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ORIGINAL ARTICLE

Clinical heterogeneity of patients with stool samples testing PCR+/Tox− from a two-step *Clostridium difficile* diagnostic algorithm

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Abstract

The clinical significance of indeterminate (PCR+/Tox−) results for patients tested with a two-step algorithm for *Clostridium difficile* infection (CDI) is uncertain. We aimed to evaluate the clinical presentation and 8-week outcomes of patients with indeterminate test results. Patients with stool samples testing positive by PCR and negative by toxin A/B immunoassay between February 1, 2017, and April 30, 2018, were assessed by antimicrobial stewardship program (ASP) clinicians and classified as colonized or infected. Retrospective chart review was performed to obtain outcomes occurring within 8 weeks of testing, including recurrent *C. difficile* diarrhea, subsequent treatment for CDI, follow-up *C. difficile* testing, all-cause mortality, and CDI-related complications. In total, 110 PCR+/Tox− patients were evaluated. ASP classified 54% of patients as infected and 46% as colonized. Patients assessed and classified as colonized did not have increased adverse outcomes by 8 weeks compared to those assessed as infected, despite not receiving treatment for CDI. We conclude that PCR+/Tox− patients are heterogeneous with respect to clinical presentation. Negative toxin A/B immunoassay in a two-step algorithm should not be interpreted in isolation to distinguish colonization from infection as many PCR+/Tox− results may be clinically significant for CDI.

Keywords Clostridium difficile · Diagnosis · PCR · Toxin · Indeterminate · Outcomes

Introduction

The diagnosis of *Clostridium difficile* infection (CDI) is complex, with variations in laboratory practice incorporating molecular testing and multi-step diagnostic algorithms. Concerns about suboptimal sensitivity have limited the role of toxin A/B enzyme immunoassay (EIA) and prompted laboratories to implement molecular assays for diagnosis [1]. However, there is mounting evidence that PCR for toxigenic *C. difficile* cannot distinguish true infection from asymptomatic colonization, potentially leading to overdiagnosis and unnecessary treatment [2]. In response, multi-step diagnostic algorithms combining glutamate dehydrogenase (GDH) and/or toxin EIA with PCR have been proposed to improve specificity and are a recommended approach for *C. difficile* diagnosis in the most recent update to the IDSA/SHEA *C. difficile* guidelines [3, 4]. One caveat with this multi-step approach is the need to interpret discordant or "indeterminate" results that arise—for example, a positive PCR followed by a negative toxin EIA—of which the clinical significance is a topic of substantial debate [5]. We sought to assess symptomatic infection compared to asymptomatic colonization at presentation among patients with indeterminate results, as well as assess their clinical outcomes 8 weeks after testing.

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Methods

Study population and laboratory testing for *C. difficile*

This study was performed at one tertiary-care hospital and one community hospital in Vancouver, Canada. Adult inpatients with stool samples submitted for *C. difficile* testing between February 1, 2017, and April 30, 2018, yielding indeterminate results were included in our study. All samples were tested using a two-step diagnostic algorithm. Briefly, an in-house developed real-time PCR targeting the *tcd*C gene was performed on all samples [6], followed by EIA for toxins A/B and GDH (C.DIFF QUIK CHEK COMPLETE®, TECHLAB®, Blacksburg, VA) on PCR-positive samples. An indeterminate result was defined as a sample testing positive by PCR and by GDH EIA but negative by EIA for toxin A/B (PCR+/GDH+/Tox−), or a sample positive by PCR and toxin A/B EIA but negative for GDH (PCR+/GDH−/Tox+). Given that the vast majority (> 98%) of indeterminate results encountered in our study were PCR+/GDH+/Tox−, we will use "PCR+/Tox−" synonymously with "indeterminate" for the remainder of this publication. If a single patient had multiple indeterminate tests within the study period, only the first indeterminate result was included in the analysis. Formed stool samples (Bristol types 1–5) submitted for testing were rejected by the laboratory. Residents of long-term care facilities and non-admitted patients tested in the emergency department or in outpatient clinics were also excluded from the study.

All *C. difficile* cases were classified as healthcare facility-onset (HO), community-onset healthcare facility associated (CO-HCFA), and community-onset (CO) in accordance with standardized definitions as outlined in the 2017 IDSA/SHEA *C. difficile* guidelines [3]. In brief, HO *C. difficile* cases were defined as those detected > 72 h after admission to our facility. If the patient was tested ≤ 72 h after admission but had a previous admission in the past 4 weeks within our hospital network lasting at least 24 h, then the patient was classified as CO-HCFA. *C. difficile* PCR results were also categorized into wild-type, or *tcd*C variants (which has been associated with the NAP1 strain), and the cycle threshold (Ct) value was recorded for each PCR performed [6].

