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TWO-STEP TESTING FOR *CLOSTRIDIOIDES DIFFICILE* IS INADEQUATE IN DIFFERENTIATING INFECTION FROM COLONIZATION IN CHILDREN

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Abstract

Objectives: Recent Infectious Disease Society of America guidelines recommend multi-step testing algorithms to diagnose *Clostridioides difficile* infection (CDI), including a combination of nucleic acid amplification-based testing (NAAT) and toxin enzyme immunoassay (EIA). However, the use of these algorithms in children, including the ability to differentiate between *C. difficile* colonization and CDI, has not been evaluated.

Methods: We prospectively enrolled asymptomatic pediatric patients with cancer, cystic fibrosis (CF), or inflammatory bowel disease (IBD) and obtained a stool sample for NAAT testing. If positive by NAAT (colonized), EIA was performed. In addition, children with symptomatic CDI

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Author's Contributions

JMP: Assisted in study concept and design, assisted in data collection and interpretation, drafted the manuscript and approved the final manuscript as submitted.

IF and RB: Assisted in study concept and design, assisted in patient enrollment and specimen collection, performed laboratory analysis, performed critical revision of the manuscript and approve the manuscript as submitted.

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MRN: Assisted in study concept and design, obtained funding for the project, designed the data collection form, assisted in patient enrollment and specimen collection, performed laboratory analysis, performed the statistical analysis and assisted in data interpretation, performed critical revision of the manuscript for important intellectual content and approves the manuscript as submitted.

who tested positive by NAAT via the clinical laboratory were enrolled, and EIA was performed on residual stool. A functional cell cytotoxicity neutralization assay (CCNA) was also applied to stool samples from both the colonized and symptomatic cohorts.

Results: Of the 225 asymptomatic children enrolled in the study, 47 (21%) were colonized with *C. difficile* including 9/59 (15.5%) with cancer, 30/92 (32.6%) with CF and 8/74 (10.8%) with IBD. An additional 41 children with symptomatic CDI were enrolled. When symptomatic and colonized children were compared, neither EIA positivity (44% versus 26%, P=0.07) nor CCNA positivity (49% versus 45%, P=0.70) differed significantly or were able to predict disease severity in the symptomatic cohort.

Conclusions: Use of a multi-step testing algorithm with NAAT followed by EIA failed to differentiate symptomatic CDI from asymptomatic colonization in our pediatric cohort. As multi-step algorithms are moved into clinical care, the pediatric provider will need to be aware of their limitations.

Keywords

inflammatory bowel disease; cystic fibrosis; cancer; enzyme immunoassay; testing

Introduction

*Clostridioides (*formerly *Clostridium) difficile* is a spore-forming, Gram-positive bacterium that is the leading cause of antibiotic-associated diarrhea and gastroenteritis-associated death in the United States.[1, 2] Despite increasing clinical importance, the detection of *C. difficile* remains a conundrum; does it reflect disease or colonization?[3]

Colonization, defined as the detection of *C. difficile* in the stool in the absence of diarrheal symptoms attributable to *C. difficile*, complicates diagnosis and is increasingly recognized in patients with comorbidities.[4] Initial studies suggested a colonization prevalence of 30%, 50%, and 17% in children with cancer, cystic fibrosis (CF), and inflammatory bowel disease (IBD), respectively.[5–7] Another study of hospitalized children identified as many asymptomatic colonized patients as symptomatic CDI cases.[8] Conversely, hospitalized children and children with comorbidities are also at higher risk of having severe and recurrent CDI and are therefore frequently tested.[9–11] Differentiation of children symptomatic with CDI from those who are colonized by *C. difficile* and have diarrheal symptoms due to another etiology is widely recognized as one of the greatest difficulties for clinicians treating patients with CDI.[3]

