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ORIGINAL ARTICLE

Stool PCR for Gastrointestinal Pathogens in Patients With and Without Immune-Mediated Intestinal Diseases

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Abstract

Background Patients with celiac disease and inflammatory bowel disease, two immune-mediated luminal conditions, have higher rates of certain infections than healthy counterparts. The prevalence of many gastrointestinal infections in these patients, however, is unknown.

Aims Using a novel clinical stool pathogen PCR test, we investigated the hypothesis that patients with celiac disease/inflammatory bowel disease had different distributions of diarrheal pathogens than other patients.

Methods We performed a retrospective cohort study of outpatients who underwent stool pathogen testing with the FilmArray Gastrointestinal PCR Panel (BioFire Diagnostics, Salt Lake City, UT) at our institution from January 1 to December 31, 2015. Rates of pathogens were measured in patients with or without celiac disease/inflammatory bowel disease.

Results Of 955 patients, 337 had positive test for any pathogen, with 465 bacterial, parasitic, or viral pathogens identified. One hundred and twenty-seven patients (13.3%) had celiac disease or inflammatory bowel disease, of which 29/127 (22.8%) had a positive test, compared to 308/828 other patients (37.2%) ($p = 0.002$). Patients with celiac disease/inflammatory bowel disease had significantly fewer viruses (1.6 vs. 8.1% of patients; $p = 0.008$) and parasites (0 vs. 3.3%; $p = 0.039$), with nonsignificant trend toward fewer bacteria (21.3 vs. 29.2%; $p = 0.063$). *Escherichia coli* species were most common in both populations.

Conclusions Stool PCR identified numerous pathogens in patients with or without celiac disease/inflammatory bowel disease. Patients with celiac disease/inflammatory bowel disease were significantly less likely to have any pathogen identified, and had significantly fewer viruses and parasites. In this population, knowledge of common pathogens can guide diagnostic evaluation and offer opportunities for treatment.

Keywords Enteric infections · Diarrhea · Celiac disease · Inflammatory bowel disease

Introduction

Celiac disease (CeD) and inflammatory bowel disease (IBD), two immune-mediated gastrointestinal diseases, are associated with alterations in both gut microbial composition and systemic immunity. Likely due in part to these qualities, patients with CeD/IBD have increased risk of a variety of systemic infections: CeD is associated with increased incidence of pneumococcal infections, tuberculosis, and influenza [1–4], while patients with IBD have higher rates of *Clostridium difficile* (newly reclassified as *Clostridioides difficile* [5]) infection than controls [6–8]. The prevalence of gastrointestinal infections due to pathogens other than *C. difficile* in patients with CeD or IBD, however, has not been well studied.

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With the introduction of improving technology to identify infections, it is possible to diagnose gastrointestinal pathogens with an increasing degree of accuracy. New multiplex PCR-based panels can detect four times as many pathogens as routine laboratory testing, with sensitivity and specificity as high as 95–100 and 97–100%, respectively [9, 10].

These new PCR-based stool tests have not yet been utilized to assess rates of gastrointestinal pathogens in patients with immune-mediated conditions, including CeD and IBD, compared to those without. Using a multiplex PCR-based gastrointestinal pathogen stool test (FilmArray Gastrointestinal Panel, BioFire Diagnostics, Salt Lake City, UT), we tested the hypothesis that the distribution of gut infectious pathogens would differ between patients with CeD or IBD and patients without these diseases. We performed a retrospective cohort study of all adults who underwent outpatient stool testing for altered bowel habits during a one-year period at our institution to compare the prevalence of a positive stool test and the distribution of specific pathogens between patients with CeD/IBD and other patients.

Methods

Study Sample

We performed a retrospective cohort study using data from the electronic medical record at New York Presbyterian-Columbia University Medical Center, a quaternary care center. A query was performed to identify adults ≥ 18 years of age who underwent stool gastrointestinal pathogen PCR testing in an outpatient setting during the study period spanning January 1, 2015, through December 31, 2015. Only outpatients were included in order to minimize clinical variability among patients. A total of 955 eligible patients were identified, all of whom were included in the study. Data collected included patient age; sex; residential zip code; race; ethnicity; presence of celiac disease, ulcerative colitis, or Crohn's disease; location of test (outpatient visit, emergency department, or at endoscopy); and results of gastrointestinal pathogen PCR stool test. In patients who received more than one outpatient gastrointestinal pathogen PCR test during the study period, only the first chronological test was included. While clinical data regarding the volume and consistency of bowel movements were not consistently available in this retrospective study, the clinical indication for this stool pathogen test at our institution is diarrhea. In order to confirm this clinical indication for testing in our study, a randomly selected sample of 22 patients (4 with IBD, 18 without CeD/IBD) was assessed; to minimize variability in the availability of clinical data, patients tested in the emergency department were used. Of the 22 patients, all were noted to have some manifestation of diarrheal illness (characterized as diarrhea, watery or loose stool, bloody stool, or increased frequency of stool) on the day of testing.

