

Original Investigation

Overdiagnosis of *Clostridium difficile* Infection in the Molecular Test Era

Christopher R. Polage, MD, MAS; Clare E. Gyorke, BS; Michael A. Kennedy, BS; Jhansi L. Leslie, BS; David L. Chin, PhD; Susan Wang, BS; Hien H. Nguyen, MD, MAS; Bin Huang, MD, PhD; Yi-Wei Tang, MD, PhD; Lenora W. Lee, MD; Kyoungmi Kim, PhD; Sandra Taylor, PhD; Patrick S. Romano, MD, MPH; Edward A. Panacek, MD, MPH; Parker B. Goodell, BS, MPH; Jay V. Solnick, MD, PhD; Stuart H. Cohen, MD

IMPORTANCE *Clostridium difficile* is a major cause of health care–associated infection, but disagreement between diagnostic tests is an ongoing barrier to clinical decision making and public health reporting. Molecular tests are increasingly used to diagnose *C difficile* infection (CDI), but many molecular test–positive patients lack toxins that historically defined disease, making it unclear if they need treatment.

OBJECTIVE To determine the natural history and need for treatment of patients who are toxin immunoassay negative and polymerase chain reaction (PCR) positive (Tox–/PCR+) for CDI.

DESIGN, SETTING, AND PARTICIPANTS Prospective observational cohort study at a single academic medical center among 1416 hospitalized adults tested for *C difficile* toxins 72 hours or longer after admission between December 1, 2010, and October 20, 2012. The analysis was conducted in stages with revisions from April 27, 2013, to January 13, 2015.

MAIN OUTCOMES AND MEASURES Patients undergoing *C difficile* testing were grouped by US Food and Drug Administration–approved toxin and PCR tests as Tox+/PCR+, Tox–/PCR+, or Tox–/PCR–. Toxin results were reported clinically. Polymerase chain reaction results were not reported. The main study outcomes were duration of diarrhea during up to 14 days of treatment, rate of CDI-related complications (ie, colectomy, megacolon, or intensive care unit care) and CDI-related death within 30 days.

RESULTS Twenty-one percent (293 of 1416) of hospitalized adults tested for *C difficile* were positive by PCR, but 44.7% (131 of 293) had toxins detected by the clinical toxin test. At baseline, Tox–/PCR+ patients had lower *C difficile* bacterial load and less antibiotic exposure, fecal inflammation, and diarrhea than Tox+/PCR+ patients ($P < .001$ for all). The median duration of diarrhea was shorter in Tox–/PCR+ patients (2 days; interquartile range, 1–4 days) than in Tox+/PCR+ patients (3 days; interquartile range, 1–6 days) ($P = .003$) and was similar to that in Tox–/PCR– patients (2 days; interquartile range, 1–3 days), despite minimal empirical treatment of Tox–/PCR+ patients. No CDI-related complications occurred in Tox–/PCR+ patients vs 10 complications in Tox+/PCR+ patients (0% vs 7.6%, $P < .001$). One Tox–/PCR+ patient had recurrent CDI as a contributing factor to death within 30 days vs 11 CDI-related deaths in Tox+/PCR+ patients (0.6% vs 8.4%, $P = .001$).

CONCLUSIONS AND RELEVANCE Among hospitalized adults with suspected CDI, virtually all CDI-related complications and deaths occurred in patients with positive toxin immunoassay test results. Patients with a positive molecular test result and a negative toxin immunoassay test result had outcomes that were comparable to patients without *C difficile* by either method. Exclusive reliance on molecular tests for CDI diagnosis without tests for toxins or host response is likely to result in overdiagnosis, overtreatment, and increased health care costs.

JAMA Intern Med. 2015;175(11):1792–1801. doi:10.1001/jamainternmed.2015.4114
Published online September 8, 2015.

← Invited Commentary
page 1801

+ Supplemental content at
jamainternalmedicine.com

+ CME Quiz at
jamanetworkcme.com and
CME Questions page 1880

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Christopher R. Polage, MD, MAS, Department of Pathology and Laboratory Medicine, University of California Davis School of Medicine, Specialty Testing Center Bldg, 3740 Business Dr, Sacramento, CA 95820 (christopher.polage@ucdmc.ucdavis.edu).

C*lostridium difficile* is one of the most common causes of health care-associated infection in US hospitals, affecting almost 1% of hospitalized patients each year.¹⁻³ Since 2000, the incidence of *C difficile* infection (CDI) has increased more than 200% while the rates of other health care-associated infections have decreased.^{1,2,4-6} More than 300 000 hospitalizations involve a CDI each year, at an annual cost of \$1.0 to \$4.9 billion to the US health care system.^{2,7}

Initial increases in the rate of CDI were attributed to the emergence of a novel, hypervirulent strain during a period when at least 95% of hospitals used toxin immunoassays for diagnosis (2000-2008).^{3,5,8-10} More recent increases have been linked to greater *C difficile* detection after the introduction of molecular tests, which are more sensitive and detect microbial DNA instead of toxin.¹⁰⁻¹⁵ Individual hospitals have reported a 50% to 100% increase in the rate of CDI after switching from toxin tests to molecular tests.^{11,12,14} Similar increases have been observed in the rate of publicly reported CDI as reporting facilities adopted molecular tests.¹⁵

For decades, toxin tests were favored over culture for diagnosis of CDI because toxins mediate disease and toxin detection was faster and provided evidence of toxin production in vivo that typically correlated better with clinical disease.^{3,10,16-18} Molecular tests such as polymerase chain reaction (PCR) target toxin genes but are similar to culture in detecting *C difficile* bacteria regardless of toxin production, making it unclear whether positive PCR results reflect clinical disease.^{3,10,19-21} The uncertain clinical significance of positive PCR results is problematic in inpatient health care facilities, where *C difficile* colonization is 5 to 10 times more common than CDI and noninfectious causes of diarrhea are also common.²²⁻²⁶ Nonetheless, concern that patients with CDI were being missed by toxin tests prompted many laboratories to switch to molecular tests in 2009, when they became available.^{10,19,27} As of the first quarter of 2014, a total of 44% of acute care hospitals participating in the National Healthcare Safety Network (NHSN) reported using molecular tests alone or in combination with other tests for diagnosis of CDI (NHSN, written communication, September 15, 2014). Therefore, there is an urgent need to determine whether patients with negative toxin test results and positive molecular test results have CDI or are simply colonized with another cause of symptoms.

To address this need, we prospectively tested hospitalized adults with suspected CDI at the University of California Davis Medical Center with molecular tests while maintaining our existing toxin test for clinical diagnosis. We then collected clinical outcome and treatment data to enable us to ask 3 related questions. First, what is the natural history of PCR-positive patients with negative toxin immunoassay results? Second, how do outcomes in these patients compare with outcomes in patients with positive toxin and PCR results or completely negative *C difficile* test results? Third, do PCR-positive patients with negative toxin results require treatment for CDI?

Methods

Study Design and Population

Hospitalized adults with a diarrheal stool sample submitted for *C difficile* testing 72 hours or longer after admission to the University of California Davis Medical Center between December 1, 2010, and October 20, 2012, were included in the study. Only the first sample was analyzed for each patient. Samples received after discharge were excluded. Patients with *C difficile* detected by culture and no other test were excluded from the study. The study protocol was approved by the University of California Davis Institutional Review Board. Informed consent was waived for the initial screening and symptom verification and overall outcome and safety analysis. A subset of patients had written informed consent obtained for additional in-person follow-up.