To accurately diagnose CDI, several tests and diagnostic strategies have been evaluated, all with notable limitations.[12] Due to concerns about the decreased specificity of nucleic acid amplification testing (NAAT), the 2017 *Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children* by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) recommended the use of multi-step testing algorithms for the diagnosis of CDI if there is no pre-agreed institutional criteria for patient stool submission. One proposed algorithm included a combination of enzyme immunoassay (EIA) and NAAT[12] and was based on prospective observational studies

evaluating optimal diagnostic strategies in symptomatic adult patients. Some adult studies have demonstrated that a positive EIA is predictive of CDI-related complications and deaths,[11, 13–15] although these results are not universal.[16] Cell cytotoxicity neutralization assays (CCNA) are considered one of the gold standards for CDI detection[17] and a large study identified significantly higher mortality in those with a + CCNA.[18]

We assessed the clinical utility of a multi-step diagnostic algorithm to differentiate *C. difficile* colonization from CDI in pediatric patients using NAAT followed by EIA. CCNA was also performed.

Methods

Study Design

Pediatric participants, ages 12 months through 18 years, were prospectively enrolled from July 2017 through December 2019 at Monroe Carell Jr. Children's Hospital at Vanderbilt after informed parental consent and patient assent when applicable. The Vanderbilt Institutional Review Board approved this study.

Thorough medical histories were obtained from all participants, including past hospitalizations, surgeries, and medications that were received 30 days prior to enrollment, with focus on immunosuppression, antibiotics, and acid blockers, and confirmed by medical record review. Data were kept strictly confidential using a REDCap database (REDCap software, Vanderbilt University).[19]

Colonized Cohort:

Asymptomatic children, defined as without diarrhea or a change in their stool pattern, and not undergoing active testing or treatment for *C. difficile*, who were between 12 months and 18 years of age with a diagnosis of cancer (solid tumor or hematologic malignancy), CF, or IBD were eligible for enrollment. Patients were recruited during outpatient visits or hospitalizations. Stool form was characterized at time of processing and patients were excluded if stool was found to be watery (Bristol Stool Scale type 7) or mushy (Bristol Stool Scale type 6). At the time of processing, an aliquot underwent testing by NAAT in the clinical laboratory. If positive by NAAT, the child was considered colonized[20, 21] and EIA and CCNA were then performed in the research laboratory. The asymptomatic participants were followed by both phone calls and chart review at 30, 60 and 90-days post-enrollment to evaluate for the development of symptomatic CDI.

Symptomatic Cohort:

Symptomatic children with diarrhea (unformed stools) between 12 months and 18 years of age who underwent clinical laboratory testing and tested positive for *C. difficile* by NAAT were enrolled. This included previously healthy children and those with additional comorbidities. Children were excluded from the analysis if they did not have both an acute change in stool character and ≥ 3 stools per 24-hour period based on IDSA/SHEA guidelines[12] or if they tested positive for an alternative enteropathogen on clinical testing by the treating provider. After consent, residual stool from the clinical laboratory was collected,

and EIA and CCNA were performed in the research laboratory. Symptomatic participants were followed with phone calls and chart review at 30- and 60-days post-enrollment to evaluate for CDI complications and recurrence.

Sample Processing and Testing:

NAAT was performed in the hospital clinical laboratory using Illumigene *C. difficile* assay (ARUP laboratories), a polymerase chain reaction test to detect the *C. difficile* gene *tcdB* encoding Toxin B (Sensitivity 90%, Specificity 96%).[22, 23] NAAT has been widely used in clinical *C. difficile* screening programs on formed samples.[24, 25] EIA testing was performed in the research laboratory in duplicate via enzyme-linked immunoassay testing using premier Toxins A and B from Meridian Biosciences per manufacturer recommendations which do not include stool form requirements (Sensitivity 80.8%, Specificity 97.5%).[22, 26]

Functional Cell Cytotoxicity Neutralization Assay/Vero Cell Rounding:

Vero cells (Green African monkey kidney epithelial cells) in DMEM (10% FBS/1% Pen/Strep) were seeded at 1.5×10^4 cells per well on a Corning (cat# 3603) 96-well flat bottom plate. Cells were incubated for 48 hours at 37°C, 5% CO2 to facilitate adherence to the plate. Stool samples were weighed, and a 10:1 dilution stock of each in sterile PBS was created. Samples were sterilized through a 0.22um filter and six, ten-fold serial dilutions were performed. For each fecal dilution, one part was mixed either with equal part PBS or equal part diluted anti-toxin (using *C. difficile* toxin/antitoxin kit provided by Techlab cat# T5000). Anti-toxin was used to confirm the presence of *C. difficile* toxin by neutralizing cell-rounding activity. The samples were incubated at room temperature for 45 min, then 10uL of sample mixture was removed and placed on cells containing 90uL of media. Vero cell rounding was visualized with a light microscope.

Statistical Methods and Sample Size calculation:

Data were managed using REDCap (REDCap software, Vanderbilt University)[19] and analyzed using Stata (Stata Corp., College Station, TX) and R (R Core Team, Vienna, Austria). Patient variables were compared using Pearson's Chi-squared test or Fisher's exact test for categorical data and Wilcoxon rank-sum test for continuous data.

Power calculation:

Power calculations were created based on prior adult data that EIA toxin tests were positive in 14% of asymptomatic *C. difficile* positive adults[27] and 45% of symptomatic adults with CDI.[28] Based on these estimates, we projected we needed 35 cases and 35 control patients to be able to reject the null hypotheses that the proportions were equal with probability (power) of 0.8 and type 1 error probability of 0.05.

Results

We enrolled 279 asymptomatic children during the study period. Children were excluded from the study due to the presence of watery stool (n=8), the presence of mushy stool (n=44), or inadequate sample for complete testing (n=2). NAAT was performed on samples from the remaining 225 children, and, if positive, the patient was included in the colonized

cohort. NAAT was positive in 47 (20.8%) children; including 9/59 (15.3%) with cancer (including 7 with a hematologic malignancy and 2 with a solid tumor malignancy), 30/92 (32.6%) with CF, and 8/74 (10.8%) with IBD.

Twenty-one (9%) of the asymptomatic children had a history of CDI, 3 of whom were positive for NAAT during the study (colonized), while the remainder were negative by NAAT. In those with prior confirmed CDI, median (IQR) time from infection to study enrollment was 19 (7 to 31) months. In the 90 days following enrollment, two of the colonized patients developed symptomatic CDI. Both were diagnosed >30 days after enrollment. There were no deaths in the asymptomatic cohort.

An additional 83 patients with symptomatic diarrhea who had + NAAT test through the routine clinical laboratory were approached. Children were excluded if they did not have both an acute change in stooling habits and ≥ 3 bowel movements per 24 hours (n=23), they tested positive for an alternative enteropathogen (n=6), or they did not have enough stool for both ELISA and CCNA (n=13). The final symptomatic cohort consisted of 41 patients. The type of CDI included 24 (58%) community-associated, 6 (15%) healthcare facility-onset, and 11 (27%) community-onset healthcare facility-associated per Center for Disease Control and Prevention definitions.[29] In the symptomatic cohort, 29 of the 41 patients included in the final cohort had additional stool testing done through the clinical lab which was performed by stool culture (n=8), giardia antigen testing (n=1), ova and parasites (n=1), rectal vancomycin-resistant enterococcus culture (n=2) and/or a PCR-based gastrointestinal panel (n=27). In the 12 patients that did not have additional testing, all had received antibiotics (n=11) and/or immunosuppression (n=6) in the 30 days prior to CDI diagnosis.

Demographics of symptomatic and colonized cohorts are compared in Table 1. Symptomatic compared with colonized children did not significantly differ in median (IQR) age (11 years (5 to 15) vs 9 years (4 to 12), P=0.06) or gender (54% male versus 49% male, P=0.66). Comorbidity profiles differed between groups based on differences in enrollment strategies. Colonized children were more likely than symptomatic children to have a history of acid blocker use in the 30 days prior to enrollment (81% versus 38%, p<0.001).