The presence of CeD/IBD was defined using the ICD9 and ICD10 diagnosis codes in the electronic medical record: celiac disease (579, K90.0), ulcerative colitis (556.*, K51.*), Crohn's disease (555.0, 555.1, 555.2, 555.9, K50.*), or none (none of these diagnosis codes). A random sample of 22 patients (2 with CeD, 2 with IBD, and 18 without either condition) was assessed to confirm that identified records were of outpatients and had correct diagnosis codes and GI pathogen PCR test dates and results. Of those 22 sampled patients, all patients were correctly classified. There were four patients who had diagnosis codes for both CeD and IBD; manual review of those four patients confirmed that all four had diagnoses of both of these conditions.

Zip codes were organized according to the following: New York City (10000–11500, 11690–11695, 11697); surrounding area: New York State excluding NYC (00501, 00544, 06390, 11500–14926 except where included in New York City), Connecticut (06000–06929), New Jersey (07000–08990); and other (all other zip codes).

This study was approved by the Columbia University Medical Center Institutional Review Board.

Gastrointestinal (GI) Pathogen PCR Testing

All samples were analyzed according to the manufacturer's instruction using the FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT). Samples were analyzed for the presence of 21 pathogens including bacteria, parasites, and viruses (see Table 3). Although the panel can also be used to identify the twenty-second pathogen, toxigenic *C. difficile*, the *C. difficile* result from this panel is not reported by our clinical laboratory as it is tested via a separate assay that is ordered separately in selected patients. For all patients who underwent separate *C. difficile* stool PCR testing within 7 days before or after the date of the GI pathogen multiplex PCR test, results of *C. difficile* testing were also collected. Where applicable, testing for *C. difficile* was performed using the Xpert C. difficile stool test (Cepheid, Sunnyvale, CA).

Statistical Analyses

We used the Chi-square test to compare characteristics of patients with positive or negative stool pathogen test and to compare prevalence of specific pathogens between patients with CeD/IBD and those without. We used multivariable analysis to identify factors independently associated with a positive stool pathogen test. The multivariable analysis included the following variables in the model a priori: immune-based disorders (IBD/CeD), sex, age, race, ethnicity, residential region (New York City, surrounding areas,

remote), and location of testing (office visit, emergency department, endoscopy suite). Statistical calculations were done using SAS version 9.4 (Cary, NC).

Results

Patient Characteristics

We identified 955 patients who underwent outpatient stool pathogen testing between January 1, 2015, and December 31, 2015 (Table 1). Of the 955 patients, 127 patients (13.3% of total) had immune-mediated diseases, including CeD (60 patients), IBD (63 patients; 38 with ulcerative colitis, 24 with Crohn's disease, and one with indeterminate colitis), or both CeD and IBD (four patients). Patients with CeD/IBD were more likely to be white (57.5 vs. 39.3%, $p = 0.001$) and to have been tested during an outpatient endoscopy (12.6 vs. 3.3%, $p < 0.001$). They were less likely to live in New York City (74.0 vs. 85.0%, $p = <0.001$) or to be tested in the emergency department (4.7 vs. 12.9%, $p < 0.001$). A total of 337 patients overall (35.3%) had a positive stool pathogen test, indicating the presence of at least one pathogen.

