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Original Article

Prevalence of Enteric Infections in Patients on Immune Checkpoint Inhibitors and Impact on Management and Outcomes

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Abstract

Background: Stool pathogen testing is recommended as part of the initial evaluation for patients with new-onset diarrhea on immune checkpoint inhibitors (ICIs), yet its significance has not been well-studied. We aimed to determine the impact of multiplex gastrointestinal (GI) pathogen PCR testing on the clinical course and use of immunosuppressive therapy in patients who develop diarrhea on ICIs.

Methods: This retrospective cohort included individuals who underwent GI pathogen panel PCR for diarrhea on ICIs at Memorial Sloan Kettering between 7/2015 and 7/2021. The primary outcome was use of immunosuppressive therapy for suspected immunotherapy-related enterocolitis (irEC). Secondary outcomes included diarrhea severity and endoscopic and histologic disease patterns.

Results: Among 521 ICI-treated patients tested for GI pathogens, 61 (11.7%) had a positive PCR. Compared to patients without detectable infections, patients with infections had more frequent grades 3-4 diarrhea (37.7% vs. 19.6%, $P < .01$) and colitis (39.3% vs. 14.7%, $P < .01$). However, patients with infections did not have higher rates of persistent or recurrent diarrhea and were less likely to receive steroids ($P < .01$) and second-line immunosuppressive agents ($P = .03$). In 105 patients with lower endoscopy, similar trends were observed and no differences in endoscopic severity or histologic patterns were noted between groups.

Conclusions: GI infections in ICI-treated patients presenting with diarrhea are linked to more severe but self-limited clinical presentations and may be optimally treated with observation and supportive care alone. Routine and timely stool pathogen testing may help avert unnecessary empiric immunosuppression for suspected irEC, which has been linked to blunted antitumor responses and numerous adverse effects.

Key words: immune checkpoint inhibitors; enteric infections; immune-related enterocolitis; GI PCR.

Implications for Practice

Stool pathogen testing is recommended for patients with new-onset diarrhea on immune checkpoint inhibitors, but the impact of results on clinical treatment decisions has not been thoroughly investigated. We found patients with superimposed enteric infections and suspected immune-related enterocolitis had more severe clinical presentations, but no higher rates of persistent or recurrent diarrhea, no differences in endoscopic severity or histologic patterns, and were less likely to receive immunosuppressive therapies. Thus, enteric infections in ICI-treated patients presenting with diarrhea are linked to more severe but self-limited presentations, which may be optimally treated with supportive care alone. Timely stool pathogen testing may help avert unnecessary empiric immunosuppression for suspected immune-related enterocolitis.

Background

The advent of immune checkpoint inhibitors (ICIs) and their unique antitumor mechanism of disinhibiting immune surveillance heralded a significant advance in the treatment of numerous malignancies. However, unlike the cytotoxic adverse events of traditional chemotherapy, ICI-induced immune stimulation commonly results in immune-related adverse events (irAEs) that can mimic an autoimmune inflammatory state. The gastrointestinal (GI) tract is a major site of toxicity with the most common location being the colon in up to 40% of patients receiving anti-CTLA-4 alone or in combination with anti-PD-1/PD-L1 therapy and up to 17%

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receiving anti-PD-1 therapy alone.[1,2] Immune-related enterocolitis (irEC), which can range from mild self-limited diarrhea to a life-threatening condition, is therefore a frequent and significant contributor to ICI-related morbidity and rarely mortality, and represents the most common irAE requiring cessation of ICI therapy.[3,4]

Current guidelines recommend stool pathogen testing in patients with new-onset diarrhea on ICIs; however, the yield of these tests and the impact on clinical course have not been well-studied.[5,6] Guidelines also recommend empiric immunosuppression for most patients with grade ≥2 diarrhea on ICIs and thus how to manage patients with confirmed infections and whether to withhold immunosuppression is an unanswered question.[2,5,6] The availability of highly sensitive multiplex molecular panels has enabled the rapid and accurate identification of previously undiagnosable enteric infections, which can often mimic or exacerbate irEC symptoms.[7] In patients with inflammatory bowel disease (IBD), such panels have been recognized as being important to inform optimal treatment decisions, including pathogen-directed antimicrobial therapies, limitation of intervention to supportive care in self-limited disease courses, and avoidance of unnecessary escalation to immunosuppressive agents.[8,9] Due to similarities between IBD and irEC, GI PCR may be instrumental in supporting clinical decision-making.[6,10]

By analyzing ICI-treated patients who had GI PCR testing performed for evaluation of new-onset diarrhea, we aim to inform clinical decision-making at this important juncture and the impact on clinical course. The primary aim of this study was to assess the clinical significance of molecular enteric pathogen detection on the use of immunosuppressive therapy for irEC. We also characterize the prevalence and types of enteric infections present in these patients, differences in diarrhea severity and outcomes, results of endoscopic and histologic evaluations, and the impact of antibiotic therapy on their clinical course and outcomes.

Methods

Study Population

This single-center, retrospective study included all individuals undergoing treatment with ICIs and presenting with new-onset diarrhea who underwent GI pathogen panel PCR testing at Memorial Sloan Kettering Cancer Center (MSKCC) between July 2015 and July 2021. We excluded patients with a prior diagnosis of IBD, HIV, celiac disease, diagnosis of *Clostridioides difficile* infection within 7 days of or concurrent with PCR, prior treatment for irEC, empiric steroids started 14 days prior to PCR for any reason, and empiric steroids after PCR for non-irEC indications.