The 47 colonized and 41 symptomatic children with + NAAT had stool tested for *C. difficile* toxin via EIA and CCNA as previously described. EIA positivity (44% vs 26%, P=0.07) or CCNA positivity (49% vs 45%, P=0.70) did not differ between symptomatic and colonized children (Table 2) although a trend toward more positive testing in symptomatic children was observed. Additionally, there was no significant difference in EIA or CCNA positivity among children when stratified by disease process or gender. Colonized children who were EIA+ were younger than children who were EIA – with a median (IQR) age of 7 years (3 to 9) versus 10 years (4 to 15) (P=0.06) but this result did not reach statistical significance. In the symptomatic cohort, no significant differences were found in median (IQR) age between EIA + and EIA − children (10.5 years (5 to 14) versus 11 years (7 to 15), P=0.51).

Colonized children who had antibiotic use in the 30 days prior to enrollment were more likely to have a + EIA when compared to children without antibiotic use (85% versus 40%, P=<0.01). There was a trend toward higher rates of + CCNA in those with prior antibiotic

history, although not statistically significant (56% versus 32%, P=0.10). In the symptomatic cohort, antibiotic use did not differ between those who were EIA + versus EIA − (72% versus 65%, P=0.632) or CCNA + versus CCNA − (75% versus 62%, P=0.37).

In the 47 colonized and 41 symptomatic pediatric patients, concordance was found between EIA and CCNA test results in 63 (72%); both were negative in 40 and both were positive in 23. When results were discordant between tests, EIA was positive and CCNA negative in 7 and EIA negative and CCNA positive in 18.

In the symptomatic cohort, the presence of a + EIA test or + CCNA did not predict more severe symptoms or clinical laboratory markers of CDI severity[30] (Table 3). No CDI-related complications were observed in the symptomatic patients. Twenty-two (54%) children were treated with oral vancomycin and 19 (46%) children were treated with oral metronidazole. Three patients were lost to follow-up. Of the 38 symptomatic children that completed study follow-up, 35 (92%) had improvement in diarrhea at the time of antibiotic cessation. Median (IQR) days until diarrhea resolved was 7 (3,10) (n=25). The 3 patients that did not have improvement in diarrhea at the time of antibiotic cessation all had a diagnosis of IBD and were later treated for a flare. One patient died, unrelated to CDI (3%), and 11 (29%) experienced an episode of recurrent CDI.

Discussion

Despite increasing incidence and evolving health consequences, the diagnosis of CDI is fraught with difficulty, much of which revolves around challenges differentiating between *C. difficile* colonization and CDI. We identified high rates of *C. difficile* colonization in our children with comorbidities; including 9/59 (15.5%) with cancer, 30/92 (32.6%) with CF and 8/74 (10.8%) with IBD, similar to rates previously reported.[5–7] These children with comorbidities also are at high risk of diarrhea from a variety of alternative etiologies including viral infections, chemotherapy, antibiotics, acid suppression, and as a manifestation of their underlying disease, in the case of IBD, which further confounds differentiation of CDI and *C. difficile* colonization.

As this differentiation remains problematic, many diagnostic approaches have been tried. Since the approval of NAAT, to detect the gene for *C. difficile* toxins, by the Food and Drug Administration in 2009, multiple centers have moved to this diagnostic approach given its superior sensitivity and fast turn-around time.[28] However, single centers noted a 50 to 100% increase in the rate of CDI after implementation of NAAT testing causing concern over the potential for colonization detection and the overdiagnosis of CDI.[31, 32] Polage et al. found that adults who were NAAT + but EIA toxin - had a lower *C. difficile* bacterial load, fewer antibiotic exposures, and less fecal inflammation and diarrhea than those who were both NAAT + and EIA toxin +. In addition, nearly all CDI-related complications in their patients were seen in those with both a positive EIA and NAAT.[28] These authors concluded that exclusive reliance on molecular tests for CDI diagnosis was resulting in overdiagnosis and treatment.[28]