Factors Associated with Positive Stool Pathogen Test

A positive stool pathogen PCR test was significantly less likely in patients with CeD/IBD than other patients: positive result was found in 29 of 127 patients with CeD/IBD (22.8%) compared to 308 of 828 patients without (37.2%) ($p = 0.002$) (Table 1). On multivariate analysis of factors independently associated with positive stool pathogen test (Table 2), patients with positive stool pathogen test were less likely to have CeD/IBD (OR 0.49; 95% CI 0.31–0.77,

Table 1 Characteristics of all outpatients at first GI pathogen PCR testing from January 1, 2015, to December 31, 2015

	All ($n = 955$)	CeD/IBD ($n = 127$)	Other ($n = 828$)	p value[c]
Patients with positive GI PCR test, no. (%)[a]	337 (35.3)	29 (22.8)	308 (37.2)	**< 0.01**
Sex, no. (%)				0.98
Female	565 (59.2)	75 (59.1)	490 (59.2)	
Male	390 (40.8)	52 (40.9)	338 (40.8)	
Age at testing, no. (%)				0.71
18–29 years	208 (21.8)	32 (25.2)	176 (21.3)	
30–49 years	277 (29.0)	38 (29.9)	239 (28.9)	
50–69 years	294 (30.8)	36 (28.3)	258 (31.2)	
≥ 70 years	176 (18.4)	21 (16.5)	155 (18.7)	
Race, no. (%)				**< 0.01**
White	398 (41.7)	73 (57.5)	325 (39.3)	
Black	80 (8.4)	8 (6.3)	72 (8.7)	
Asian	26 (2.7)	0 (0.0)	26 (3.1)	
Other	451 (47.2)	46 (36.2)	405 (48.9)	
Ethnicity, no. (%)				0.12
Hispanic	153 (16.0)	14 (11.0)	139 (16.8)	
Non-Hispanic	370 (38.7)	58 (45.7)	312 (37.7)	
Other	432 (45.2)	55 (43.3)	377 (45.5)	
Residential zip code, no. (%)				**< 0.01**
New York City	798 (83.6)	94 (74.0)	704 (85.0)	
Surrounding area[b]	134 (14.0)	24 (18.9)	110 (13.3)	
Other	23 (2.4)	9 (7.1)	14 (1.7)	
Location test performed, no. (%)				**< 0.01**
Office visit	799 (83.7)	105 (82.7)	694 (83.8)	
Emergency department	113 (11.8)	6 (4.7)	107 (12.9)	
Endoscopy	43 (4.5)	16 (12.6)	27 (3.3)	

Bold values are statistically significant ($p < 0.05$)

[a]Positive test indicates identification of any pathogen(s) on GI pathogen PCR testing. Toxigenic *C. difficile* was excluded from positive test results, as this result is not reported as part of the GI pathogen PCR at our institution

[b]Living in states of New York, New Jersey, or Connecticut, excluding New York City

[c]Distribution among patients with CeD/IBD compared to other patients

Table 2 Multivariable analysis of factors independently associated with a positive GI pathogen PCR result

	Odds ratio (95% CI)	*p* value
Diagnosis		
Other	1.00	
CeD/IBD	0.49 (0.31–0.77)	< **0.01**
Sex		
Female	1.00	
Male	1.44 (1.10–1.90)	< **0.01**
Age at testing		
18–29 years	1.00	
30–49 years	1.03 (0.70–1.50)	0.90
50–69 years	0.78 (0.53–1.14)	0.20
≥ 70 years	0.68 (0.44–1.05)	0.08
Race		
White	1.00	
Black	1.04 (0.62–1.74)	0.89
Asian	0.70 (0.28–1.73)	0.44
Other	1.09 (0.74–1.59)	0.68
Ethnicity		
Non-Hispanic	1.00	
Hispanic	1.27 (0.83–1.95)	0.26
Other	0.99 (0.66–1.47)	0.96
Residential zip code		
New York City	1.00	
Surrounding area[a]	0.83 (0.55–1.26)	0.38
Other	2.10 (0.89–4.98)	0.09
Location test performed		
Office visit	1.00	
Emergency department	1.58 (1.04–2.40)	**0.03**
Endoscopy	1.15 (0.58–2.27)	0.69

Bold values are statistically significant ($p < 0.05$)

[a]Living in states of New York, New Jersey, or Connecticut, excluding New York City

$p = 0.002$), more likely to be male (OR 1.44; 95% CI 1.10–1.90 $p = 0.009$), and more likely to have been tested in the emergency department than other locations (OR compared to office visit 1.58; 95% CI 1.04–2.40, $p = 0.032$). Among the 113 patients tested in the emergency department, 6 had CeD/IBD (5.3%); of the 54 patients tested in the emergency department who had a positive test result, two had CeD/IBD (3.7%).