Data Collection

Data regarding demographics, PCR results, diarrhea, immunosuppressive therapy, antibiotics, clinical outcomes, and endoscopic evaluations were collected. Diarrhea and colitis were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 5.0. Endoscopic scoring was adapted from the Mayo endoscopic scoring system.[4] Colitis histological subtypes were categorized from pathology reports as normal colonic mucosa, acute colitis, chronic active colitis, microscopic colitis (lymphocytic or collagenous), and GVHD-like (apoptosis-predominant) colitis according to previously-described patterns.[11]

Gastrointestinal PCR Panel Stool Test

Two PCR panels were used over the course of the study. The Luminex xTAG GI Pathogen Panel (Luminex Corporation), an FDA-cleared panel targeting 14 pathogens was used from 2014 to 2017. The FilmArray Gastrointestinal Panel (BioFire Diagnostics), a panel capable of detecting 22 GI pathogens was implemented in 2017. The equivalent analytical performance of the 2 panels was previously described.[12] A full list of covered pathogens is shown in Supplementary Table S1.

Outcomes

The primary outcome was use of immunosuppressive therapy for irEC, including corticosteroids and second-line biologic agents, such as infliximab and vedolizumab. Secondary outcomes included prevalence and types of enteric infections, differences in clinical, endoscopic, and histologic assessments, and the impact of antibiotic and steroid therapy on clinical outcomes of ICI-treated patients with diagnosed GI infections. Time from diarrhea onset to clinical response was defined as the first observed decrease to grade ≤1 symptoms with at least one grade decrease from initial diagnosis. Time from diarrhea onset to resolution was defined as grade ≤1 symptoms for at least 30 days duration. Overall survival (OS) was calculated from the date of ICI initiation to date of death or last follow-up and landmarked at 6 months from ICI initiation.

Statistical Analysis

Continuous variables were summarized as mean and SDs if normally distributed and as median and interquartile range (IQR) if not normally distributed. Categorical variables were summarized as counts and percentages. Student's *t*-tests and Wilcoxon's rank sum tests were used to compare continuous variables while chi-square tests and Fisher's exact tests were used for categorical variables. Multivariable logistic regression models were used to evaluate the independent effect of potential predictors of steroids or second-line immunosuppressive agent usage. Median OS was estimated using Kaplan-Meier curves with log-rank tests to compare durations between groups. To ensure that differences in survival between patients based on GI PCR results or antibiotic treatment status were not due to immortal time bias, we used a 6-month landmark analysis to examine patients who developed diarrhea within 6 months of ICI initiation (the median onset time in our study cohort) and remained alive after the landmark time. Patients were stratified by GI PCR status in one analysis and antibiotic treatment status in another. For both, outcome events were only considered if they occurred after the 6-month landmark. An alpha of 0.05 was considered significant. Statistical calculations were performed using Stata Statistical Software: Release 17 (StataCorp). This study was approved by the Institutional Review Board at MSKCC.

Results

Study Cohort

Of 992 ICI-treated adult patients presenting with diarrhea who underwent a GI PCR within the study period, 521 patients met the inclusion criteria (Fig. 1). Within this group, 61 (11.7%) tested positive for a GI pathogen. The

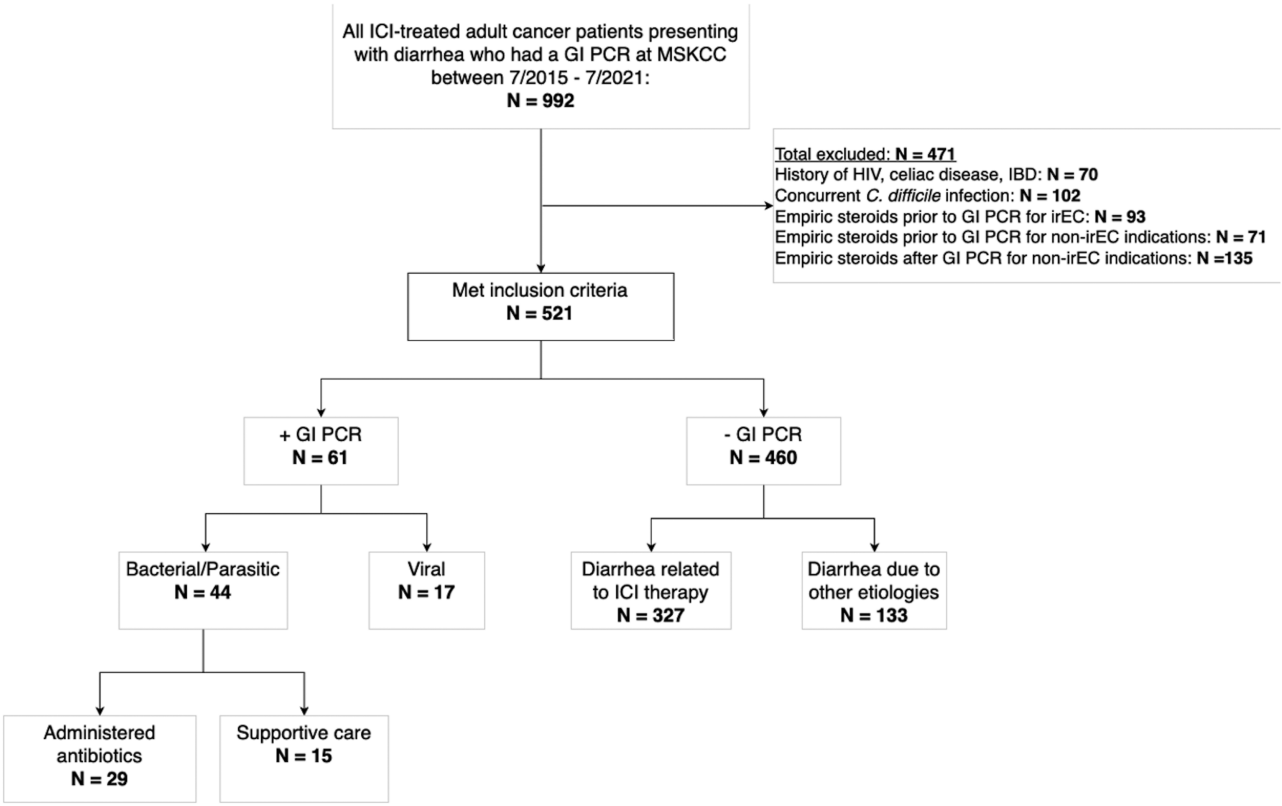

Figure 1. Patient allocation by inclusion criteria and PCR result.