Laboratory Testing

All stool samples had a US Food and Drug Administration (FDA)-approved *C difficile* toxin immunoassay (*C difficile* Premier toxins A and B; Meridian Biosciences) performed and reported clinically. Formed stools were rejected. Eligible samples also had 1 or more FDA-approved molecular *C difficile* tests (Xpert *C. difficile*/Epi; Cepheid; and illumigene *C. difficile*; Meridian Biosciences) performed but not reported, allowing patients to be grouped by *C difficile* toxin immunoassay and PCR results as toxin immunoassay positive and PCR positive (Tox+/PCR+), Tox-/PCR+, or Tox-/PCR-. Additional tests were performed to characterize the nature of the *C difficile* colonization and host inflammatory response. The PCR-positive samples had toxin quantitated (xCELLigence System for Real-Time Cellular Analysis, version 2; ACEA Biosciences) and the concentration of *C difficile* DNA determined as a measure of bacterial load (Xpert *C. difficile*/Epi; Cepheid).²⁸⁻³⁰ The Tox-/PCR+ samples were tested by a cell cytotoxin assay (*C. difficile* Tox-B; TechLab), the more sensitive historical standard for *C difficile* toxin detection and diagnosis, to determine the number of samples that would have been positive if this test had been used instead of the toxin immunoassay. Culture was performed to recover *C difficile* isolates for ribotyping and verification of capacity to produce toxins. Lactoferrin was measured in PCR+ samples and random PCR- samples as a marker of inflammation (Leuko EZ Vue; TechLab; and IBD-Scan; TechLab). Lactoferrin results were classified as high if they exceeded the 95th percentile of results in PCR- patients. See the eMethods in the Supplement for additional details.

Clinical Data Collection

Diarrheal symptoms were verified at the time of *C difficile* testing. Patients were considered to have diarrhea if they had at least 3 unformed bowel movements or at least 600 mL of rectal or colostomy output recorded in the electronic health record (EHR) within 24 hours on the day of or before sample collection. Patients not meeting the threshold for diarrhea in the EHR had their nurse called to verify diarrheal status. Other data were obtained from laboratory, EHR, and adminis-

trative databases. See the eMethods in the Supplement for additional details.

Outcomes and Clinical Case Attribution

The primary outcome was duration of diarrhea for the 15-day period encompassing the day of sample collection (day 1) and up to 14 days of treatment. Secondary outcomes included rate of CDI-related complications (ie, megacolon, colectomy for fulminant colitis, and intensive care unit [ICU] care related to CDI) and CDI-related deaths within 30 days. The CDI-related complications and deaths were analyzed separately to distinguish patients with complicated CDI disease of the colon from patients with CDI as a contributing cause of death but not necessarily complicated CDI of the colon. Repeat *C difficile* tests and treatment were analyzed within 14 days of day 1 as an indication of ongoing clinical suspicion or empirical treatment for CDI in Tox-/PCR+ patients and to determine how many became positive with repeat testing. *Clostridium difficile* tests and treatment 15 to 30 days after day 1 were analyzed as a proxy for recurrent or prolonged CDI occurring after the initial treatment period. Ten or more days of metronidazole or oral vancomycin therapy was considered full treatment. Duration of diarrhea was determined from nurse-recorded stool counts and rectal or colostomy outputs in the EHR, excluding formed stools. Each day was categorized as a diarrhea day if at least 3 unformed stools or at least 600 mL of fecal output was recorded. Days with less stool output were categorized as a no-diarrhea day. Duration of diarrhea was the sum of days from day 1 to the last day with diarrhea, followed by 2 or more days without diarrhea. Cases of CDI-related megacolon and colectomies were identified by searching for patients with a procedure or billing code for abdominal radiology, colonoscopy, colectomy, or diagnosis of megacolon or pseudomembranous colitis within 30 days (eTable 1 in the Supplement). Clinical and surgical notes and radiology, endoscopy, and pathology reports were reviewed to confirm or exclude CDI-related megacolon or colectomy. Partially treated complications diagnosed before day 1 were excluded. Intensive care unit care related to CDI was determined as follows. First, patients located in or transferred into the ICU on day 1 (± 1 day) were identified. The ICU care was then determined to be CDI related (ie, attributable to or contributed to by CDI) or unrelated by blinded EHR review by 2 board-certified infectious diseases physicians (H.H.N., L.W.L., J.V.S., or S.H.C.). The physician adjudicators were blinded to PCR results but otherwise were provided with all relevant clinical, procedural, diagnostic, and outcome information available in the EHR. Disagreements were resolved by a third infectious diseases physician (H.H.N., L.W.L., J.V.S., or S.H.C.). Deaths were identified by discharge disposition codes and EHR review of PCR-positive patients with unknown mortality status at 30 days. Attribution of deaths as CDI related or unrelated was determined by blinded infectious diseases physician EHR review (L.W.L., J.V.S., or S.H.C.) in the same manner as for ICU care.

Statistical Analysis

Baseline data were summarized and tested for differences. The Kruskal-Wallis test was used for continuous variables except

for age, which was compared with an analysis of variance. For categorical variables, including outcomes, a χ^2 test or Fisher exact test was used. Kaplan-Meier estimates were used to show time to resolution of diarrhea for each group, with censoring of patients who were discharged or died during the follow-up, and compared with the log-rank test. A Cox proportional hazards model was used to estimate the effect of Tox+/PCR+ or Tox-/PCR+ status compared with Tox-/PCR- status on the duration of diarrhea, adjusting for age, comorbidities, ICU status on day 1 (± 1 day), prior antibiotic days, prior metronidazole or oral vancomycin exposure, maximum white blood cell count on day 1 (± 1 day), *C difficile* ribotype, and fecal lactoferrin level. See the eMethods in the Supplement for additional details.

Results

Patient Cohort and Baseline Characteristics

An overview of the study design, patient cohort, and follow-up is shown in Figure 1. In total, 1416 hospitalized adults were analyzed, including 131 Tox+/PCR+ patients (9.3%), 162 Tox-/PCR+ patients (11.4%), and 1123 Tox-/PCR- patients (79.3%).