Pathogens Identified

Among 337 total patients with positive tests, the majority were positive for one pathogen, but 69 (20.5%) were positive for two pathogens, 26 were positive for three pathogens, one patient was positive for 4 pathogens, and one patient was positive for 5 pathogens. A total of 465 bacterial, parasitic, or viral pathogens were identified.

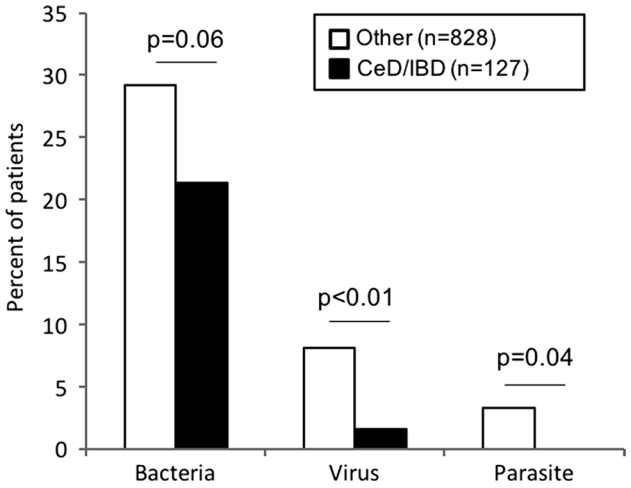

Fig. 1 Patients without or with CeD/IBD who tested positive for bacteria, viruses, or parasites. Values represent percent of patients within a diagnosis category (CeD/IBD or other)

Rate of positive test was assessed based on microbe type—bacterial, viral, or parasitic—in patient with CeD/IBD compared to other patients (Fig. 1). For bacterial pathogens, there were 269 patients (28.2%) with a positive result, comprised of 27 of 127 patients with CeD/IBD (21.3%) compared to 242 of 828 patients without (29.2%), a difference that did not meet significance ($p = 0.063$). For viral pathogens, patients with CeD/IBD had significantly fewer positive results: there were 69 patients (7.2% of total) with a positive viral test result, comprised of 2 of 127 patients with CeD/IBD (1.6%) compared to 67 of 828 patients without these conditions (8.1%) ($p = 0.008$). For parasitic pathogens, no patients with CeD/IBD had a positive result, compared to 27 patients without (2.8% of total patients, 3.3% of patients without CeD/IBD), a difference that was also statistically significant ($p = 0.039$). Analysis of CeD and IBD separately compared to controls showed the same trends, but differences in microbe type (bacterial, viral, or parasitic) were not statistically significant.

When distribution of individual species was assessed (Table 3), the combined diarrheagenic *Escherichia coli* and Shigella species—including Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC), *E. coli* O157, and *Shigella*/Enteroinvasive *E. coli* (EIEC)—accounted for 296 of 465 overall pathogens. EPEC was the most prevalent bacterial pathogen identified in all patients ($n = 143$), as well as in both patient groups separately. The most common viral and parasitic pathogens overall were *Norovirus GI/GII* ($n = 38$) and *Giardia lamblia* ($n = 13$), respectively. Among all 127 patients with CeD/IBD, 8

Table 3 Species identified among patients with positive GI pathogen PCR test based on diagnosis

