Sorrentino D, Nguyen VQ. Diagnostic modality of *Clostridioides difficile* infection predicts treatment response and outcomes in inflammatory bowel disease. *Dig Dis Sci* 2021;**66**:547-553.
21. Nitzan O, Elias M, Chazan B, Raz R, Saliba W. *Clostridium difficile* and inflammatory bowel disease: role in pathogenesis and implications in treatment. *World J Gastroenterol* 2013;**19**:7577-7585.
22. Khanna S, Shin A, Kelly CP. Management of *Clostridium difficile* infection in inflammatory bowel disease: expert review from the clinical practice updates committee of the AGA Institute. *Clin Gastroenterol Hepatol* 2017;**15**:166-174.
23. Abu-Sbeih H, Herrera LN, Tang T, et al. Impact of antibiotic therapy on the development and response to treatment of immune checkpoint inhibitor-mediated diarrhea and colitis. *J Immunother Cancer* 2019;**7**:242.
24. Singh H, Nugent Z, Yu BN, Lix LM, Targownik LE, Bernstein CN. Higher incidence of *Clostridium difficile* infection among individuals with inflammatory bowel disease. *Gastroenterology* 2017;**153**:430-438.
25. Sheflin AM, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. *Curr Oncol Rep* 2014;**16**:406.
26. Wang Y, Wiesnoski DH, Helmink BA, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med* 2018;**24**:1804-1808.
27. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* 2021;**371**:602-609.
28. Davar D, Dzutsev AK, McCulloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* 2021;**371**:595-602.

Supplementary material

Supplementary Table 1 Baseline clinical characteristics of cancer patients on ICI therapy and with CDI (N=41) identified by NAAT

ID	Age	Sex	Primary Cancer	ICI type	ECOG	Cancer Status at time of CDI	Immunosuppressant use[a]	PPI use[b]	Antibiotic use[c]	CDI Antibiotic treatment	ICI colitis treatment
1	22	M	Hodgkin's lymphoma	PD-1	1	Response	Yes	Yes	Yes	Yes	Yes
2	25	M	Melanoma	PD-1	1	Progression	Yes	Yes	Yes	Yes	Yes
3	26	M	Acute myeloid leukemia	PD-1	1	Remission	Yes	No	Yes	Yes	No
4	31	M	Head & neck squamous	PD-1	3	Progression	Yes	Yes	No	Yes	Yes
5	36	M	Melanoma	PD-1, CTLA-4	2	Progression	Yes	Yes	Yes	No	Yes
6	39	M	Melanoma	PD-1	4	Progression	No	Yes	No	Yes	No
7	45	F	Lung adenocarcinoma	PD-1, CTLA-4	1	Progression	No	Yes	No	Yes	Yes
8	46	F	Cervical	PD-L1	2	Progression	No	No	Yes	Yes	No
9	49	M	Melanoma	PD-1, CTLA-4	4	Progression	Yes	Yes	Yes	Yes	No
10	49	M	Renal cell	PD-1	1	Progression	No	No	No	Yes	Yes
11	55	F	Large cell neuroendocrine	PD-1	1	Progression	Yes	Yes	No	Yes	No
12	56	F	Colon	PD-1	3	Progression	No	No	No	Yes	No
13	57	M	Small cell	PD-L1	2	Progression	No	No	No	Yes	No
14	58	M	Melanoma	PD-1, CTLA-4	1	Response	Yes	No	No	Yes	Yes
15	58	F	Melanoma	PD-1, CTLA-4	3	Progression	Yes	Yes	No	Yes	Yes
16	60	F	Melanoma	PD-1, CTLA-4	1	Progression	Yes	Yes	Yes	Yes	Yes
17	60	M	Renal cell	PD-1, CTLA-4	1	Progression	No	No	No	Yes	No
18	61	F	Urothelial	PD-1, CTLA-4	1	Response	Yes	Yes	Yes	No	Yes
19	63	F	Renal cell	PD-1	1	Progression	No	No	No	Yes	No
20	64	F	Cervical	PD-L1	3	Progression	Yes	No	Yes	No	No
21	65	F	Renal cell	PD-1	1	Stable	Yes	Yes	No	Yes	No
22	65	F	Lung adenocarcinoma	PD-1	4	Progression	No	No	No	Yes	Yes
23	65	M	Urothelial	PD-1	1	Progression	No	No	No	Yes	No
24	67	F	Renal cell	PD-1, CTLA-4	3	Progression	Yes	Yes	Yes	Yes	Yes
25	69	F	Urothelial	PD-L1	4	Progression	No	Yes	Yes	Yes	No
26	69	F	Ovarian	PD-L1	1	Stable	No	No	No	Yes	Yes
27	70	M	Prostate	PD-1	2	Progression	Yes	Yes	Yes	Yes	No
28	71	M	Prostate	CTLA-4	1	Stable	Yes	Yes	Yes	Yes	No
29	71	M	Renal	PD-1	1	Progression	No	No	No	No	Yes
30	72	M	Renal	PD-1, CTLA-4	1	Progression	No	No	Yes	Yes	No

(Contd...)