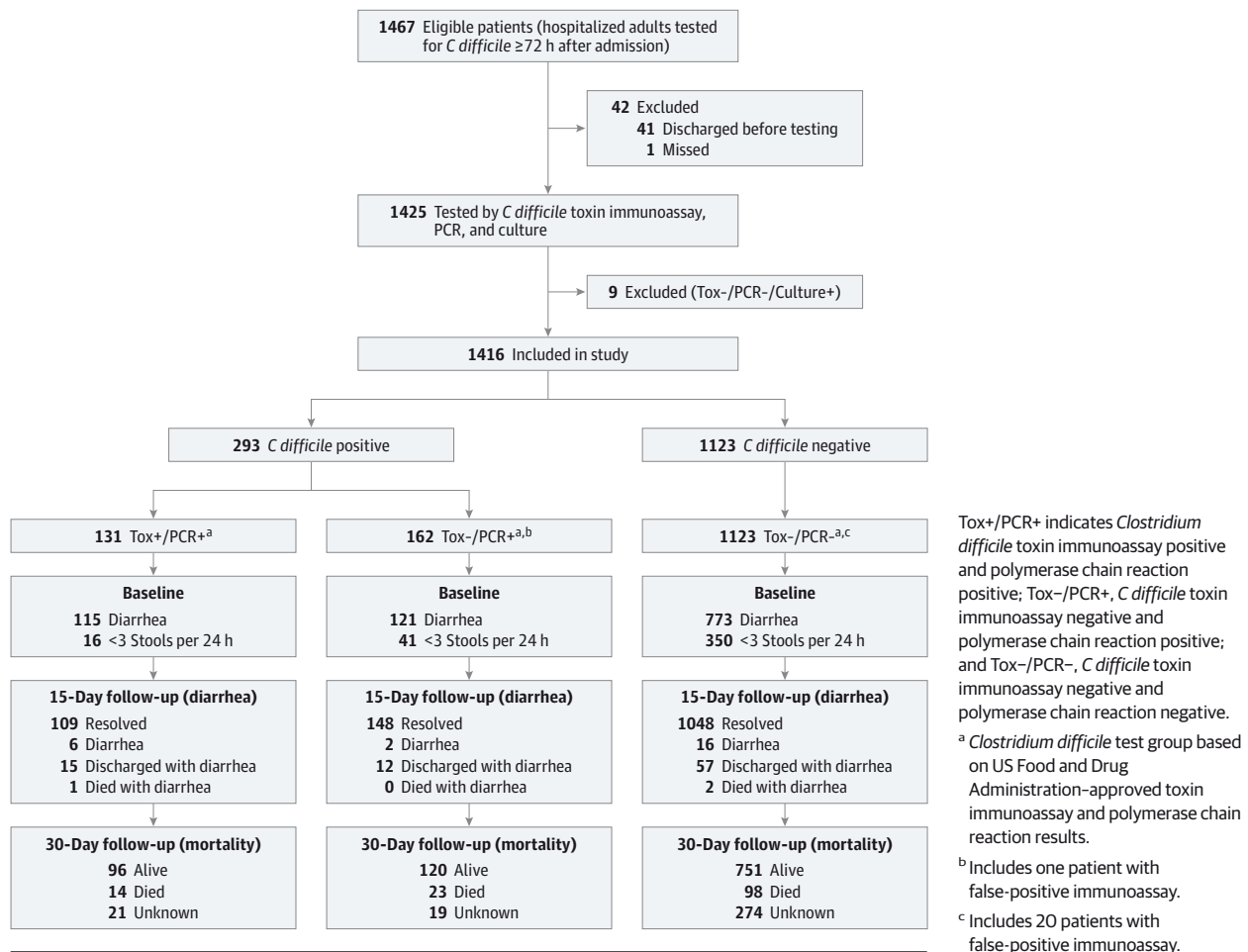
The groups were similar in age, sex, number of comorbidities, nonantibiotic medication exposures, and proportions with leukopenia, renal insufficiency, and hypoalbuminemia except for fewer comorbidities in Tox-/PCR- patients (Table 1 and eTable 2 in the Supplement). However, the Tox+/PCR+ group had more prior antibiotic exposure, more patients with leukocytosis, and more diarrhea on day 1. In feces, Tox+/PCR+ patients had an increased *C difficile* bacterial load, higher toxin concentration, and greater frequency of hypervirulent *C difficile* strain than Tox-/PCR+ patients. Correspondingly, Tox+/PCR+ patients had significantly more fecal lactoferrin than Tox-/PCR+ patients, and 36.8% (43 of 117) had a lactoferrin level greater than the 95th percentile of Tox-/PCR- patients. In contrast, few Tox-/PCR+ patients (13.4% [19 of 142]) had a lactoferrin level above the 95th percentile of Tox-/PCR- patients, and 79.0% (15 of 19) of these patients had an alternative explanation for fecal inflammation, a previous diagnosis of CDI, or anti-*C difficile* treatment before testing (eTable 3 in the Supplement).

Duration of Diarrhea

The Tox+/PCR+ patients had a longer duration of diarrhea than Tox-/PCR+ patients and Tox-/PCR- patients ($P < .001$) and had a greater risk of diarrhea during the follow-up (Figure 2 and Table 2). In contrast, Tox-/PCR+ patients and Tox-/PCR- patients had a similar risk of diarrhea on most days.

In the multivariable model, Tox+/PCR+ status had the strongest effect on duration of diarrhea, decreasing the probability of diarrhea being resolved by 37% each day relative to the Tox-/PCR- reference group (hazard ratio, 0.63; 95% CI, 0.48-0.83). Age, white blood cell count, and lactoferrin level were also significant predictors of duration of diarrhea, but their relative contribution was small ($\leq 2\%$ each) (eTable 4 in the Supplement). The Tox-/PCR+ status and pretest exposure to

Figure 1. Flow of Patients Through Testing and Follow-up



metronidazole or oral vancomycin were not significant predictors in the multivariable model.

CDI-Related Complications and Mortality Within 30 Days

The frequency of CDI-related complications (ie, megacolon, colectomy for fulminant colitis, and ICU care related to CDI) and deaths is summarized in Table 3. The Tox+/PCR+ patients had more CDI-related complications than Tox-/PCR+ patients and Tox-/PCR- patients (10 [7.6%] of 131 vs 0 [0%] of 162 vs 3 [0.3%] of 1123, $P < .001$). In contrast, the rate of CDI-related complications was similar between Tox-/PCR+ patients and Tox-/PCR- patients (0% vs 0.3%, $P > .99$). The Tox+/PCR+ patients also had more CDI-related deaths than Tox-/PCR+ patients and Tox-/PCR- patients (11 [8.4%] of 131 vs 1 [0.6%] of 162 vs 0 [0%] of 1123, $P < .001$) while the rate was similar between Tox-/PCR+ patients and Tox-/PCR- patients (0.6% vs 0%, $P = .13$). Two deaths in the Tox+/PCR+ group were directly attributable to CDI, and 9 had CDI as a contributing factor. One Tox-/PCR+ patient (patient 1641 in eTable 3 in the Supplement) had an uncomplicated, recurrent CDI that resolved before care was withdrawn for severe underlying illness, but CDI was considered a contributing factor to death.

Repeat *C difficile* Testing and Treatment Within 14 Days

Repeat *C difficile* testing and treatment within 14 days of day 1 was analyzed as an indication of ongoing clinical suspicion or empirical treatment for CDI in Tox-/PCR+ patients (Table 3). During this period, 61 Tox-/PCR+ patients (37.7%) were retested, and 13 (8.0%) had toxins detected (mean time to positive result, 5.7 days; 95% CI, 3.2-8.2 days). None of these patients developed a *C difficile*-related complication. However, one patient (patient 1641 in eTable 3 in the Supplement) had CDI that was considered a contributing factor to death, although symptoms had resolved before care was withdrawn for other reasons. During the same period, most Tox-/PCR+ patients (59.3% [96 of 162]) received no treatment, 45 patients (27.8% [45 of 162]) received partial treatment (1-9 days), and 21 patients (13.0% [21 of 162]) received the equivalent of full treatment (≥ 10 days).

Clostridium difficile Testing and Treatment Between 15 and 30 Days

Clostridium difficile tests and treatment 15 to 30 days after day 1 were analyzed as a proxy for recurrent or prolonged CDI (Table 3). During this period, Tox+/PCR+ patients were retested almost twice as often as Tox-/PCR+ patients (19.8% vs

Table 1. Baseline Characteristics of the Study Population by *Clostridium difficile* Test Group