	All ($N = 337$)	CeD/IBD ($n = 29$)	Other ($n = 308$)	p value
Bacteria, no. (% of patients)				
Campylobacter (jejuni, coli, and upsaliensis)	44	6 (20.7)	38 (12.3)	0.20
Plesiomonas shigelloides	3	0 (0.0)	3 (1.0)	0.59
Salmonella	9	1 (3.4)	8 (2.6)	0.79
Yersinia enterocolitica	15	0 (0.0)	15 (4.9)	0.22
Vibrio (parahaemolyticus, vulnificus, and cholerae)	0	0 (0.0)	0 (0.0)	N/A
Vibrio cholerae	0	0 (0.0)	0 (0.0)	N/A
Enteroaggregative *E. coli* (EAEC)	75	4 (13.8)	71 (23.1)	0.25
Enteropathogenic *E. coli* (EPEC)	143	18 (62.1)	125 (40.6)	0.06
Enterotoxigenic *E. coli* (ETEC)	27	1 (3.4)	26 (8.4)	0.34
Shiga-like toxin-producing *E. coli* (STEC)	24	0 (0.0)	24 (8.4)	0.12
E. coli O157	3	0 (0.0)	3 (1.0)	0.41
Shigella/Enteroinvasive *E. coli* (EIEC)	24	2 (6.9)	22 (7.1)	0.96
Parasites, no. (% of patients)				
Cryptosporidium	12	0 (0.0)	12 (4)	0.28
Cyclospora cayetanensis	3	0 (0.0)	3 (1.0)	0.59
Entamoeba histolytica	0	0 (0.0)	0 (0.0)	N/A
Giardia lamblia	13	0 (0.0)	13 (4.2)	0.26
Viruses, no. (% of patients)				
Adenovirus F 40/41	4	0 (0.0)	4 (1.3)	0.54
Astrovirus	5	0 (0.0)	5 (1.6)	0.49
Norovirus GI/GII	38	1 (3.4)	37 (12.0)	0.16
Rotavirus A	11	0 (0.0)	11 (3.6)	0.30
Sapovirus (I, II, IV, and V)	12	1 (3.4)	11 (3.6)	0.97

Statistics refer to number of positive results in patients with CeD/IBD vs. other patients for each pathogen.

Percentage shown is percentage of patients who were positive for given pathogen; as some patients were positive for more than one pathogen, sum of percentages for a given diagnosis (column) may exceed 100%

N/A not applicable

unique pathogens were identified, compared to 18 unique pathogens among all 828 patients without immune-mediated disease. Comparing the rate of each individual pathogen in patients with CeD/IBD versus other patients, there was no significant difference in any pathogen.

Rates of *C. difficile* Infection

In order to fully evaluate infectious etiologies of diarrhea, rate of *C. difficile* positivity was also assessed. Of 955 patients who underwent stool GI pathogen PCR testing, 358 (37.5%) underwent *C. difficile* testing within 7 days before or after the GI pathogen PCR test. Of the 358 patients tested for *C. difficile*, 61 had CeD/IBD and 297 did not. Seven patients with CeD/IBD (11.5% of those tested) had positive *C. difficile* test, all of whom had IBD, not CeD. Comparatively, 30 patients without immune-mediated diseases (10.1% of those tested) had positive *C. difficile* test ($p = 0.748$). Of patients tested for *C. difficile* who had a negative stool pathogen PCR test, 6 of 98 with CeD/IBD (6.1%) and 27 of 520 without immune-mediated diseases (5.2%) tested positive for *C. difficile* ($p = 0.916$). One patient with CeD/IBD and one patient without these diseases were positive both for *C. difficile* and for a pathogen on the multiplex stool PCR.

Discussion

Although patients with CeD and IBD, two immune-mediated gastrointestinal diseases, have been shown to have different rates of pneumococcal infections, tuberculosis, influenza, and *Clostridium difficile* than other patients [1–4, 6], rates of gastrointestinal infections other than *C. difficile* in these patients have not previously been extensively studied. In this retrospective cohort study of all outpatients who underwent stool pathogen testing at our institution with a novel highly sensitive and -specific PCR-based test, we found that PCR-based stool testing identified a wide range of gastrointestinal pathogens in patients with and without CeD/IBD, with diarrheagenic *E. coli* and *Shigella* species most common in both

patient groups. When compared to other patients, patients with CeD/IBD had significantly fewer positive tests, with significantly reduced rates of viruses and parasites. There was no significant difference in relative rate of any individual pathogen between the two groups. Especially in patients with CeD or IBD, who are at risk to present with diarrhea due to the nature of their underlying disease, the ability to rapidly and accurately identify diarrheal pathogens and a knowledge of common pathogens may limit invasive diagnostic workup, even precluding the need for endoscopy in some cases, and may provide opportunities for treatment.

Symptoms of underlying disease flare such as abdominal pain, increased stool frequency, or watery or bloody diarrhea may be indistinguishable from gastrointestinal infection, so patients with chronic immune-mediated luminal disorders are often evaluated for infection during an exacerbation of symptoms in order to determine appropriate treatment. Our finding that patients with CeD/IBD were less likely to have a positive stool pathogen test than other patients might therefore be expected, as patients with CeD or IBD are more likely to have diarrhea from a non-infectious cause. Interestingly, the test location that was most common among positive tests was the emergency department, suggesting that patients self-presenting due to acute or severe symptoms were most likely to have a positive test. Although it might be expected that patients with CeD/IBD would be more likely to seek evaluation of diarrheal illness, even with milder symptoms, these patients represented only 5% of all patients tested in the emergency department and only 2% of patients with positive tests in the emergency department.