Supplementary Table 1 (*Continued*)

ID	Age	Sex	Primary Cancer	ICI type	ECOG	Cancer Status at time of CDI	Imm unosuppressant use[a]	PPI use[b]	Antibiotic use[c]	CDI Antibiotic treatment	ICI colitis treatment
31	73	M	Melanoma	CTLA-4	2	Progression	Yes	Yes	Yes	Yes	Yes
32	74	M	Lung adenocarcinoma	PD-1	2	Progression	Yes	Yes	Yes	Yes	Yes
33	76	F	Urothelial	PD-1	2	Progression	Yes	Yes	Yes	Yes	No
34	78	M	Urothelial	CTLA-4	1	Stable	Yes	No	No	Yes	Yes
35	79	M	Melanoma	PD-1	2	Progression	Yes	No	Yes	Yes	No
36	79	F	Neuroendocrine	PD-1	3	Progression	No	Yes	Yes	Yes	No
37	79	F	Follicular lymphoma	PD-L1	2	Response	No	No	Yes	Yes	Yes
38	81	F	Melanoma	PD-1	3	Response	Yes	Yes	Yes	Yes	Yes
39	81	F	Acute myeloid leukemia	PD-1	1	Progression	No	Yes	Yes	Yes	No
40	83	M	Melanoma	PD-1	3	Progression	No	Yes	Yes	Yes	No
41	84	F	Lung adenocarcinoma	PD-1, CTLA-4	2	Progression	No	Yes	No	Yes	Yes

[a]3 months before CDI

[b]6 months before CDI

[c]3 months before CDI

ECOG, Eastern Cooperative Oncology Group; CDI, Clostridioides difficile infection CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-(L)-1, programmed death-1/programmed death-1 ligand; NAAT, nucleic acid amplification test; ICI, immune checkpoint inhibitor; PPI, proton pump inhibitor

Supplementary Table 2 Clinical characteristics of patients with and without recurrence of CDI (N=41)

Characteristic	Recurrence (N=10)	No recurrence (N=31)	P-value
ICI			
CTLA-4	1 (10)	2 (6)	>0.99
PD-1/L1	7 (70)	20 (65)	>0.99
Combination	2 (20)	9 (29)	0.700
PPI use <3 months before CDI, no. (%)	5 (50)	19 (61)	0.714
Antibiotic use <3 months before CDI, no. (%)	4 (40)	19 (61)	0.289
Cancer progression at time of CDI, no. (%)	7 (70)	24 (77)	0.683
Concurrent IMDC, no. (%)	8 (80)	19 (61)	0.448
Patients with negative EIA, no. (%)	9 (90)	25 (81)	0.660
CTCAE grade of diarrhea, no. (%)			
1-2	4 (40)	24 (77)	0.911
3-4	6 (60)	6 (19)	
CTCAE grade of colitis			
1-2	10 (100)	26 (84)	0.310
3-4	0 (0)	5 (16)	
Median duration of symptoms, days (IQR), N=41	19 (13-30)	10 (5-20)	0.128
ANC <1.5 K/μL within 7 days of CDI, no. (%)	1 (10)	5 (16)	>0.99
Peak Cr >1.5 within 7 days of CDI, no. (%)	0 (0)	2 (6)	>0.99
Hospitalization/emergency room requirement related to CDI, no. (%)	7 (70)	17 (55)	0.480
Antibiotic treatment for CDI, no. (%)	10 (100)	27 (87)	0.556
Treatments for the initial CDI episode, no. (%)			
Metronidazole monotherapy	1 (10)	7 (23)	0.653
Vancomycin monotherapy	6 (60)	20 (65)	>0.99
Fidaxomicin monotherapy	0 (0)	0 (0)	n/a
Combination	3 (30)	0 (0)	0.011
Symptom improvement after treatment no. (%)	8 (80)	26 (84)	>0.99
Antibiotic use for non-CDI related infection after CDI treatment	6 (60)	24 (77)	0.413
Immunosuppressant use after CDI treatment	7 (70)	17 (55)	0.480

Fisher's exact test was used given the small number of categories

CTCAE, common terminology criteria for adverse events; CDI, Clostridioides difficile infection CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-(L)-1, programmed death-1/programmed death-1 ligand; ICI, immune checkpoint inhibitor; PPI, proton pump inhibitor; EIA, enzyme immunoassay; ICI, immune checkpoint inhibitor; IMDC, immune-mediated diarrhea or colitis; IQR, interquartile range; ANC, absolute neutrophil count; Cr, creatinine mg/dL

Supplementary Table 3 Clinical characteristics of patients with immune checkpoint inhibitor therapy that were tested for CDI (N=41) by NAAT and EIA