Characteristic	<i>C difficile</i> Positive		<i>C difficile</i> Negative	
	Tox+/PCR+ ^b (n = 131)	Tox-/PCR+ ^{b,c} (n = 162)	Tox-/PCR- ^{b,d} (n = 1123)	P Value ^a
Age, median (IQR), y	64 (52-71)	58 (48-68)	59 (47-71)	.12
Female sex, No. (%)	64 (48.9)	83 (51.2)	530 (47.2)	.61
Comorbidities, median (IQR)	4 (2-6)	4 (2-5)	3 (2-5)	.01
APR-DRG risk of mortality subclass 3 or 4, No. (%)	104 (79.4)	128 (79.0)	787 (70.1)	.008
Intensive care unit care on day 1 ± 1 d, No. (%) ^e	30 (22.9)	57 (35.2)	435 (38.7)	.002
Hospital days before day 1, median (IQR) ^e	10 (6-24)	8 (5-12)	8 (5-12)	<.001
Admitted from health care facility, No. (%)	40 (30.5)	34 (21.0)	160 (14.2)	<.001
<i>C difficile</i> positive within 90 d before day 1 ^e	5 (3.8)	10 (6.2)	13 (1.2)	<.001
Antibiotic days within 90 d before day 1, median (IQR) ^e	16 (7-32)	10 (4-27)	8 (4-18)	<.001
Other diarrheal or gastrointestinal inflammatory process, No. (%) ^f	8 (6.1)	27 (16.7)	161 (14.3)	.02
Metronidazole or oral vancomycin within 48 h before day 1, No. (%) ^e	3 (2.3)	32 (19.8)	184 (16.4)	<.001
WBC count ≥15 000 cells/μL on day 1 ± 1 d, No./total No. tested (%) ^e	54/129 (41.9)	50/154 (32.5)	323/1101 (29.3)	.01
WBC count <4000 cells/μL on day 1 ± 1 d, No./total No. tested (%) ^e	20/129 (15.5)	32/154 (20.8)	200/1101 (18.2)	.52
Creatinine level >1.5 mg/dL on day 1 ± 1 d, No./total No. tested (%) ^e	36/127 (28.3)	45/156 (28.8)	297/1102 (27.0)	.85
Albumin level <2.5 g/dL on day 1 ± 1 d, No./total No. tested (%) ^e	29/48 (60.4)	50/70 (71.4)	318/475 (66.9)	.46
Diarrhea present on day 1 ± 1 d, No. (%) ^e	121 (92.4)	143 (88.3)	927 (82.5)	.004
Stool count on day 1, median (IQR) ^e	5 (3-6)	3 (2-5)	3 (2-5)	<.001
<i>C difficile</i> toxin B, median (IQR), ng/mL	640.8 (172.5-1194.0)	1.1 (0.3-2.5)	NA	<.001
Hypervirulent <i>C difficile</i> ribotype RT027/078, No. (%)	68 (51.9)	39 (24.1)	NA	<.001
<i>C difficile</i> binary toxin positive, No. (%)	71 (54.2)	45 (27.8)	NA	<.001
Log ₁₀ <i>C difficile</i> DNA copies/mL, median (IQR)	7.3 (6.6-7.7)	4.9 (4.4-6.2)	NA	<.001
Fecal lactoferrin level, median (IQR), μg/mL,	37.7 (8.8-261.5)	20.1 (5.0-50.3)	7.8 (0.5-32.6)	<.001
Normal lactoferrin level, No./total No. tested ^g	25/117 (21.4)	44/142 (31.0)	89/188 (47.3)	<.001
High lactoferrin level, No./total No. tested ^h	43/117 (36.8)	19/142 (13.4)	9/188 (4.8)	<.001

Abbreviations: APR-DRG, all-patient refined diagnosis-related group; IQR, interquartile range; NA, not applicable; Tox+/PCR+, *C difficile* toxin immunoassay positive and polymerase chain reaction positive; Tox-/PCR+, *C difficile* toxin immunoassay negative and polymerase chain reaction positive; Tox-/PCR-, *C difficile* toxin immunoassay negative and polymerase chain reaction negative; WBC, white blood cell.

SI conversion factors: To convert WBC count to ×10⁹/L, multiply by 0.001; to convert creatinine level to micromoles per liter, multiply by 88.4; to convert albumin level to grams per liter, multiply by 10.

^a P value for significance across 3 groups except for characteristics not applicable to Tox-/PCR- group.

^b *Clostridium difficile* test group based on US Food and Drug Administration-approved toxin immunoassay and PCR results.

^c Includes one patient with false-positive toxin immunoassay.

^d Includes 20 patients with false-positive toxin immunoassay.

^e Day 1 is the day of sample collection for the *C difficile* toxin test.

^f Includes inflammatory bowel diseases, functional diarrheal disorders, diverticulitis, appendicitis, ischemic colitis, other infectious or noninfectious enterocolitis, graft-vs-host disease, and peritoneal, mesenteric, or retroperitoneal infections.

^g Normal fecal lactoferrin level defined as within the upper limit of a healthy person's reference range per the manufacturer's package insert.

^h High fecal lactoferrin level defined as exceeding the 95th percentile fecal lactoferrin level in Tox-/PCR- patients (>89.05 μg/mL).

11.1%, $P = .04$) and were positive 3 times more often (10.7% vs 3.1%, $P < .001$). During the same period, most Tox-/PCR+ patients (78.4% [127 of 162]) received no treatment, while 13 patients (8.0% [13 of 162]) received treatment for at least 10 days.

Additional Analyses to Evaluate the Robustness of the Study Findings

Outcome differences between the Tox-/PCR+ and Tox+/PCR+ groups remained significant when comparisons were limited to the subgroup of Tox-/PCR+ patients who received full or partial treatment within 14 days ($P = .04$ for time to resolution of diarrhea and $P = .004$ for CDI-related complication or death) or no treatment ($P = .003$ for time to resolution of diarrhea and

$P < .001$ for CDI-related complication or death). No significant outcome differences were observed between the Tox-/PCR- group and individual Tox-/PCR+ subgroups with or without treatment.

If the historical cell cytotoxin assay had been used for diagnosis instead of a toxin immunoassay, 48 additional Tox-/PCR+ patients (29.6%) would have been reported positive. However, this subgroup had a low toxin concentration (median, 10 ng/mL; interquartile range, 2-81 ng/mL) and outcomes that were similar to cell cytotoxin-negative Tox-/PCR+ patients ($P = .47$ for time to resolution of diarrhea and $P = .30$ for CDI-related complication or death), with no difference in treatment ($P = .61$), and better than Tox+/PCR+ pa-

tients ($P < .001$ for time to resolution of diarrhea and $P = .03$ for CDI-related complication or death).

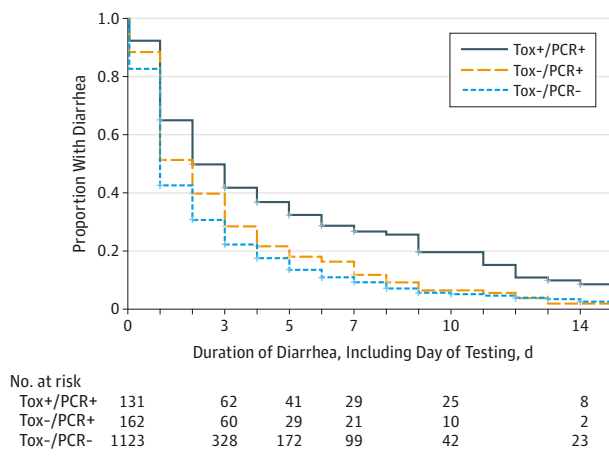
Discussion

This study addresses an important question for physicians, hospitals, and policy makers: do toxin-negative patients with a positive *C difficile* PCR test result require treatment? To

answer this question, we prospectively tested 1416 hospitalized patients with FDA-approved PCR tests while maintaining our existing toxin test for clinical diagnosis to determine the natural history of toxin-negative patients with positive PCR results. We found that 55.3% (162 of 293) of patients with a positive *C difficile* PCR test result lacked toxin by the clinical toxin immunoassay test and had outcomes that were comparable to patients with no *C difficile* detected. These Tox-/PCR+ patients had milder symptoms at the time of testing and a shorter duration of diarrhea than toxin-positive patients. In total, 58.7% (95 of 162) were never retested, and only 13.0% (21 of 162) received the equivalent of a full course of treatment. Repeat analyses with the treated Tox-/PCR+ patients removed did not change our conclusions. Overall, 18 of 19 *C difficile*-related complications and deaths (94.7%) occurred in toxin-positive patients. Only one of 162 toxin-negative patients (0.6%) was considered to have CDI as a contributing factor to death.