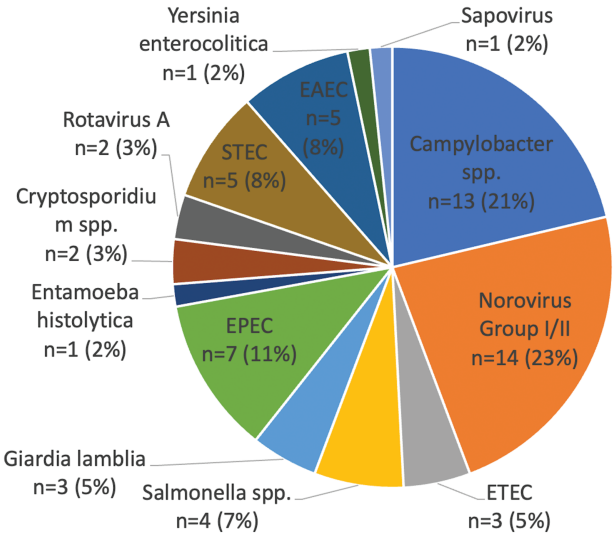

Figure 2. Enteric infection type and prevalence.

3 most commonly detected pathogens were Norovirus (n = 14), *Campylobacter* spp. (n = 13), and enteropathogenic *Escherichia coli* (n = 7) (Fig. 2). Demographic, cancer, and ICI characteristics are summarized in Table 1. Patients testing positive on PCR tended to be younger than patients testing negative (56.6 vs. 61.3 years, P = .02). Most of the patients were male (n = 283, 54.3%), White (n = 418, 80.2%), and non-Hispanic (n = 479, 91.9%). The most common individual cancer type was genitourinary in 125 patients (24.0%), and the median duration of ICI treatment was comparable between groups (6.9 vs. 5.5 months, P = .99).

Of 460 patients testing negative on GI PCR, 190 (41.3%) had diarrhea etiologies attributed to irEC that was biopsy-proven and/or of high enough clinical suspicion to warrant prescription of immunosuppressive therapy while 137 (29.8%) had clinical suspicion of ICI-related diarrhea but were not given steroids due to the diarrhea's transient or low-grade nature (Table 2). Among the remaining patients, 58 (12.6%) had diarrhea due to other drugs and 75 (16.3%) due to other non-drug etiologies. For subsequent analyses, we excluded individuals with these other identified etiologies and only included the 327 PCR-negative patients with suspicion for irEC with the 61 PCR-positive patients (388 total).

Clinical Course and Treatment

The time from ICI initiation to diarrhea onset and median duration of diarrhea were similar between groups (PCR positive vs. negative; 101.4 vs. 82.1 days, P = .78; and 8.1 vs. 8.1 days, P = .80, respectively) (Table 3). However, patients with infections had more severe clinical presentations than those without identified infections, including higher rates of grades 3-4 diarrhea (37.7% vs. 19.6%, P < .01), grades 3-4 colitis (39.3% vs. 14.7%, P < .01), and hospitalization (49.2% vs. 35.2%, P = .04). PCR-positive patients were also more likely to report abdominal pain (59.0% vs. 26.0%, P < .01) and fever (39.3% vs. 16.2%, P < .01).

Patients with infections were less likely than those without infections to receive either steroid treatment (22.9% vs. 45.0%, P < .01) or second-line immunosuppressives (8.2% vs. 19.9%, P = .03). A breakdown of treatment strategies and escalation to immunosuppressive therapies by GI PCR result status is shown in Supplementary Fig. 1. In patients receiving steroids, treatment duration was similar regardless

Table 1. Characteristics of ICI-treated patients undergoing GI PCR testing for new-onset diarrhea.

Characteristics	Total patients *n* = 521	GI PCR + *n* = 61	GI PCR - *n* = 460	*P*-value
Age at time of GI PCR (years), mean (SD)	60.7 (14.1)	56.6 (16.1)	61.3 (13.7)	.02
Sex, *n* (%)				.56
Male	283 (54.3)	31 (50.8)	252 (54.8)	
Female	238 (45.7)	30 (49.2)	208 (45.2)	
Race, *n* (%)				.75
White	418 (80.2)	50 (82.0)	368 (80.0)	
Black	33 (6.3)	3 (4.9)	30 (6.5)	
Asian	41 (7.9)	6 (9.8)	35 (7.6)	
Other/unknown	29 (5.6)	2 (3.3)	27 (5.9)	
Ethnicity, *n* (%)				.61
Non-Hispanic	479 (91.9)	58 (95.1)	421 (91.5)	
Hispanic	31 (6.0)	2 (3.3)	29 (6.3)	
Unknown	11 (2.1)	1 (1.6)	10 (2.2)	
Cancer type, *n* (%)				.77
Genitourinary	125 (24.0)	13 (21.3)	112 (24.4)	
Lung	52 (10.0)	6 (9.8)	46 (10.0)	
Gynecologic	50 (9.6)	7 (11.5)	43 (9.4)	
Melanoma	38 (7.3)	7 (11.5)	31 (6.7)	
GI/hepatobiliary	26 (5.0)	2 (3.3)	24 (5.2)	
Other[a]	230 (44.2)	26 (42.6)	204 (44.4)	
Cancer stage, *n* (%)				.36
I	73 (14.3)	8 (16.0)	65 (14.1)	
II	27 (5.3)	2 (4.0)	25 (5.4)	
III	96 (18.8)	5 (10.0)	91 (19.8)	
IV	314 (61.6)	35 (70.0)	279 (60.7)	
Chemotherapy within 90 days prior to GI PCR, *n* (%)				.79
Yes	273 (52.4)	31 (50.8)	242 (52.6)	
No	248 (47.6)	30 (49.2)	218 (47.4)	
Type of ICI, *n* (%)				.43
PD-(L)1	428 (82.2)	50 (82.0)	378 (82.2)	
CTLA-4	11 (2.1)	0 (0)	11 (2.4)	
Combination	82 (15.7)	11 (18.0)	71 (15.4)	
Total duration of ICI treatment (m), median (IQR)	5.6 (1.8-16.1)	6.9 (2.1-16.4)	5.5 (1.8-16.0)	.99
Reason for stopping ICI, *n* (%)				.80
ICI-related GI adverse events	136 (26.1)	20 (32.8)	116 (25.2)	
Progression of disease	41 (7.9)	5 (8.2)	36 (7.8)	
Other ICI-related adverse events	11 (2.1)	1 (1.6)	10 (2.2)	
Death or lost to follow up	16 (3.1)	2 (3.3)	14 (3.0)	
Complete remission	0 (0)	0 (0)	0 (0)	
Resumed ICI	312 (59.9)	33 (54.1)	279 (60.7)	
Completion of treatment protocol	5 (1.0)	0(0)	5 (1.1)	