Clinical assessment

Patients with an indeterminate result were reviewed by either the antimicrobial stewardship (ASP) physician or pharmacist to assess whether the patient was colonized or infected, based on new-onset ≥ 3 loose stools in 24 h without an alternate diagnosis [3, 7]. ASP review also included a review of the chart (including stool charts), medications (e.g., laxatives or other medications associated with diarrhea such as metformin), and laboratory results. A discussion would then be initiated with the most responsible physician (MRP). Patients were classified by ASP as colonized or infected based on ASP clinical assessment. If after consultation with ASP, the MRP decided to continue treatment for a patient assessed as colonized by ASP, then cases would be classified as infected.

Clinical data collection

Clinical data were obtained by a retrospective chart review. Data related to the patients' baseline clinical status at the time of testing were obtained, including relevant biochemical abnormalities (WBC > 15 or < 4×10^9 cells/L, creatinine > 130 μmol/L, albumin < 25 g/L), presence of comorbidities, patient location (ICU or other ward), and any completed treatment for CDI during their admission (metronidazole or oral vancomycin). Outcomes occurring within 8 weeks (56 days) after testing were also recorded and included development of new-onset *C. difficile* associated diarrhea, need for subsequent CDI treatment (metronidazole or oral vancomycin), follow-up *C. difficile* testing, all-cause mortality, and development of complications (colectomy, toxic megacolon, ICU admission). Patients with multiple follow-up tests performed within the 8-week follow-up period were classified according to their most significant test result, with positive results taking precedence over indeterminate or negative results. Only patients whose follow-up tests were all negative were classified as having negative follow-up testing within 8 weeks.

Comorbidities recorded at time of testing and during the 8-week follow-up period included diabetes mellitus, chronic cardiovascular disease, chronic pulmonary disease, chronic renal disease (including hemodialysis), chronic liver disease (cirrhosis), malignancy, cerebrovascular disease/dementia/immobility, inflammatory bowel disease (IBD), ischemic colitis/intestinal vascular insufficiency, functional diarrheal disorders, intraabdominal infections, solid organ transplant, bone marrow transplant, non-transplant immunosuppression (systemic corticosteroids, HIV infection), or viral enteritis.

Statistical analysis

We stratified patients with indeterminate test results by ASP assessment (infected versus colonized) and compared them based on clinical data described above. Categorical variables were compared using the chi-square or Fisher exact test, as appropriate. Continuous variables were compared using Student's *t* test. Two-tailed tests were used for all comparisons, and results were considered significant if $p < 0.05$. Statistical analyses were performed using OpenEpi [8].

Results

In total, 2102 unique stool samples were sent for *C. difficile* testing during the study period. There were 288 (13.7%) positive by PCR, and of those, 174 (60.4%) were indeterminate (Fig. 1). One hundred ten indeterminate patients met the inclusion criteria in our study.

Baseline clinical characteristics and patient comorbidities were compared between patients assessed as colonized and patients assessed as infected. A higher proportion of patients assessed as infected were immunosuppressed (non-transplant related) at baseline compared to those classified as colonized (33.9% vs. 15.7%, $p = .049$). There were no other significant differences in baseline clinical characteristics or comorbidities. The majority of these cases had no known association with healthcare facilities and were defined as CO (55.4%). HO cases made up 35.5% of cases in our study, and a minority of cases were CO-HCFA (9.1%). No significant differences were noted between HO, CO-HCFA, and CO cases when comparing colonized and infected groups. Wild-type *tcd*C strains were predominant (83.2%). There were no significant differences in the proportion of *tcd*C variants, or in mean Ct value between colonized and infected groups.