In contrast, Humphries et al. did not identify differences in EIA positivity in adult patients with mild versus severe disease (49% vs 58%, *P*=0.31). They concluded that the poor sensitivity of toxin EIA does not support its use in a testing algorithm and recommended the use of NAAT as the primary diagnostic laboratory test for CDI.[16] More recently, an ultrasensitive quantitative toxin immunoassay was also unable to differentiate adults with CDI from those with asymptomatic carriage,[21] but quantitation of serum cytokines and anti-toxin immunoglobulin levels differentiated colonization from CDI in another cohort of adult patients.[20]

Despite these conflicting findings, the 2017 *Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children* systematically reviewed diagnostic approaches and proposed the use of a stool toxin test as part of a multistep algorithm in the absence of a pre-agreed institutional criteria for patient stool submission. The authors noted this was a weak recommendation based on low quality of evidence.[12] However, many institutions were swift to institute this approach based on these guidelines.[33]

To our knowledge this is the first attempt to apply a multi-step testing approach including NAAT and EIA to a cohort of pediatric patients to evaluate its ability to differentiate between *C. difficile* colonization and CDI. In our pediatric cohort, NAAT followed by EIA failed to differentiate symptomatic disease and colonization (EIA + in 44% vs 26%, respectively, *P*=0.07) although there was a non-significant trend toward increased positivity in symptomatic patients. Pediatric patients who were EIA + were also no more likely to have more severe symptoms or clinical laboratory markers of CDI severity when compared with those who were EIA − (Table 3).

We also performed a functional CCNA via vero cell rounding on stool samples to evaluate if this performed better than EIA testing. Using similar methodology, Planche et al. evaluated clinical data and outcomes in adult patients who were positive by CCNA versus positive by culture and negative by CCNA. Patients with positive CCNA had significantly higher mortality than those who were CCNA negative and culture positive (16.6% vs 9.7%, *P*=0.04) on univariate analysis.[18] However, we could not replicate these findings in our cohort of pediatric patients. Like our EIA findings, children positive by CCNA were no more likely to be symptomatic than colonized (49% versus 45%, *P*=0.70)) or have more severe symptoms or clinical laboratory abnormalities (Table 3).

The reason why children can have functionally active *C. difficile* toxin and not develop symptomatic disease remains unclear and warrants additional study. Perhaps the adaptive immune response, differences in the intestinal microbiome, or alterations in the intestinal toxin receptors or the intestinal mucus layer can protect the host from developing CDI in the setting of *C. difficile* colonization. Although we did not identify a difference in EIA results when stratified by age, perhaps dynamic changes that occur in the intestinal flora in early childhood may be clinically relevant in children with CDI and *C. difficile* colonization and warrant additional study. Interestingly, in the colonized cohort, children who had received an antibiotic in the 30 days prior to enrollment were more likely to be EIA + than those who did not have a recent antibiotic exposure (*P*=<0.01). The reason why children with both identifiable toxin and recent antibiotic exposure do not develop symptomatic CDI warrants

study and may elucidate additional protective mechanisms. Alterations in the intestinal mucus layer may be particularly relevant in pediatric patients with CF, where altered mucus is well recognized,[34] high rates of colonization are described, and symptomatic disease is rare.[6]

Limitations of this study include the relatively small sample size, although appropriate based on our power calculations, and a nonsignificant trend toward increased EIA positivity in the symptomatic cohort. Continued collection of larger numbers of stool samples from both symptomatic and colonized patients may reveal small but important differences between *C. difficile* colonization and CDI and strengthen the associations with EIA testing. A second limitation is the risk of misclassification bias, with potentially symptomatic patients being classified as colonized and vice versa. To limit this, we excluded asymptomatic patients with watery or mushy stool at the time of enrollment and required symptomatic patients to report an acute change in diarrhea or ≥3 bowel movements in 24 hours per current IDSA/SHEA definitions.[12] We excluded patients with an additional enteropathogen detected but there remains the possibility that some of the children in the symptomatic cohort were infected by an alternative pathogen as 12 (29%) of the 41 did not have additional testing. Notably, all 12 patients had received either an antibiotic or immunosuppression in the 30 days prior to CDI diagnosis, increasing the likelihood of CDI. In addition, the clinical significance of co-infections remains poorly defined and may not preclude active CDI.[35]