[a]Includes non-melanoma skin cancer, head and neck/endocrine, hematologic, breast, sarcoma, neuroendocrine, brain/nervous system, and unknown primary.
Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated protein 4; GI, gastrointestinal; ICI, immune checkpoint inhibitor; IQR, interquartile range; PD-(L)1, programmed death-(ligand)1.

of infection status (43.6 vs. 41.6 days, $P = .65$). Moreover, despite more severe presentations in patients with infections, clinical outcomes were similar between groups with no significant differences noted in diarrhea recurrence (18.0% vs. 10.2%, $P = .08$) or time from diarrhea onset to recurrence (75.5 vs. 88.2 days, $P = .69$). Time from diarrhea onset to clinical response and to resolution were also similar between groups (7.1 vs. 8.1 days, $P = .63$; and 8.1 vs 8.1 days, $P = .80$, respectively).

Survival Analysis

In a landmark survival analysis, 50 PCR-positive and 366 PCR-negative patients were alive at the 6-month landmark (Supplementary Fig. 2). PCR-positive patients did not display

Table 2. Diarrhea etiologies of ICI-treated patients with negative GI PCR results.

Diarrhea etiology, *n* (%)	Total patients *n* = 460
Biopsy-proven or high clinical suspicion of ICI-colitis	190 (41.3)
Moderate clinical suspicion of ICI colitis without biopsy	137 (29.8)
Other drug-related[†]	58 (12.6)
Other identified cause[‡]	75 (16.3)

[†]Includes antibiotics, tyrosine kinase inhibitors, chemotherapy, and laxatives.
[‡]Includes progression of disease, adrenal insufficiency, procedures, diverticulitis, or other non-colitis GI pathologies.
Abbreviations: GI, gastrointestinal; ICI, immune checkpoint inhibitor.

significantly different OS from the PCR-negative group (HR 0.70, 95% CI, 0.46-1.07, *P* = .10). Median OS was 44 months (95% CI, 33 to not reached [NR]) in PCR-positive patients and 31 months (95% CI, 25-37) in PCR-negative patients. Furthermore, among the PCR-positive patients, 20 receiving antibiotics and 30 not receiving antibiotics were included in a separate 6-month landmark analysis (Supplementary Fig. 3). Compared to patients not receiving antibiotics, there was no significant difference in OS (HR 1.71, 95% CI, 0.76-3.82, *P* = .20); the median OS was NR (95% CI, 41-NR) for patients not receiving antibiotics and 44 months (95% CI, 12-NR) for patients receiving antibiotics.

Therapy for PCR-Positive Patients

Of 61 PCR-positive patients, 27 of 38 (71%) with bacterial infections were treated with antibiotics (Supplementary Table S2). Two of 6 (33%) with parasitic infections and no patients with viral infections received antibiotics. No significant differences between those treated with or without antibiotics were noted in diarrhea severity (*P* = .72), diarrhea duration (*P* = .72), hospitalization (*P* = 1.00), steroid treatment (*P* = .59), steroid duration (*P* = .32), or second-line immunosuppressive treatment (*P* = .65). Recurrence of diarrhea, however, was more common in patients treated with antibiotics (29.6% vs. 8.8%, *P* = .05) with a median interval to recurrence of 83.1 days. Demographic, cancer, and ICI characteristics were also similar between groups although ICI treatment duration was numerically shorter in those receiving antibiotics (3.0 months vs. 9.7 months, *P* = .36) (Supplementary Table S3). A restricted analysis of patients with bacterial infections comparing the 27 patients treated with antibiotics and the 11 patients not receiving antibiotics was consistent with the lack of significant differences between groups (Supplementary Table S4).

In an exploratory analysis among patients with a positive PCR, patients subsequently treated with steroids were observed to experience worse clinical outcomes with higher rates of persistent and chronic diarrhea, hospitalization, second-line immunosuppressive use, and longer time to diarrhea resolution compared to those not receiving steroids (Supplementary Table S5).