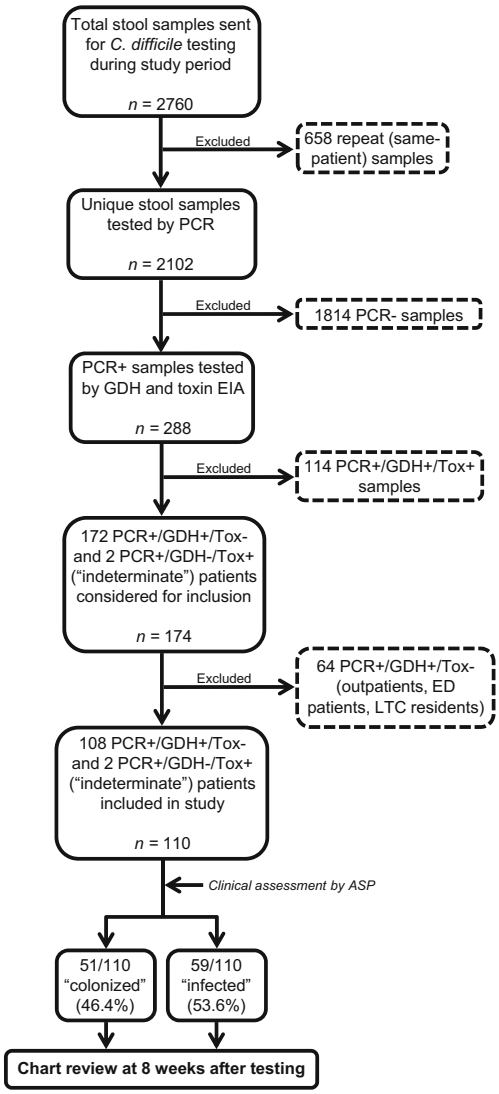

Fig. 1 Outline of study population and clinical classification. Abbreviations: *EIA*, enzyme immunoassay; *PCR*, polymerase chain reaction for *C. difficile tcd*C gene; *GDH*, *C. difficile* glutamate dehydrogenase; *Tox*, *C. difficile* toxin A/B; *ED*, emergency department; *LTC*, long-term care; *ASP*, antimicrobial stewardship program

ASP assessment classified 53.6% of patients with indeterminate tests as infected, and the remaining 46.4% as colonized. Clinical outcomes developing within 8 weeks of testing are listed in Table 1 and did not significantly differ between colonized and infected patients.

As a post hoc analysis, we evaluated outcomes of patients with IBD included in our study since asymptomatic colonization with *C. difficile* and true infection are difficult to distinguish in this population, potentially resulting in IBD patients with underlying colonization being classified as infected due to empiric CDI therapy. Out of 110 patients, 14 had IBD (12.7%), of which 9 were classified as infected. There were no significant differences in any 8-week outcomes when comparing IBD patients assessed as colonized to those assessed as infected. Compared to patients without IBD classified as infected, infected patients with IBD had a higher incidence of all-cause colectomy within 8 weeks (22% vs. 0%, $p = 0.04$) but no differences in other 8-week outcomes.

Discussion

Clinical assessment by ASP revealed that a substantial proportion (53.6%) of those with indeterminate *C. difficile* laboratory results at our institution were diagnosed with and treated for CDI. Despite recent studies of two-step algorithms suggesting there are minimal clinical implications of a PCR-positive, toxin EIA-negative result, our study highlights heterogeneity in clinical presentation among PCR+/Tox− patients and suggests that further individualized clinical evaluation of these patients is warranted [2, 9–12]. This is consistent with the recommendations in the current European *C. difficile* diagnostic guidelines, which suggests that PCR+/Tox− results may represent true infection or colonization and require further clinical evaluation [13].

Importantly, the remaining 46.4% of patients assessed by ASP were classified as being colonized with *C. difficile* and having other underlying causes of diarrhea. Despite none of these patients receiving CDI-specific therapy, we did not observe a significant increase in clinical relapse, all-cause mortality, complications of CDI, or other adverse outcomes by 8 weeks compared to the infected group that received treatment. These balancing measures showing a lack of adverse outcomes suggest that individualized clinical assessment of colonization or infection by ASP at the time of testing could

Table 1 Clinical outcomes 8 weeks after initial testing, stratified by ASP clinical assessment

Outcome	Colonized ($n = 51$)	Infected ($n = 59$)	Total ($n = 110$)	P value
Follow-up *C. difficile* testing performed, no. (%)	17 (33.3)	23 (39.0)	40 (36.4)	.68
Negative repeat testing, no./total no. tested (%)	9/17 (52.9)	8/23 (34.8)	17/40 (42.5)	.41
Indeterminate repeat testing, no./total no. tested (%)	7/17 (41.2)	8/23 (34.8)	15/40 (37.5)	.93
Positive repeat testing, no./total no. tested (%)	1/17 (5.9)	7/23 (30.4)	8/40 (20.0)	.12
New-onset *C. difficile* related diarrhea, no. (%)	1 (2.0)	7 (11.9)	8 (7.3)	.10
All-cause colectomy, no. (%)	2 (3.9)	2 (3.4)	4 (3.6)	> .99
All-cause megacolon, no. (%)	0 (0)	0 (0)	0 (0)	–
All-cause ICU care, no. (%)	3 (5.9)	3 (5.1)	6 (5.5)	> .99
Subsequent treatment with metronidazole or oral vancomycin, no. (%)	1 (2.0)	7 (11.9)	8 (7.3)	.10
All-cause mortality, no. (%)	6 (11.8)	5 (8.5)	11 (10.0)	.80