Enrollment strategies differed between the colonized and symptomatic cohorts. We enrolled only children with comorbidities in the colonized cohort since healthy children are less commonly colonized,[36] while the symptomatic cohort included children with and without comorbidities. This was done based on achievability of cohort enrollment, but future studies should focus on children with and without comorbidities to elucidate additional differences. In our study, we found that EIA and CCNA positivity was not altered by the presence of a comorbidity in either the symptomatic or colonized cohorts. Finally, discrepancies between EIA and CCNA results in our cohort may reflect technical difficulties in CCNA or differences in test sensitivity and specificity.

In conclusion, we found that the use of a multi-step testing algorithm using NAAT and EIA, one approach recommended by current IDSA guidelines,[12] was unable to accurately differentiate colonization versus CDI in our cohort of children, many with comorbidities. NAAT followed by CCNA did not improve the ability to differentiate between CDI and C. *difficile* colonization. It is possible that other multi-step approaches as mentioned by the IDSA guidelines,[12] such as glutamate dehydrogenase (GDH) plus toxin or GDH plus toxin arbitrated by NAAT or additional testing using serum cytokines and anti-toxin antibodies may yield improved diagnostic strategies.

Identifying characteristics that distinguish *C. difficile* colonization from infection is critical to limit unnecessary antibiotic use and prevent delayed and missed diagnoses and may also help identify important components in *C. difficile* pathophysiology. Future research will need to investigate other possible diagnostic targets in CDI. As multi-step algorithms move into greater clinical use, pediatricians should be aware of the limitations that still exist for *C. difficile* diagnosis. With knowledge of these limitations, the clinical context, including

likelihood of colonization, must be strongly considered when testing for and diagnosing CDI.

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WHAT IS KNOWN

- *C. difficile* is the most common cause of gastroenteritis-associated death in the United States.
- *C. difficile* infection (CDI) is difficult to diagnose, and a variety of testing strategies have been suggested.
- *C. difficile* colonization confounds the diagnosis of CDI.

WHAT IS NEW

- Children with cystic fibrosis, cancer, and inflammatory bowel disease have high rates of *C. difficile* colonization.
- Nucleic acid amplification-based testing (NAAT) followed by toxin enzyme immunoassay (EIA) failed to differentiate *C. difficile* infection from colonization in children.
- Children with + EIA did not have more severe *C. difficile* symptoms or clinical laboratory abnormalities.

Table I.

Demographic summary of symptomatic versus colonized patients positive for *C. difficile* by nucleic acid amplification-based testing

	Symptomatic (n=41)	Colonized (n=47)	*P* value
Age (median, IQR)	11 (5, 15)	9 (4, 12)	0.06
Gender			0.66
Male (n=45)	22 (54%)	23 (49%)	
Female (n=43)	19 (46%)	24 (51%)	
Comorbidity			
CF (n=30)	0	30 (64%)	<0.001
Cancer (n=21)	12 (29%)	9 (19%)	0.27
IBD (n=17)	9 (22%)	8 (17%)	0.60
None/other	20 (49%)	0	<0.001
Location at time of enrollment			0.68
Inpatient (n=43)	21 (51%)	22 (47%)	
Outpatient/ER (n=45)	20 (49%)	25 (53%)	
Antibiotic use in 30 days prior to enrollment (n=53)	28 (68%)	25 (53%)	0.15
Acid blocker use in 30 days prior to enrollment (n=54)	16 (39%)	38 (81%)	<0.001
Hospital stay in 90 days prior to enrollment (n=41)	17 (41%)	24 (51%)	0.37
Surgery in 90 days prior to enrollment (n=26)	12 (29%)	14 (30%)	0.96

CF, cystic fibrosis; IBD, inflammatory bowel disease; IQR, interquartile range

Table II.