Endoscopic and Histologic Assessment

Among 23 PCR-positive patients and 82 negative patients who underwent endoscopic assessment within 60 days after the PCR date, similar patterns to the overall cohort were observed with increased colitis clinical severity in the positive group (*P* < .01) but no significant differences in diarrhea recurrence (*P* = .82), time to clinical response (*P* = .25), or time to resolution (*P* = .16) (Table 4). Time from diarrhea onset to endoscopic evaluation (*P* = .67) and Mayo endoscopic score (*P* = .65) were not significantly different between groups. The most common colitis histological subtypes were acute colitis (47.8% in positive vs. 31.7% in negative, *P* = .46) followed by chronic active colitis (30.4% vs. 28.1%). Twenty-one (20.0%) patients were found to have normal colonic mucosa on biopsy. Furthermore, PCR-positive patients were less likely than negative patients to receive steroids (26.1% vs. 52.4%, *P* = .03) or second-line immunosuppressives (13.0% vs. 35.4%, *P* = .04).

Predictors of Immunosuppressive Therapy

Multivariable analysis of predictors of steroid and second-line immunosuppressive treatment for biopsy-proven irEC was performed in the overall cohort (Table 5). A positive GI PCR result was independently associated with a lower likelihood of receiving steroids (OR 0.29, *P* < .01) and second-line immunosuppressive agents (OR 0.18, *P* < .01). Clinical severity was also found to be predictive with higher grades of diarrhea (grades 3-4 vs. 1-2) associated with increased steroid use (OR 3.99, *P* < .01) and second-line immunosuppressive treatment (OR 2.30, *P* = .01). Higher grades of colitis (grades 3-4 vs. 1-2) were also associated with increased second-line immunosuppressive therapy (OR 4.70, *P* < .01), though not increased steroid use.

Multivariable analysis was also performed in the endoscopic assessment subgroup (Supplementary Table S6). A positive PCR continued to be an independent negative predictor of steroid treatment (OR 0.21, *P* = .02) and second-line immunosuppressive therapy (OR 0.16, *P* = .02). Endoscopic severity was a strong predictor of immunosuppression, with moderate-severe colitis endoscopically (Mayo scores 2-3) associated with increased steroid (OR 4.00, *P* = .02) and second-line immunosuppressive use (OR 3.76, *P* = .03), compared to normal or mild colitis activity (Mayo 0-1). Active inflammation on histology was predictive of increased steroid utilization (OR 2.69, *P* < .01) but not increased second-line immunosuppressives (OR 2.73, *P* = .25). While higher colitis grades continued to be associated with increased second-line immunosuppressive use (OR 5.99, *P* < .01), diarrhea severity notably was not associated with immunosuppression in this subgroup.

Discussion

In our study, GI infections were observed in 11.7% of ICI-treated patients presenting with diarrhea and may serve as important clinical predictors for the need for immunosuppressive therapy in these individuals. Despite more severe clinical presentations, patients testing positive on PCR were less likely to receive steroids and second-line immunosuppressive agents and did not have higher rates of persistent or recurrent diarrhea. Additionally, a 6-month landmark analysis did not reveal any significant differences in OS in patients stratified by PCR result. While endoscopic and histologic findings were associated with the use of immunosuppression, they were unable discern between infectious and ICI-related colitis. These results highlight that patients with infections detected on PCR only transiently display worse symptoms

Table 3. Clinical characteristics and treatment outcomes of ICI-treated patients undergoing GI PCR testing for new-onset diarrhea.

Characteristics	Total patients *n* = 388	GI PCR + *n* = 61	GI PCR – *n* = 327	*P*-value
Time from ICI therapy initiation to diarrhea onset (day), median (IQR)	82.63 (32.4-203.8)	101.4 (38.5-298.1)	82.1 (30.4-194.7)	.78
Presenting associated symptoms, *n* (%)				
Abdominal pain	121 (31.2)	36 (59.0)	85 (26.0)	<.01
Fever	77 (19.9)	24 (39.3)	53 (16.2)	<.01
Bloody stools	20 (5.2)	1 (1.6)	19 (5.8)	.18
Highest grade of diarrhea, *n* (%)				<.01
I/II	301 (77.6)	38 (62.3)	263 (80.4)	
III/IV	87 (22.4)	23 (37.7)	64 (19.6)	
Highest grade of colitis, *n* (%)				<.01
I/II	316 (81.4)	37 (60.7)	279 (85.3)	
III/IV	72 (18.6)	24 (39.3)	48 (14.7)	
Duration of diarrhea (day), median (IQR)	8.1 (4.1-17.2)	8.1 (5.1-17.2)	8.1 (4.1-14.2)	.80
Duration of diarrhea categories, *n* (%)				.36
Acute (<14 days)	262 (67.5)	43 (70.5)	219 (67.0)	
Persistent (14-30 days)	61 (15.7)	6 (9.8)	55 (16.8)	
Chronic (>30 days)	65 (16.8)	12 (19.7)	53 (16.2)	
Hospitalization for symptoms, *n* (%)				.04
Yes	145 (37.4)	30 (49.2)	115 (35.2)	
No	243 (62.6)	31 (50.8)	212 (64.8)	
Laboratory tests, mean (SD)				
Hemoglobin (g/dL) (*n* = 387)	11.5 (2.4)	11.7 (2.6)	11.4 (2.4)	.33
Albumin (g/dL) (*n* =3 85)	3.5 (0.7)	3.6 (0.7)	3.5 (0.7)	.30
CRP (mg/dL) (*n* = 61)	6.8 (7.2)	6.2 (8.2)	6.8 (7.1)	.82
Endoscopic assessment, *n* (%)				.06
Yes	105 (27.1)	23 (37.7)	82 (25.1)	
No	283 (72.9)	38 (62.3)	245 (74.9)	
Steroid treatment, *n* (%)				<.01
Yes	161 (41.5)	14 (22.9)	147 (45.0)	
No	227 (58.5)	47 (77.1)	180 (55.0)	
Duration of steroid treatment (day), median, (IQR)	41.6 (24.3-75.0)	43.6 (20.3-77.1)	41.6 (25.3-75.0)	.65
Second-line immunosuppressives, *n* (%)				.03
Yes	70 (18.0)	5 (8.2)	65 (19.9)	
No	318 (82.0)	56 (91.8)	262 (80.1)	
Time to diarrhea response (day), median (IQR)	8.1 (4.1-16.2)	7.1 (4.1-13.2)	8.1 (4.1-16.2)	.63
Time to resolution (day), median (IQR)	8.1 (4.1-17.2)	8.1 (4.1-14.2)	8.1 (5.1-17.2)	.80
Recurrence of diarrhea, *n* (%)				.07
Yes	44 (11.4)	11 (18.0)	33 (10.2)	
No	341 (88.6)	50 (82.0)	291 (89.8)	
Time from diarrhea onset to recurrence (day), median (IQR)	86.2 (61.8-105.4)	75.5 (66.9-88.7)	88.2 (57.8-123.7)	.69