As patients with CeD and IBD are known to have higher rates of *C. difficile* than other patients [6, 11, 12], we explored the hypothesis that *C. difficile* infection may offer an alternative explanation for diarrhea among patients with CeD/IBD. Patients with CeD/IBD were more likely to be tested for *C. difficile* compared to controls (48 and 36% of patients, respectively). However, among patients with negative stool GI pathogen PCR test, similar proportions of patients with CeD/IBD and patients without these conditions were found to have *C. difficile* infection (6.1 and 5.2%, respectively). This analysis was limited by the low number of tested patients, as only one-third of the overall study population had been tested for *C. difficile*.

The reduction in identified viruses and parasites among patients with CeD/IBD was significant, while bacteria showed a similar but nonsignificant trend. This study did not address mechanisms of acquisition of or immune response to infections, but two factors that may contribute are altered systemic immunity and altered gut microbiota in patients with CeD/IBD. The associations of CeD and IBD with altered systemic immunity are well established: CeD is closely associated with HLA-DQ2 and/or DQ8, and leukocytes of patients with CeD and IBD have been shown to have abnormal IFN-gamma generation and IL-18 response, among other immune mediators [13–15]. These associations have been implicated in certain clinical phenomena such as an attenuated T and B cell-mediated immune response to Hepatitis B vaccination among patients with CeD [16–19]. It is difficult to predict how this type of dysregulation differentially impacts viral or parasitic pathogens compared to bacterial ones: prior studies have shown increased rates of both viral and bacterial infections in patients with CeD or IBD [1, 3–7], while our data show a significant difference in rates of viruses and parasites but not bacteria. Both CeD and IBD have also been associated with altered gut microbiota. Patients with CeD have increased numbers of gram-negative and decreased numbers of gram-positive bacteria, but may have increased prevalence of certain Clostridial species [20]. Patients with Crohn's disease have higher prevalence of Proteobacteria [21], and patients with Crohn's disease and ulcerative colitis each have increased numbers of certain genera of Firmicutes [22]. The manner in which these microbiome deviations impact susceptibility to gastrointestinal infections remains to be elucidated.

As this was a retrospective cohort study, clinical symptoms such as number of bowel movements or the presence of bloody bowel movements were not consistently available for all patients. Assessment of 22 randomly selected patients tested in the emergency room did demonstrate that, in all of those patients, clinical indication for stool pathogen testing was diarrheal illness. However, given limited and variable clinical data, the severity of diarrhea was not measured, and no assessment was possible among patients with diarrhea of varying severity. It would be useful to collect detailed data regarding clinical symptoms in the future studies to confirm a correlation between identified pathogens and clinical symptoms, as the presence of certain pathogens as colonizers cannot be excluded. Our study was based on a PCR-based pathogen test, without confirmation by culture data. The limitations of a single assay were mediated by the fact that the stool pathogen test in our study has been shown to have high sensitivity and specificity in large-scale studies [8, 9]. Finally, data for this study were collected at a single center, and the absolute rate of positive stool pathogen test in patients with CeD/IBD in our study was low. It is therefore possible that the different rate of bacterial pathogens was nonsignificant because the study was underpowered to detect a statistical difference. Similarly, the ability to detect statistical differences in the organism-specific analysis was limited by low numbers of positive test results for each specific pathogen. As PCR-based multiplex stool pathogen tests become more widely available, prospective, multicenter analyses should be performed to confirm our findings in other patient populations.

Despite these limitations, this study demonstrates that among patients tested with a PCR-based stool pathogen

panel, patients with two immune-mediated diseases, CeD and IBD, have significantly fewer viral and parasitic pathogens identified than patients without these conditions, with a nonsignificant trend toward fewer bacterial pathogens. While this result can be partially explained by the fact that patients with CeD/IBD have more non-infectious causes of diarrhea than other patients, it may also represent an impact of altered systemic immunity on distribution of gastrointestinal pathogens. More generally, the identification of any stool pathogen may offer an important therapeutic opportunity in patients with CeD/IBD. New PCR-based stool pathogen tests offer the opportunity to study the distribution of infectious pathogens with unprecedented sensitivity and specificity and may have important clinical implications.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflicts of interest and nothing to declare.