ASP antimicrobial stewardship program, *ICU* intensive care unit

safely reduce inappropriate CDI-specific antimicrobial use. This also supports the notion that a one-step algorithm involving PCR alone can lead to overdiagnosis and inappropriate treatment, and as such a one-step approach may have falsely classified these cases as infected. Reducing such "false-positives" after a positive PCR, through the use of a two-step algorithm and clinical assessment, can help limit the potential harm associated with unnecessary antibiotic exposure in patients asymptomatically colonized with *C. difficile*, as well as any untoward effects of being labeled CDI-positive. In addition to direct medication related side effects, metronidazole and vancomycin have the potential to result in unintended consequences due to the disruption of the normal gastrointestinal microbiota. The normal flora of the gastrointestinal tract, specifically obligate anaerobic bacteria and non-toxigenic strains of *C. difficile*, have been reported to be protective for CDI [14, 15]. In mice models, microbiota disruption has also been associated with acquisition of nosocomial pathogens such as vancomycin-resistant Enterococci [16].

Our study has limitations. This study was conducted in two hospitals in a single city and may not be generalizable to other regions or healthcare facilities. In addition, the classification of an infection was based on the clinical assessment by ASP and a discussion with the MRP, but if there were discordance in clinical impression, classification was deferred to the MRP. This likely resulted in some colonized patients being categorized as infected, which may have underestimated the differences between colonized and infected patients with indeterminate results. This represents a conservative approach to diagnosis that reflects inherent challenges in the clinical diagnosis of CDI, namely difficulties in interpreting non-specific signs and symptoms and the presence of confounding factors such as IBD. The inclusion of patients with IBD in the infected group may also have underestimated differences between colonized and infected patients, as IBD-associated diarrhea is often difficult to distinguish clinically from CDI-related diarrhea. However, our post hoc analysis of IBD patients assessed as infected showed that they did not have outcomes that differed significantly from those of infected patients without IBD, though this comparison is limited by the low number of infected IBD patients. Inclusion of immunosuppressed patients, in which there may be a bias towards empiric CDI therapy, may also have contributed to overestimation of the number of infected patients. However, in a separate post hoc analysis, removal of all immunosuppressed patients (solid organ transplant, bone marrow transplant, and non-transplant immunosuppressed; $n = 37$) did not change any of our outcomes significantly when comparing colonized and infected groups ($p > 0.05$). In addition, post-discharge follow-up is an inherent challenge of surveillance and ideally would include direct follow-up with the patient at 8 weeks post-diagnosis. However, follow-up after the initial positive result based on repeat encounters at our healthcare facilities or repeat *C. difficile* testing at another laboratory would have captured the majority of complications in our study.

Implementation of a two-step algorithm for *C. difficile* diagnostics identified a significant proportion of PCR-positive samples which are GDH or toxin-negative (60.4%, 174/288). The collaboration between ASP and Medical Microbiology supported clinicians in the interpretation of the diagnostic algorithm, and clinical review of indeterminate results identified 54% of patients had CDI. Cost-effectiveness was not evaluated in this study, but will be important to address given the importance of clinical evaluation for the diagnosis of colonization or infection. This study reinforces the need for clinical assessment of PCR+/Tox− patients as this population can be clinically heterogeneous and not all PCR+/Tox− results are clinically significant. The two-step algorithm and review of all *C. difficile* indeterminates also provides an opportunity to steward anaerobic agents for colonized patients. Although limited evidence exists for infection control precautions for colonized patients, current recommendations advise against

placing asymptomatic patients on precautions [3]. With in-the-moment assessment of colonization status by ASP, the Infection Prevention and Control team could also appropriately manage indeterminate patients, particularly since private rooms are a scarce resource in many older healthcare facilities.

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Compliance with ethical standards

Ethics approval Ethics approval was obtained for this study by the University of British Columbia – Providence Health Care Research Institute research ethics board.

Conflicts of interest The authors declare that they have no conflicts of interest.

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