Abbreviations: GI, gastrointestinal; HR, hazard ratio; ICI, immune checkpoint inhibitor; IQR, interquartile range.

and may be optimally treated with observation and supportive care.

Avoidance of unnecessary immunosuppression is of particular importance among patients on ICI, as data suggest that high-dose steroids and second-line immunosuppressives may inhibit optimal ICI antitumor responses.[13,14] Therefore, proper identification of pathogens may enable clinicians to avoid unnecessary empiric immunosuppression and related morbidity, especially in patients who may present with transiently worse clinical pictures associated with enteric infections.

There are numerous similarities between IBD and irEC in clinical presentation, endoscopic findings, and treatment approaches.[15] Previous studies in IBD patients have demonstrated similar trends to our study in the influence of enteric

Table 4. Clinical characteristics and treatment outcomes of ICI-treated patients with endoscopic assessment within 60 days after GI PCR by test result status.

Characteristics	Total patients *n* = 105	GI PCR + *n* = 23	GI PCR - *n* = 82	*P*-value
Time from ICI therapy initiation to diarrhea onset (days), median (IQR)	108.5 (46.5-206.8)	197.7 (57.8-358.9)	95.3 (32.4-170.3)	.12
Presenting associated symptoms, *n* (%)				
Abdominal pain	42 (40.0)	16 (69.6)	27 (31.7)	<.01
Fever	22 (21.0)	11 (47.8)	11 (13.4)	<.01
Bloody stools	13 (12.4)	1 (4.4)	12 (14.6)	.03
Highest grade of diarrhea, *n* (%)				.07
I/II	63 (60.0)	10 (43.5)	53 (64.6)	
III/IV	42 (40.0)	13 (56.5)	29 (35.4)	
Highest grade of colitis, *n* (%)				<.01
I/II	74 (70.5)	11 (47.8)	63 (76.8)	
III/IV	31 (29.5)	12 (52.2)	19 (23.2)	
Duration of diarrhea (days), median (IQR)	12.2 (6.1-32.4)	8.1 (4.1-27.4)	13.2 (6.1-33.5)	.08
Duration of diarrhea categories, *n* (%)				.26
Acute (<14 days)	58 (55.2)	16 (69.6)	42 (51.2)	
Persistent (14-30 days)	18 (17.1)	2 (8.7)	16 (19.5)	
Chronic (>30 days)	29 (27.6)	5 (21.7)	24 (29.3)	
Hospitalization for symptoms, *n* (%)				.70
Yes	51 (48.6)	12 (52.2)	39 (47.6)	
No	54 (51.4)	11 (47.8)	43 (52.4)	
Laboratory tests, mean (SD)				
Hemoglobin (g/dL)	11.7 (2.4)	12.3 (2.6)	11.5 (2.3)	.14
Albumin (g/dL)	3.5 (0.7)	3.7 (0.7)	3.5 (0.7)	.16
CRP (mg/dL) (*n* = 21)	5.2 (5.3)	3.0 (5.1)	5.6 (5.3)	.45
Time to endoscopic assessment (days), median (IQR)	13.2 (4.1-35.5)	12.2 (6.1-38.5)	15.2 (3.0-35.5)	.67
Type of endoscopic procedure, *n* (%)				.08
Colonoscopy	66 (62.9)	18 (78.3)	48 (58.5)	
Sigmoidoscopy	39 (37.1)	5 (21.7)	34 (41.5)	
Mayo endoscopic score, *n* (%)				.65
Score 0	19 (18.1)	4 (17.4)	15 (18.3)	
Score 1	51 (48.6)	13 (56.5)	38 (46.3)	
Score 2	31 (29.5)	6 (26.1)	25 (30.5)	
Score 3	4 (3.8)	0 (0)	4 (4.9)	
Colitis histologic subtype, *n* (%)				.46
Acute colitis	37 (35.2)	11 (47.8)	26 (31.7)	
Chronic active colitis	30 (28.6)	7 (30.4)	23 (28.1)	
Microscopic colitis	13 (12.4)	1 (4.4)	12 (14.5)	
GVHD-like (apoptosis)	4 (3.8)	1 (4.4)	3 (3.7)	
Normal mucosa	21 (20.0)	3 (13.0)	18 (22.0)	
Steroid treatment, *n* (%)				.03
Yes	49 (46.7)	6 (26.1)	43 (52.4)	
No	56 (53.3)	17 (73.9)	39 (47.6)	
Duration of steroid treatment (days), median, (IQR)	50.7 (29.4-119.6)	43.6 (23.3-77.1)	50.7 (29.4-119.6)	.70
Second-line immunosuppressives, *n* (%)				.04
Yes	32 (30.5)	3 (13.0)	29 (35.4)	
No	73 (69.5)	20 (87.0)	53 (64.6)	
Recurrence of diarrhea, *n* (%)				.82
Yes	20 (19.1)	4 (17.4)	16 (19.5)	
No	85 (80.9)	19 (82.6)	66 (80.5)	