Our findings are consistent with the conventional view that CDI is a toxin-mediated inflammatory disease preceded by antibiotic exposure and *C difficile* overgrowth.³ Toxin-negative patients had less antibiotic exposure, *C difficile* DNA, and inflammation and manifested milder symptoms and no complications, despite minimal or no treatment. These findings strongly suggest that most patients with negative toxin test results and *C difficile* detected by PCR do not need treatment for CDI. We suspect that most of these patients were colonized with *C difficile* and had another cause of diarrhea. This hypothesis is supported by studies^{22-26,31} showing that *C difficile* colonization and immunity are common in hospitalized patients and most nosocomial diarrhea is noninfectious. It is possible that some toxin-negative patients have mild or early infection because clinical toxin tests can miss toxin at low concentrations, and occasional toxin-negative patients become positive on repeat testing.^{3,10,18,32-35} Correspondingly, we detected toxin in 29.6% (48 of 162) of Tox-/PCR+ patients by the historical cell cytotoxin assay, and 8.0% (13 of 162) of

Figure 2. Kaplan-Meier Curves of Time to Resolution of Diarrhea by *Clostridium difficile* Test Group



The median duration of diarrhea for patients with at least 1 day was 3 days (interquartile range, 1-6 days) for Tox+/PCR+ (121 of 131), 2 days (interquartile range, 1-4 days) for Tox-/PCR+, and 2 days (interquartile range, 1-3 days) for Tox-/PCR- (927 of 1123) ($P < .001$). Log-rank P values are $P < .001$ for all groups, $P = .003$ for Tox+/PCR+ vs Tox-/PCR+, (143 of 162) $P < .001$ for Tox+/PCR+ vs Tox-/PCR-, and $P < .001$ for Tox-/PCR+ vs Tox-/PCR-. Tox+/PCR+ indicates *C difficile* toxin immunoassay positive and polymerase chain reaction positive; Tox-/PCR+, *C difficile* toxin immunoassay negative and polymerase chain reaction positive; Tox-/PCR-, *C difficile* toxin immunoassay negative and polymerase chain reaction negative.

Table 2. Relative Risk (95% CI) of Diarrhea Each Day

Day	Comparison		
	Tox+/PCR+ vs Tox-/PCR+	Tox+/PCR+ vs Tox-/PCR-	Tox-/PCR+ vs Tox-/PCR-
1	1.05 (0.97-1.13)	1.12 (1.06-1.18)	1.07 (1.01-1.14)
2	1.27 (1.03-1.56)	1.46 (1.29-1.73)	1.18 (0.99-1.40)
3	1.28 (0.98-1.67)	1.62 (1.32-1.98)	1.27 (1.02-1.58)
4	1.51 (1.07-02.13)	1.87 (1.46-2.40)	1.24 (0.93-1.66)
5	1.75 (1.15-2.65)	2.04 (1.53-2.73)	1.17 (0.82-1.67)
6	1.88 (1.17-3.02)	2.31 (1.67-3.20)	1.23 (0.81-1.85)
7	1.71 (1.02-2.85)	2.51 (1.73-3.64)	1.47 (0.95-2.29)
8	2.30 (1.25-4.22)	2.72 (1.82-4.06)	1.18 (0.69-2.04)
9	3.09 (1.54-6.20)	3.90 (2.52-6.03)	1.26 (0.66-2.42)
10	3.18 (1.37-7.38)	3.67 (2.18-6.19)	1.16 (0.53-2.53)
11	3.18 (1.37-7.38)	4.06 (2.39-6.90)	1.28 (0.58-2.81)
12	2.89 (1.14-7.30)	3.64 (2.00-6.62)	1.26 (0.54-2.96)
13	3.09 (0.99-9.63)	3.30 (1.63-6.68)	1.07 (0.38-3.02)
14	4.95 (1.07-22.90)	2.98 (1.36-6.53)	0.60 (0.14-2.53)
15	3.71 (0.76-18.08)	3.22 (1.28-8.07)	0.87 (0.20-3.73)

Abbreviations: Tox+/PCR+, *Clostridium difficile* toxin immunoassay positive and polymerase chain reaction positive; Tox-/PCR+, *C difficile* toxin immunoassay negative and polymerase chain reaction positive; Tox-/PCR-, *C difficile* toxin immunoassay negative and polymerase chain reaction negative.

Table 3. Nondiarrheal Outcomes and Treatment by *Clostridium difficile* Test Group

Outcome	<i>C difficile</i> Positive		<i>C difficile</i> Negative		P Value ^a
	Tox+/PCR+ (n = 131)	Tox-/PCR+ (n = 162)	Tox-/PCR- (n = 1123)		
<i>C difficile</i>-Related Complication or Death Within 30 d, No. (%)					
Complication ^b	10 (7.6)	0	3 (0.3)		<.001
Death ^c	11 (8.4)	1 (0.6)	0		<.001
Complication or death	18 (13.7)	1 (0.6)	3 (0.3)		<.001
Repeat <i>C difficile</i> Testing Within 14 d, No. (%)					
Retested	14 (10.7)	61 (37.7)	374 (33.3)		<.001
Positive toxin test result	3 (2.3)	13 (8.0)	17 (1.5)		<.001
Repeat <i>C difficile</i> Testing at 15-30 d, No. (%)					
Tested	26 (19.8)	18 (11.1)	106 (9.4)		.001
Positive toxin test result	14 (10.7)	5 (3.1)	10 (0.9)		<.001
Treatment Within 14 d					
Metronidazole or oral vancomycin, No. (%) ^d	131 (100)	66 (40.7)	361 (32.1)		<.001
Duration of metronidazole or oral vancomycin, if treated, median (IQR), d	14 (11-14)	6 (3-11)	5 (2-9)		<.001
Non- <i>C difficile</i> antibiotic, No. (%)	98 (74.8)	141 (87.0)	912 (81.2)		.03
Duration of non- <i>C difficile</i> antibiotic, if treated, median (IQR), d	11 (3-14)	10 (4-14)	10 (4-14)		.13
Treatment at 15-30 d					
Metronidazole or oral vancomycin, No. (%)	75 (57.3)	35 (21.6)	137 (12.2)		<.001
Duration of metronidazole or oral vancomycin, if treated, median (IQR), d	9 (3-14)	4 (3-15)	6 (3-9)		<.001

Abbreviations: IQR, interquartile range; Tox+/PCR+, *C difficile* toxin immunoassay positive and polymerase chain reaction positive; Tox-/PCR+, *C difficile* toxin immunoassay negative and polymerase chain reaction positive; Tox-/PCR-, *C difficile* toxin immunoassay negative and polymerase chain reaction negative.

^a P value for significance across 3 groups.

^b Intensive care unit care, colectomy, or megacolon related to *C difficile* infection. The Tox+/PCR+ complications included 3 fulminant colitis or megacolon and 7 intensive care unit care related to *C difficile* infection. Two Tox-/PCR+ patients with partially treated complications diagnosed as having a positive toxin test result before day 1 were excluded. The Tox-/PCR-

complications included 3 intensive care unit care related to *C difficile* infection. $P < .001$ for Tox+/PCR+ vs Tox-/PCR+ and $P > .99$ for Tox-/PCR+ vs Tox-/PCR-.

^c All-cause mortality within 30 days was 14 (10.7%), 23 (14.2%), and 98 (8.7%), respectively, for the 3 groups. $P = .08$ for all groups and $P = .21$ for Tox+/PCR+ vs Tox-/PCR+. For *C difficile* infection-related death, $P < .001$ for Tox+/PCR+ vs Tox-/PCR+ and $P = .13$ for Tox-/PCR+ vs Tox-/PCR-.

^d Full treatment (≥ 10 days) and partial treatment (1-9 days) values were 119 (90.8%) and 12 (9.2%), respectively; 21 (13.0%) and 45 (27.8%), respectively; and 82 (7.3%) and 279 (24.8%), respectively, for the 3 groups.

Tox-/PCR+ patients retested positive by the clinical toxin immunoassay in a subsequent sample. However, the relative lack of adverse events in this subgroup suggests that these patients are also at lower risk of complications than clinical toxin immunoassay-positive patients and routine treatment is unnecessary.