References

1. Ludvigsson JF, Wahlstrom J, Grunewald J, Ekbom A, Montgomery SM. Coeliac disease and risk of tuberculosis: a population based cohort study. *Thorax*. 2007;62:23–28.
2. Peters U, Askling J, Gridley G, Ekbom A, Linet M. Causes of death in patients with celiac disease in a population-based swedish cohort. *Arch Intern Med*. 2003;163:1566–1572.
3. Thomas HJ, Wotton CJ, Yeates D, Ahmad T, Jewell DP, Goldacre MJ. Pneumococcal infection in patients with coeliac disease. *Eur J Gastroenterol Hepatol*. 2008;20:624–628.
4. Marild K, Fredlund H, Ludvigsson JF. Increased risk of hospital admission for influenza in patients with celiac disease: a nationwide cohort study in Sweden. *Am J Gastroenterol*. 2010;105:2465–2473.
5. Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prevot 1938. *Anaerobe*. 2016;40:95–99.
6. Nguyen GC, Kaplan GG, Harris ML, Brant SR. A national survey of the prevalence and impact of *Clostridium difficile* infection among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol*. 2008;103:1443–1450.
7. Saidel-Odes L, Borer A, Odes S. *Clostridium difficile* infection in patients with inflammatory bowel disease. *Ann Gastroenterol*. 2011;24:263–270.
8. Pascarella F, Martinelli M, Miele E, Del Pezzo M, Roscetto E, Staiano A. The impact of *Clostridium difficile* infection on pediatric inflammatory bowel disease. *J Pediatr*. 2009;154:854–858.
9. Khare R, Espy MJ, Cebelinski E, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol*. 2014;52:3667–3673.
10. Buss SN, Leber A, Chapin K, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol*. 2015;53:915–925.
11. Ananthakrishnan AN, McGinley EL, Binion DG. Excess hospitalisation burden associated with *Clostridium difficile* in patients with inflammatory bowel disease. *Gut*. 2008;57:205–210.
12. Lebwohl B, Nobel YR, Green PHR, Blaser MJ, Ludvigsson JF. Risk of *Clostridium difficile* infection in patients with celiac disease: a population-based study. *Am J Gastroenterol*. 2017;112:1878–1884.
13. Al-Toma A, Goerres MS, Meijer JW, Pena AS, Crusius JB, Mulder CJ. Human leukocyte antigen-DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma. *Clin Gastroenterol Hepatol*. 2006;4:315–319.
14. Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, Buckner JH. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. *J Immunol*. 2007;179:4704–4710.
15. Jarry A, Malard F, Bou-Hanna C, et al. Interferon-alpha promotes Th1 response and epithelial apoptosis via inflammasome activation in human intestinal mucosa. *Cell Mol Gastroenterol Hepatol*. 2016;3:72–81.
16. Park SD, Markowitz J, Pettei M, et al. Failure to respond to hepatitis B vaccine in children with celiac disease. *Pediatr Gastroenterol Nutr.*. 2007;44:431–435.
17. Nemes É, Lefler É, Szegedi L, et al. Gluten intake interferes with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease. *Pediatrics*. 2008;121:e1570–e1576.
18. Noh KW, Poland GA, Murray JA. Hepatitis B vaccine nonresponse and celiac disease. *Am J Gastroenterol*. 2003;98:2289–2292.
19. Di Sabatino A, Rosado MM, Cazzola P, et al. Splenic hypofunction and the spectrum of autoimmune and malignant complications in celiac disease. *Clin Gastroenterol Hepatol*. 2006;4:179–186.
20. Girbovan A, Sur G, Samasca G, Lupan I. Dysbiosis a risk factor for celiac disease. *Med Microbiol Immunol*. 2017;206:83–91.
21. Wlodarska M, Kostic AD, Xavier RJ. An integrative view of microbiome-host interactions in inflammatory bowel disease. *Cell Host Microbe*. 2015;17:577–591.
22. Fyderek K, Strus M, Kowalsa-Duplaga K, et al. Mucosal bacterial microflora and mucus later thickness in adolescents with inflammatory bowel disease. *World J Gastroenterol*. 2009;15:5287–5294.