Table 4. Continued

Characteristics	Total patients *n* = 105	GI PCR + *n* = 23	GI PCR - *n* = 82	*P*-value
Time from diarrhea onset to recurrence (days), median (IQR)	92.3 (65.9-127.8)	89.2 (66.9-92.3)	95.3 (64.9-133.3)	.25
Time to clinical response (days), median (IQR)	10.1 (5.1-17.2)	8.1 (4.1-14.2)	11.2 (6.1-19.3)	.25
Time to resolution (days), median (IQR)	12.2 (6.1-32.4)	8.1 (4.1-27.4)	13.2 (6.1-33.5)	.16

Abbreviations: GI, gastrointestinal; HR, hazard ratio; ICI, immune checkpoint inhibitor; IQR, interquartile range.

Table 5. Multivariable logistic regression of predictors of immunosuppressive therapy for immune-related enterocolitis.

	Steroids		Second-line immunosuppressives	
Characteristics	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)2	*P*-value3
Age at time of GI PCR	1.00 (0.98-1.02)	.91	1.00 (0.98-1.02)	.84
Sex				
Male	1 (reference)		1 (reference)	
Female	0.89 (0.57-1.39)	.62	0.71 (0.39-1.28)	.25
Time from ICI therapy initiation to diarrhea onset	1.00 (0.99-1.00)	.84	1.00 (0.99-1.00)	.29
Chemotherapy within 90 days prior to GI PCR				
No	1 (reference)		1 (reference)	
Yes	0.63 (0.39-0.89)	.08	0.94 (0.52-1.68)	.82
Type of ICI				
PD-(L)1	1 (reference)		1 (reference)	
CTLA-4	2.41 (0.64-9.03)	.19	1.17 (0.22-6.16)	.85
Combination	2.11 (1.19-3.73)	.01	1.19 (0.58-2.44)	.64
GI PCR status				
Negative	1 (reference)		1 (reference)	
Positive	0.29 (0.15-0.59)	<.01	0.18 (0.06-0.53)	<.01
Highest grade of diarrhea				
I/II	1 (reference)		1 (reference)	
III/IV	3.99 (2.18-7.30)	<.01	2.30 (1.19-4.45)	.01
Highest grade of colitis				
I/II	1 (reference)		1 (reference)	
III/IV	1.19 (0.86-1.69)	.27	4.70 (2.32-9.48)	<.01

Abbreviations: CTLA-4: cytotoxic T-lymphocyte associated protein 4; GI, gastrointestinal; ICI, immune checkpoint inhibitor; PD-(L)1: programmed death-(ligand)1.

infection testing on clinical decision-making, with patients testing positive on GI PCR being less likely to have IBD therapies added or escalated.[8,9,16,17] Physicians' awareness of concomitant enteric infections in patients on ICI and similar conditions may lead to therapeutic approaches that emphasize antibiotics, observation, and supportive care over immunosuppressive agents. Furthermore, in our study as well in existing literature on IBD populations, even with decreased utilization of immunosuppressive treatments, the presence of GI pathogens did not impact long-term outcomes with no differences in complication, surgical, or hospitalization rates between patients with or without infections.[16,17] In evaluating OS, we recognized the potential confounding influence of the immortal time bias in which the Kaplan-Meier curves may have appeared different due to variation in time from ICI initiation to irEC onset between compared groups. To account for this bias, we conducted a landmark analysis which confirmed no significant difference in OS between patients stratified by PCR result. Our findings are also consistent with a previous retrospective study of 22 patients with irEC and superimposed GI infections that found infections to be associated with more severe clinical symptoms, no increased risk of recurrence or mortality, and no improvement with antibiotics.[18] However, most patients with GI infections in the aforementioned study did receive immunosuppressive therapies (only 14% were solely treated with supportive care), which contrasts with 77% of PCR-positive patients being managed without immunosuppressive therapies in our cohort. While these differences likely reflect different study inclusion criteria and diverging institutional practice patterns, our data suggest routine immunosuppression is likely not needed in these patients.

To perform a more rigorous assessment of the clinical significance of GI infections in ICI-treated patients, we analyzed

data from a subset of these patients who underwent endoscopy. There have been few studies examining the endoscopic and histological characteristics of irEC[4,19-21]; however, these features and their potential utility in predicting disease management have not yet been described in irEC patients with superimposed GI infections. Our results are consistent with previously described positive associations between endoscopic severity and the use of biological therapy, emphasizing the importance of endoscopy not only in irEC diagnosis but also in guiding appropriate immunosuppressive agent employment.[22,23] Furthermore, despite having more severe diarrhea by grade and symptoms, patients with infections had similar endoscopic and histologic findings to those without infections in our study, which supports prior literature describing poor correlations between irEC symptoms and endoscopy.[11,22,23] On histology, while there were more diagnoses of acute colitis (resembling infectious colitis) over chronic active colitis (resembling inflammatory colitis such as irEC) in the PCR-positive group, this difference was non-significant and therefore histological findings were unable to clearly distinguish between infectious and ICI-related colitis in our cohort. With endoscopy's inability to reliably differentiate between inflammatory and infectious colitis in this setting, patients with suspected irEC should first be evaluated with GI PCR testing to guide further evaluation and therapy and spare unnecessary endoscopic intervention and associated procedural risks if PCR testing is positive. Obtaining an endoscopy with biopsy remains an important component of the workup for suspected irEC; however, it is not sufficient by itself nor alongside evaluation of clinical symptoms to properly inform treatment strategies in patients with ICI-related diarrhea and possible GI infections. Combination of clinical and endoscopic evaluation with non-invasive objective tests, such as inflammatory biomarkers, and, notably, PCR test results, will maximize acute care regimens and long-term outcomes in patients with suspected irEC.