These results are consistent with a large retrospective study³⁶ that found no *C difficile*-related complications and lower mortality among hospitalized patients with negative toxin results. Our findings also agree with several smaller studies^{11,14,37-41} and one large, multicenter study²¹ that reported milder symptoms or a lower mortality rate in toxin-negative patients with positive PCR results. Other studies⁴²⁻⁴⁵ that have investigated clinical characteristics of Tox-/PCR+ patients were generally underpowered or not designed to compare outcomes. Finally, there are reports of patients with severe or complicated CDI missed by toxin tests,^{43,46} but our data suggest that such patients are rare.

Strengths of our study include the prospective study design, large sample size, nonreporting of PCR results, measurement of duration of diarrhea, inclusion of patients without *C difficile* for comparison, and rigorous evaluation of *C difficile*-

related complications and deaths. We quantified fecal *C difficile* DNA, toxins, and inflammation to provide mechanistic insight into the reasons for the different test results and outcomes. The primary weakness of the study was the inability to achieve equivalent risk allocation between groups. In addition, we cannot exclude the possibility that empirical treatment affected outcomes in some Tox-/PCR+ patients, but the outcome differences we observed remained when these patients were removed. It is also possible that our outcome adjudicators were influenced by positive toxin results, but 26 of 42 Tox+/PCR+ patients with ICU care or death (61.9%) were judged not to have a CDI-related outcome, indicating that the adjudication was a highly discriminatory process overall. Finally, we cannot exclude the possibility that systematic underrecording of stools in patients with negative toxin results could account for the shorter duration of diarrhea in these patients. However, our requirement of 2 or more days without diarrhea to end an episode would make it unlikely that underrecording by individual nurses would have a significant effect on our diarrhea measure.

Molecular tests have the potential benefits of decreasing the need for repeat testing and empirical treatment because

of their high negative predictive value and may have a role in infection prevention if Tox−/PCR+ patients contribute to the spread of *C difficile* in health care facilities.^{34,43,47} However, our results offer compelling evidence that as many as half of the patients with positive *C difficile* PCR test results are likely to be overdiagnosed and exposed to unnecessary treatment at institutions using molecular tests. The number of patients potentially affected by this issue is massive. Most institutions experience a 50% to 100% increase in reported CDI after switching to molecular tests, and the proportion of institutions using molecular *C difficile* tests has increased dramatically since initiation in 2009 of the first FDA-approved molecular test.¹¹⁻¹⁵ In 2014, almost 44% of NHSN acute care facilities reported using molecular tests for CDI diagnosis (NHSN, written communication, September 15, 2014).

Therefore, there is an urgent need to educate physicians that molecular tests are not specific for CDI, even in the presence of symptoms, and patients with positive PCR results do not necessarily need treatment. Similarly, while underdiagnosis may occur with lack of testing,⁴⁸ policy makers should be aware that molecular *C difficile* tests are a major cause of overdiagnosis and consider the potential costs of overtreatment in recommendations and analyses. Laboratories need to be aware that rejection of formed stool samples is not suffi-

cient to ensure that all positive molecular *C difficile* results represent disease.

We concur with authors in the United Kingdom that molecular tests should not be used as a stand-alone diagnostic test for CDI and diagnostic recommendations should move back in the direction of defining clinical disease as a positive toxin result in patients with diarrhea.^{21,49} Most toxin-negative patients with *C difficile* do not need specific treatment, although there may be a role for identifying carriers to prevent transmission.^{21,43} Future studies should focus on developing diagnostic approaches to accurately distinguish patients with active infection vs colonization, which may include quantitation of *C difficile* DNA, toxins, or host response. In the meantime, 2-step testing with a screening test, such as PCR or glutamate dehydrogenase antigen detection, followed by a toxin test to confirm active infection is a reasonable diagnostic strategy.^{21,49}

Conclusions

Up to half of the patients with positive molecular test results for *C difficile* do not experience adverse events without treatment and do not need treatment for CDI. Exclusive reliance on molecular tests for *C difficile* diagnosis is likely to result in overdiagnosis, unnecessary treatment, and increased health care costs.

ARTICLE INFORMATION

Accepted for Publication: May 26, 2015.

Published Online: September 8, 2015.
doi:10.1001/jamainternmed.2015.4114.

Author Affiliations: Department of Pathology and Laboratory Medicine, University of California Davis School of Medicine, Sacramento (Polage, Gyorke, Kennedy, Leslie, Wang); Division of Infectious Diseases, Department of Internal Medicine, University of California Davis School of Medicine, Sacramento (Polage, Nguyen, Lee, Solnick, Cohen); Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor (Leslie); Center for Healthcare Policy and Research, University of California Davis, Sacramento (Chin, Romano); Yolo County Health Department, Woodland, California (Wang); Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York (Huang, Tang); Department of Clinical Laboratory, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (Huang); Weill Medical College of Cornell University, New York, New York (Tang); Division of Biostatistics, Department of Public Health Sciences, University of California Davis School of Medicine, Sacramento (Kim, Taylor); Division of General Medicine, Department of Internal Medicine, University of California Davis School of Medicine, Sacramento (Romano); Division of General Pediatrics, Department of Pediatrics, University of California Davis School of Medicine, Sacramento (Romano); Department of Emergency Medicine, University of California Davis School of Medicine, Sacramento (Panacek, Goodell); Department of Medical Microbiology and Immunology, University of California Davis School of Medicine, Sacramento (Solnick).

Author Contributions: Dr Polage had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis.

Study concept and design: Polage, Chin, Romano, Cohen.

Acquisition, analysis, or interpretation of data: Polage, Gyorke, Kennedy, Leslie, Chin, Wang, Nguyen, Huang, Tang, Lee, Panacek, Goodell, Solnick, Cohen.

Drafting of the manuscript: Polage.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Polage, Kim, Taylor.

Obtained funding: Polage, Romano.

Administrative, technical, or material support: Polage, Panacek.

Study supervision: Polage.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Polage reported receiving research materials (ie, *Clostridium difficile* diagnostic test kits and reagents) from Meridian Biosciences, Cepheid, TechLab, and Alere; and honoraria for a webinar about diagnostic testing for *C difficile* and nosocomial diarrhea from Alere. Dr Nguyen reported receiving research funding for pharmaceutical clinical trials of treatment for patients with *C difficile* from Merck, Cubist, and Viropharma. Dr Tang reported receiving research funding and materials from ACEA Biosciences. Dr Cohen reported serving on an advisory board for Merck and receiving research funding for pharmaceutical clinical trials of treatment for patients with *C difficile* from Merck, Viropharma, Seres Health, and Actelion. No other disclosures were reported.

Funding/Support: This study was supported by grants UL1 RRO24146 and TL1 RRO24145-06 from the National Center for Research Resources (Dr Polage), grant UL1T000002 and linked award TL1T000133 from the National Center for Advancing Translational Sciences (Dr Chin), and grant T32HS022236 from the Agency for Healthcare Research and Quality through the Quality Safety Comparative Effectiveness Research Training Program (Dr Chin). *Clostridium difficile* diagnostic test kits and reagents were received from Meridian Biosciences, Cepheid, TechLab, and Alere.