Characteristic	NAAT+/EIA- (N=34)	NAAT+/EIA+ (N=7)
CTCAE grade of diarrhea, no. (%)		
1-2	23 (68)	5 (71)
3-4	10 (29)	2 (29)
CTCAE grade of colitis, no. (%)		
1-2	30 (88)	6 (86)
3-4	4 (12)	1 (14)
Median duration of symptoms, days (IQR), N=41	13 (5-29)	10 (7-15)
ANC <1.5 K/μL within 7 days of CDI, no. (%)	6 (18)	0 (0)
Peak Cr >1.5 within 7 days of CDI, no. (%)	1 (3)	1 (14)
Immunosuppression use within 60 days of CDI, no. (%)	17 (50)	5 (71)
Antibiotic use within 60 days of CDI, no. (%)	17 (50)	6 (86)
Treatments for the initial CDI episode, no. (%)		
Metronidazole monotherapy	6 (18)	1 (14)
Vancomycin monotherapy	21 (62)	5 (71)
Fidaxomicin monotherapy	0 (0)	0 (0)
Combination	0 (0)	1 (14)
Fecal microbiota transplantation	3 (9)	2 (29)
Concurrent IMDC, no. (%)	18 (53)	4 (57)
Immunosuppressant treatment for IMDC, no. (%)	20 (59)	4 (57)
Concurrent use of antibiotic and immunosuppressants, no. (%)	18 (53)	3 (43)
Symptom improvement after treatment, no. (%)	28 (82)	5 (71)

CDI treatments include antibiotics and fecal transplantation

CDI, Clostridioides difficile infection; FMT, fecal microbiota transplantation; IQR, interquartile range; IMDC, immune-mediated diarrhea and colitis; CTCAE, common terminology criteria for adverse events; NAAT, nucleic acid amplification test; EIA, enzyme immunoassay; ANC, absolute neutrophil count; Cr, creatinine mg/dL

Supplementary Table 4 Clinical characteristics of patients with CDI treated with FMT vs. antibiotic/IMS group among IMDC patients

Characteristic	FMT treated group (N=5)	Antibiotic/ IMS treated group (N=19)
ECOG, no. (%)		
0-2	4 (80)	13 (68)
3-4	1 (20)	6 (32)
Cancer type, no. (%)		
Melanoma	1 (20)	7 (37)
Genitourinary	2 (40)	3 (16)
Other	2 (40)	9 (47)
ANC <1.5 K/μL within 7 days of CDI, no. (%)	0 (0)	5 (26)
Hospitalization for diarrhea, no. (%)	4 (80)	12 (63)
CTCAE grade of diarrhea, no. (%)		
3-4	5 (100)	6 (32)
CTCAE grade of colitis, no. (%)		
1-2	4 (80)	12 (63)
3-4	1 (20)	7 (37)
Endoscopic findings		
Ulcers	2 (40)	2 (11)
Non-ulcer inflammation	2 (40)	6 (32)
Normal	0 (0)	5 (26)
Concurrent IMDC, no. (%)	5 (100)	12 (63)
Symptom improvement, no. (%)	4 (80)	16 (84)
CDI eradicated, no. (%)	5 (100)	14 (74)
Recurrent CDI by last follow up	0 (0)	5 (26)
Recurrent diarrhea due to IMDC	1 (20)	6 (32)
Overall mortality, no. (%)*	1 (20)	9 (50)

*All deaths were due to underlying malignancy

ANC, absolute neutrophil count; CDI, Clostridioides difficile infection; ECOG, Eastern Cooperative Oncology Group; FMT, fecal microbiota transplantation; IMS, immunosuppressant; IQR, interquartile range; SD, standard deviation; WBC, white blood cell; IMDC, immune-mediated diarrhea and colitis; CTCAE, common terminology criteria for adverse events

Supplementary Table 5 FMT treated patients (N=5)

Characteristic	Value
Age, mean (SD), years	55 (20)
Male sex, no. (%)	2 (40)
Concomitant comorbidities, no. (%)	3 (60)
ECOG status, no. (%)	
0-2	4 (80)
3-4	1 (20)
Cancer type, no. (%)	
Melanoma	1 (20)
Genitourinary	3 (60)
Lung	1 (20)
Hematologic	1 (20)
Cancer status at time of CDI, no. (%)	
Remission	0 (0)
Stable disease	4 (80)
Progression	1 (20)
Immunosuppressant use 3 months before CDI, no. (%)	3 (60)
PCR+/EIA-, no. (%)[a]	3 (60)
Median duration from CDI to last encounter, (IQR), months	7 (6-16)
IMDC improved/resolved, no. (%)	4 (80)
Overall mortality no. (%)	1 (20)

[a]The other 2 cases were PCR+/EIA+

FMT, fecal microbiota transplantation; SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; CDI, Clostridioides difficile infection; IQR, interquartile range; PCR, polymerase chain reaction; EIA, enzyme immunoassay; IMDC, immune-mediated diarrhea and colitis