GI pathogens have been theorized to contribute to more severe irEC symptoms by their effects on inflammatory and immune mechanisms, such as expansion of T-cell populations, increased release of cytokines, and alterations of the native gut microbiome.[24,25] Of our 61 patients with GI infections, the types and prevalence of enteric pathogens identified on PCR differed from a previous study in 22 irEC patients with superimposed infections.[18] Relative to Ma et al's findings in which *E. coli* constituted all bacterial infections and 77% of all cases, we observed fewer numbers of *E. coli* (33% of all cases) and the presence of several other bacterial strains, including *Campylobacter*, *Salmonella*, and *Yersinia*. Our study demonstrated similar frequency (23% in Ma et al's study vs. 28% in our study) and types of viral infections with norovirus being the most common. Our study is also the first to report on concomitant parasitic infections in irEC patients, which made up 10% of total cases in our cohort. Alongside the largest sample size to date, the greater variation of enteric pathogens identified in our study may support the increased generalizability of our results across multiple infectious agent subtypes. Further research is needed to examine potential differences in the impact of various enteric pathogen types in the pathogenesis of ICI colitis.

The role of antibiotics in treating non-*C. difficile* gastrointestinal pathogens is another area of uncertainty and of particular interest in patients on immunotherapy, where the microbiome-disrupting effects of antibiotics have been suggested to increase the risk of and worsen outcomes in irEC.[26] Antibiotic therapy for GI infections was not associated with changes in diarrhea severity, use of immunosuppressive therapy, steroid duration, hospitalization, symptom resolution, or OS in our landmark survival analysis but was linked to higher rates of diarrhea recurrence. These results are consistent with a previous study and support the potential for antibiotic-induced immune overactivity and autoimmunity against the native microbiome leading to decreased protection against recurrent diarrhea.[18] That patients undergoing antibiotic therapy in our cohort had ICI treatment durations that were nearly 7 months shorter than those not receiving antibiotics echoes prior studies that have described associations of antibiotic-induced dysbiosis with increased risks of severe irEC, need for immunosuppressive therapy, and hospitalization.[26,27] Our findings highlight the importance of avoiding unnecessary antibiotic regimens to reduce possible complications such as provoking more severe irEC courses,[26] increasing the risk of diarrhea recurrence, and possibly leading to shorter duration of ICI therapy. As such, similar to avoiding unnecessary escalation of immunosuppressive therapies, increased antibiotic stewardship may improve patient outcomes and lower the downsides of over-treatment at an individual and population level. While the decision to prescribe antibiotics often considers numerous aspects of a patient's individualized illness presentation, the risks of exacerbating dysbiosis in patients with irEC and concomitant GI infections should be kept in mind while determining management options.

Our study is the largest analysis of stool pathogen testing in patients with diarrhea on ICI, the first to examine all-comers within this group undergoing initial GI PCR testing for the workup of new onset diarrhea and the first to assess endoscopic and histological associations with treatments within this group. By analyzing patients at the time of presentation for diarrhea and initial GI PCR testing, we were uniquely able to capture the clinical reality of irEC evaluations and in turn maximize the applicability of our results to real world practice settings.

There are several limitations to our study. Due to its retrospective design, our results are unable to describe causal relationships between GI infections and diarrhea severity, treatment regimens, and patient outcomes. The PCR panel is unable to distinguish between active infection, colonization, or contamination and does not assess for all potential infectious agents, especially less-common pathogens. Furthermore, patients with *C. diff* were excluded due to the pathogen's exclusion from the PCR panel and distinct risk factors, associations with colitis, and outcomes. However, *C. diff* remains a major infectious cause of diarrhea in cancer patients, and we plan to study patients with suspected irEC and superimposed *C. diff* infection and their unique considerations in a dedicated future analysis. Treatment protocols for diarrhea on ICI are not standardized, and so the decision for therapy escalation or endoscopic evaluation were at the discretion of the treating gastroenterologist and/or oncologist.

Conclusion

We observed that GI infections in patients with diarrhea on ICI therapy led to more severe, but often transient illness, and may be optimally treated with observation and supportive care without averse clinical outcomes. We recommend the routine

and timely use of PCR tests to screen for GI infections, ideally prior to initiating empiric immunosuppressive treatment or to guide early cessation of empiric treatment. A combination of PCR testing with clinical evaluation, endoscopic assessment, and other inflammatory biomarkers will maximize acute care regimens and long-term outcomes in patients with suspected irEC. Our findings emphasize the need for further studies to investigate the interactions between enteric infections, immunosuppressive treatment, and antibiotic therapies in the pathogenesis of ICI colitis and the differential effects of various types of pathogens at a granular level. Further prospective studies are also needed to examine the impact and utility of GI PCR testing alongside other biomarkers of colonic inflammation on irEC management and outcomes.

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Conflict of Interest

Mini Kamboj has received consulting fees from Regeneron; speaker fees from Web MD & Medscape and MjH Life Sciences. Michael A. Postow has received consulting fees from BMS, Merck, Novartis, Eisai, Pfizer, and Chugai; and institutional support from RGenix, Infinity, BMS, Merck, and Novartis. David M. Faleck has received consulting fees from AzurRx, Equillium, Janssen, Mallinckrodt Pharmaceuticals, and OnQuality Pharmaceuticals. The other authors indicated no financial relationships.

Author Contributions

Conception/design: P.M., D.S., M.P., M.L., D.F. Provision of study material or patients: M.L., D.F. Collection and/or assembly of data: P.M., D.S., N.B., M.K., M.P., M.L., D.F. Data analysis and interpretation: P.M., D.S., M.P., M.L., D.F. Manuscript writing: P.M., M.L., D.F. Final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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