Role of the Funder/Sponsor: The industry contributors to this study had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Additional Contributions: We thank the following for their contributions without compensation: staff of the University of California Davis Medical Center Clinical Microbiology Laboratory for assistance with testing and processing samples; Jennifer Brown, MD (University of California Davis School of Medicine) for blinded electronic health record reviews; and all the volunteers who participated as undergraduates at the University of California Davis or California State University, Sacramento, in patient enrollment, clinical data collection, or sample processing, including Sameer Aggarwal, BS, Robert Araiza, BS, Adeel Ashfaq, BS, Mercy Bechtold, BS, Joel Breck, BA, MPH, Jenna DeYoung, BS, Tu Dinh, BS, Timothy Fong, BS, MPH, Kaitlin Gee, BS, Megan Gilbert, BS, Breana Hill, BS, Chae Ji Kim, BS, Bryan Lee, BS, Dan O'Brien, BS, Thomas Osterberg-Diess, BS, Erik Pennell, BS, Roberto Ramos, BS, Daniel Sadoma, BS, Natalie Telis, BS, Joanna Tripet-Diel, BS, MPH, Andrew Tubbs, BS, William Valcheck, BS, and Alvin Au Yeung, BS.

REFERENCES

- Magill SS, Edwards JR, Bamberg W, et al; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. 2014;370(13):1198-1208.
- Lucado J, Gould C, Elixhauser A. *Clostridium difficile* Infections (CDI) in Hospital Stays, 2009: Statistical Brief #124. Rockville, MD: Agency for Health Care Policy and Research; 2006-2012. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs.
- Rupnik M, Wilcox MH, Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol*. 2009;7(7):526-536.
- McDonald LC, Lessa F, Sievert D, et al; Centers for Disease Control and Prevention (CDC). Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb Mortal Wkly Rep*. 2012;61(9):157-162.
- Zilberberg MD, Shorr AF, Kollef MH. Increase in adult *Clostridium difficile*-related hospitalizations and case-fatality rate, United States, 2000-2005. *Emerg Infect Dis*. 2008;14(6):929-931.
- Kang J, Sickbert-Bennett EE, Brown VM, Weber DJ, Rutala WA. Changes in the incidence of health care-associated pathogens at a university hospital from 2005 to 2011. *Am J Infect Control*. 2014;42(7):770-775.
- Dubberke ER, Olsen MA. Burden of *Clostridium difficile* on the healthcare system. *Clin Infect Dis*. 2012;55(suppl 2):S88-S92.
- McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353(23):2433-2441.
- Pépin J, Valiquette L, Alary ME, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ*. 2004;171(5):466-472.
- Burnham CA, Carroll KC. Diagnosis of *Clostridium difficile* infection: an ongoing conundrum for clinicians and for clinical laboratories. *Clin Microbiol Rev*. 2013;26(3):604-630.
- Longtin Y, Trottier S, Brochu G, et al. Impact of the type of diagnostic assay on *Clostridium difficile* infection and complication rates in a mandatory reporting program. *Clin Infect Dis*. 2013;56(1):67-73.
- Moehring RW, Lofgren ET, Anderson DJ. Impact of change to molecular testing for *Clostridium difficile* infection on healthcare facility-associated incidence rates. *Infect Control Hosp Epidemiol*. 2013;34(10):1055-1061.
- Fong KS, Fatica C, Hall G, et al. Impact of PCR testing for *Clostridium difficile* on incident rates and potential on public reporting: is the playing field level? *Infect Control Hosp Epidemiol*. 2011;32(9):932-933.
- Koo HL, Van JN, Zhao M, et al. Real-time polymerase chain reaction detection of asymptomatic *Clostridium difficile* colonization and rising *C. difficile*-associated disease rates. *Infect Control Hosp Epidemiol*. 2014;35(6):667-673.
- Gould CV, Edwards JR, Cohen J, et al; *Clostridium difficile* Infection Surveillance Investigators, Centers for Disease Control and Prevention. Effect of nucleic acid amplification testing on population-based incidence rates of *Clostridium difficile* infection. *Clin Infect Dis*. 2013;57(9):1304-1307.
- Bartlett JG, Taylor NS, Chang T, Dzink J. Clinical and laboratory observations in *Clostridium difficile* colitis. *Am J Clin Nutr*. 1980;33(1)(suppl):2521-2526.
- Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr. *Clostridium difficile*-associated diarrhea and colitis. *Infect Control Hosp Epidemiol*. 1995;16(8):459-477.
- Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis*. 2008;46(suppl 1):S12-S18.
- Wilcox MH, Planche T, Fang FC, Gilligan P. What is the current role of algorithmic approaches for diagnosis of *Clostridium difficile* infection? *J Clin Microbiol*. 2010;48(12):4347-4353.
- Dubberke ER, Han Z, Bobo L, et al. Impact of clinical symptoms on interpretation of diagnostic assays for *Clostridium difficile* infections. *J Clin Microbiol*. 2011;49(8):2887-2893.
- Planche TD, Davies KA, Coen PG, et al. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C difficile* infection. *Lancet Infect Dis*. 2013;13(11):936-945.
- Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med*. 2000;342(6):390-397.
- Samore MH, DeGirolami PC, Tlucko A, Lichtenberg DA, Melvin ZA, Karchmer AW. *Clostridium difficile* colonization and diarrhea at a tertiary care hospital. *Clin Infect Dis*. 1994;18(2):181-187.
- Leekha S, Aronhalt KC, Sloan LM, Patel R, Orenstein R. Asymptomatic *Clostridium difficile* colonization in a tertiary care hospital: admission prevalence and risk factors. *Am J Infect Control*. 2013;41(5):390-393.
- Alasmari F, Seiler SM, Hink T, Burnham CA, Dubberke ER. Prevalence and risk factors for asymptomatic *Clostridium difficile* carriage. *Clin Infect Dis*. 2014;59(2):216-222.
- Polage CR, Solnick JV, Cohen SH. Nosocomial diarrhea: evaluation and treatment of causes other than *Clostridium difficile*. *Clin Infect Dis*. 2012;55(7):982-989.
- Brecher SM, Novak-Weekley SM, Nagy E. Laboratory diagnosis of *Clostridium difficile* infections: there is light at the end of the colon. *Clin Infect Dis*. 2013;57(8):1175-1181.
- Ryder AB, Huang Y, Li H, et al. Assessment of *Clostridium difficile* infections by quantitative detection of *tcdB* toxin by use of a real-time cell analysis system. *J Clin Microbiol*. 2010;48(11):4129-4134.
- Leslie JL, Cohen SH, Solnick JV, Polage CR. Role of fecal *Clostridium difficile* load in discrepancies between toxin tests and PCR: is quantitation the next step in *C. difficile* testing? *Eur J Clin Microbiol Infect Dis*. 2012;31(12):3295-3299.
- Huang B, Jin D, Zhang J, et al. Real-time cellular analysis coupled with a specimen enrichment accurately detects and quantifies *Clostridium difficile* toxins in stool. *J Clin Microbiol*. 2014;52(4):1105-1111.
- Viscidi R, Laughon BE, Yolken R, et al. Serum antibody response to toxins A and B of *Clostridium difficile*. *J Infect Dis*. 1983;148(1):93-100.
- Cardona DM, Rand KH. Evaluation of repeat *Clostridium difficile* enzyme immunoassay testing. *J Clin Microbiol*. 2008;46(11):3686-3689.
- Borek AP, Aird DZ, Carroll KC. Frequency of sample submission for optimal utilization of the cell culture cytotoxicity assay for detection of *Clostridium difficile* toxin. *J Clin Microbiol*. 2005;43(6):2994-2995.
- Khanna S, Pardi DS, Rosenblatt JE, Patel R, Kammer PP, Baddour LM. An evaluation of repeat stool testing for *Clostridium difficile* infection by polymerase chain reaction. *J Clin Gastroenterol*. 2012;46(10):846-849.
- Deshpande A, Pasupuleti V, Patel P, et al. Repeat stool testing to diagnose *Clostridium difficile* infection using enzyme immunoassay does not increase diagnostic yield. *Clin Gastroenterol Hepatol*. 2011;9(8):665-669.e1. doi:10.1016/j.cgh.2011.04.030.
- Polage CR, Chin DL, Leslie JL, Tang J, Cohen SH, Solnick JV. Outcomes in patients tested for *Clostridium difficile* toxins. *Diagn Microbiol Infect Dis*. 2012;74(4):369-373.
- Gerding DN, Olson MM, Peterson LR, et al. *Clostridium difficile*-associated diarrhea and colitis in adults: a prospective case-controlled epidemiologic study. *Arch Intern Med*. 1986;146(1):95-100.
- Walker AS, Eyre DW, Wyllie DH, et al; Infections in Oxfordshire Research Database. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. *Clin Infect Dis*. 2013;56(11):1589-1600.
- de Jong E, de Jong AS, Bartels CJ, van der Rijt-van den Biggelaar C, Melchers WJ, Sturm PD. Clinical and laboratory evaluation of a real-time PCR for *Clostridium difficile* toxin A and B genes. *Eur J Clin Microbiol Infect Dis*. 2012;31(9):2219-2225.
- Beaulieu C, Dionne LL, Julien AS, Longtin Y. Clinical characteristics and outcome of patients with *Clostridium difficile* infection diagnosed by PCR versus a three-step algorithm. *Clin Microbiol Infect*. 2014;20(10):1067-1073.
- Baker I, Leeming JP, Reynolds R, Ibrahim I, Darley E. Clinical relevance of a positive molecular test in the diagnosis of *Clostridium difficile* infection. *J Hosp Infect*. 2013;84(4):311-315.
- Kaltsas A, Simon M, Unruh LH, et al. Clinical and laboratory characteristics of *Clostridium difficile* infection in patients with discordant diagnostic test results. *J Clin Microbiol*. 2012;50(4):1303-1307.
- Guerrero DM, Chou C, Jury LA, Nerandzic MM, Cadnum JC, Donskey CJ. Clinical and infection control implications of *Clostridium difficile* infection with negative enzyme immunoassay for toxin. *Clin Infect Dis*. 2011;53(3):287-290.
- Humphries RM, Uslan DZ, Rubin Z. Performance of *Clostridium difficile* toxin enzyme immunoassay and nucleic acid amplification tests stratified by patient disease severity. *J Clin Microbiol*. 2013;51(3):869-873.
- Berry N, Sewell B, Jafri S, et al. Real-time polymerase chain reaction correlates well with clinical diagnosis of *Clostridium difficile* infection. *J Hosp Infect*. 2014;87(2):109-114.
- Lashner BA, Todorczuk J, Sahn DF, Hanauer SB. *Clostridium difficile* culture-positive toxin-negative diarrhea. *Am J Gastroenterol*. 1986;81(10):940-943.