Comparison of toxin enzyme immunoassay and vero cell rounding results in pediatric patients positive for *C. difficile* on nucleic acid amplification-based testing

	EIA + (N=30)	EIA – (N=58)	Vero cell + (N=41)	Vero cell – (N=47)
Symptomatic	18 (60%)	23 (40%)	20 (49%)	21 (45%)
Colonized	12 (40%)	35 (60%)	21 (51%)	26 (55%)
Age in years (median, IQR)	8 (4, 13)	10.5 (5, 15)	9 (4, 13)	10 (5, 16)
Gender				
Male	17 (57%)	28 (48%)	24 (59%)	21 (45%)
Female	13 (43%)	30 (52%)	17 (41%)	26 (55%)
CF	8 (27%)	22 (38%)	13 (32%)	17 (36%)
Non-CF	22 (73%)	36 (62%)	28 (68%)	30 (64%)
Symptomatic IBD	2 (7%)	7 (12%)	4 (10%)	5 (11%)
Symptomatic Non-IBD	16 (53%)	16 (28%)	16 (39%)	16 (34%)
Colonized IBD	1 (3%)	7 (12%)	4 (10%)	4 (9%)
Colonized Non-IBD	11 (37%)	28 (48%)	17 (41%)	22 (47%)
Symptomatic Cancer	4 (13%)	8 (14%)	4 (10%)	8 (17%)
Symptomatic Non-cancer	14 (47%)	15 (26%)	16 (39%)	13 (28%)
Colonized Cancer	3 (10%)	6 (10%)	4 (10%)	5 (11%)
Colonized Non-cancer	9 (30%)	29 (50%)	17 (41%)	21 (45%)

CF, cystic fibrosis; EIA, enzyme immunoassay; IBD, inflammatory bowel disease; IQR, interquartile range

Table III.

Toxin immunoassay and Vero cell rounding results as markers of severity in patients with symptomatic CDI

	EIA + (n=18)	EIA – (n=23)	Vero cell + (n=20)	Vero cell – (n=21)
Symptoms				
Fever	4 (22%)	9 (39%)	4 (20%)	9 (43%)
Blood in stools	8 (44%)	7 (30%)	7 (35%)	8 (38%)
Number of stools[1]	10.4	8.5	8 (6–12)	7 (4–10)
Vomiting	5 (28%)	10 (43%)	8 (40%)	7 (33%)
Abdominal pain	16 (89%)	16 (70%)	16 (80%)	16 (76%)
Laboratory Values				
WBC (median, IQR) (n=34)	6.6 (4.6, 10.5)	6.2 (2.9, 10.7)	7.2 (4.6, 12)	5.8 (3.9, 8.5)
CRP (median, IQR) (n=20)	6.7 (2.8, 9.8)	8.5 (1.3, 73.3)	7.6 (2, 10)	8.5 (2.3, 129.2)
ESR (median, IQR) (n=19)	9.5 (5.5, 16.5)	30 (12, 37)	12.5 (6, 19)	30 (7, 37)
Albumin (median, IQR) (n=28)	4.2 (3.5, 4.2)	3.8 (3.5, 4.2)	4.2 (3.5, 4.2)	3.8 (3.5, 4.2)
Relapse in 60 days following CDI (n=11)	6 (33%)	5 (22%)	7 (35%)	4 (19%)

[1]Maximum number of stools in 24 hours; mean.

CDI, *C. difficile* infection; CRP, C-reactive protein; EIA, enzyme immunoassay; ESR, erythocyte sedimentation rate; IQR, interquartile range; WBC, white blood cell count