47. Sydnor ER, Lenhart A, Trollinger B, et al. Antimicrobial prescribing practices in response to different *Clostridium difficile* diagnostic methodologies. *Infect Control Hosp Epidemiol*. 2011; 32(11):1133-1136.

48. Davies KA, Longshaw CM, Davis GL, et al. Underdiagnosis of *Clostridium difficile* across Europe: the European, Multicentre, Prospective, Biannual, Point-Prevalence Study of *Clostridium difficile* Infection in Hospitalised Patients With Diarrhoea (EUCLID). *Lancet Infect Dis*. 2014;14(12):1208-1219.

49. Department of Health, United Kingdom. Updated guidance on the diagnosis and reporting of *Clostridium difficile*. <https://www.gov.uk/government/publications/updated-guidance-on-the-diagnosis-and-reporting-of-clostridium-difficile>. Accessed July 8, 2015.

Invited Commentary

Diagnosis of *Clostridium difficile* Infection Treat the Patient, Not the Test

Erik R. Dubberke, MD, MSPH; Carey-Ann D. Burnham, PhD

No laboratory test can diagnose *Clostridium difficile* infection (CDI). Rather, CDI is a clinical diagnosis that can be supported by laboratory data. While a diagnostic assay may indicate the absence or presence of the organism or its toxins, the test by itself does not determine who does or does not have CDI.

One of the challenges in diagnosing CDI is that there are more asymptomatic *C difficile* carriers than there are people with CDI in the community and in the hospital. It is estimated 3% to 7% of the healthy adult population are colonized with *C difficile*.¹ Asymptomatic colonization is more common among individuals with inpatient health care exposures. Between 4.4% and 15% of people are colonized with *C difficile* on admission to the hospital, with as many as 50% of persons living in long-term care facilities colonized.¹ To add to the dilemma, diarrhea is frequent among those with health care exposures. Although *C difficile* is the most common health care-associated pathogen in the United States and CDI is a major cause of morbidity and mortality, it typically affects less than 1% of hospitalized patients and is the cause of diarrhea in only 5% to 10% of hospitalized people who have diarrhea and are tested for *C difficile*.¹

In the absence of clinical data, it is impossible to determine if a positive *C difficile* diagnostic assay represents asymptomatic *C difficile* colonization or CDI. To date, few high-quality studies have determined the true performance of *C difficile* diagnostic assays when the clinical picture is taken into account. This dilemma is why knowledge of the clinical context of the patient is so important when evaluating *C difficile* assays, as illustrated by Polage et al² in this issue of *JAMA Internal Medicine*.

Polage et al² demonstrate that patients who were *C difficile* negative by toxin enzyme immunoassay (EIA) but positive by polymerase chain reaction (PCR) (Tox-/PCR+) had significantly less severe diarrhea at the time of testing, more rapid resolution of diarrhea, and fewer CDI-related complications or deaths ($P < .001$ for all) compared with patients who were positive by toxin EIA and PCR (Tox+/PCR+). The presentation and outcomes of patients with specimens that were Tox-/PCR+ were no different from those of patients with specimens that were negative by both toxin EIA and PCR. The strengths of this study are its large sample size, the inclusion of high-quality data about patients before and after the testing episode, the

longitudinal follow-up of patients after discharge from the hospital, and the fact that the results of the PCR-based diagnostic assay were not reported to clinicians and so could not influence their treatment decisions.

The findings by Polage et al² are consistent with what is known about CDI, toxin EIAs, and PCR-based diagnostics for *C difficile*. Fecal samples positive for toxin have higher bacterial and toxin levels in stool and higher levels of inflammatory markers than stool samples positive by PCR alone, indicating that those positive by PCR alone are more likely to have asymptomatic *C difficile* carriage than CDI. The largest *C difficile* diagnostic comparison to date reported test results from 6522 inpatient episodes and found that people positive for toxin were more likely to die compared with people colonized with *C difficile* but without detectable toxin in stool.³ Planche et al³ also observed that mortality among individuals who were colonized with *C difficile* but without detectable toxin was no different from that among individuals who were not colonized with *C difficile*.

Another important observation by Polage et al,² which is consistent with prior literature, is that more of their patients were Tox-/PCR+ than Tox+/PCR+. The real-world performance of PCR when clinical data were taken into account in this study yielded a sensitivity of 100% and a specificity of 87.4%, for a positive predictive value of 44.7%. In a much smaller study⁴ in which our group prospectively interviewed and examined all patients who had stool samples included in a *C difficile* assay comparison, it was found that the specificity of PCR-based diagnostics ranged from 84.3% to 86.1% when the clinical presentation of patients was considered, with a corresponding positive predictive value of 44.0% to 47.1%. With such low positive predictive values, it is not surprising that there are numerous false positives with PCR testing. Indeed, the Centers for Disease Control and Prevention⁵ found that the CDI incidence increased by 43% to 67% at hospitals that change from toxin EIAs to PCR-based assays for detection.

The increase in *C difficile* detection associated with PCR-based diagnostics has serious implications at the hospital and patient levels. More positive assays result in more patients in contact precautions. Such precautions are costly in that they require resources such as single rooms and gowning. *Clostridium difficile* infection incidence data are available to the public (<http://www.hospitalcompare.hhs.gov/>), and these data may be used for value-based purchasing decisions (<http://www>