Diagnostic Microbiology and Infectious Disease xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Effective utilization of *C. difficile* PCR and identification of clinicopathologic factors associated with conversion to a positive result in symptomatic patients

Mohamed E. Mostafa, Timothy Flynn, Christopher P. Hartley, Nathan A. Ledeboer, Blake W. Buchan *

Department of Pathology, The Medical College of Wisconsin, Milwaukee, WI

ARTICLE INFO

Article history:
Received 30 June 2017
Received in revised form 21 November 2017
Accepted 29 November 2017
Available online xxxx

Keywords:
Clostridium difficile
PCR
Test utilization
Cost-savings

ABSTRACT

Objectives: We assess the diagnostic yield of repeat testing for *C. difficile* using molecular methods within 7 days of a negative test and identify specific factors associated with conversion from negative to positive test result within a 7-day period to aid in selective test utilization.
Methods: A retrospective chart review of 20,866 laboratory test orders for *C. difficile* PCR was conducted. The test result, clinicopathologic patient features, and previous test results were recorded. Univariate and multivariate analysis was conducted to compare patients with initial and repeat negative results ($n = 248$) to a group of patients with conversion from negative to positive results within 7 days.
Results: Univariate analysis demonstrated a history of *C. difficile* infection, receipt of antibiotics within 14 days, and duration of hospital stay as factors significantly different between patients with repeat negative and conversion to positive *C. difficile* test result. Only history of *C. difficile* infection was significantly different upon multivariate analysis.
Conclusions: Identification of prior *C. difficile* infection as the only factor significantly correlated with conversion from negative to positive *C. difficile* test result within 7 days aids in selective test utilization and reduces the costs associated with unnecessary laboratory testing.

1. Introduction

Clostridium difficile is a gram-positive, anaerobic, spore-forming bacillus that was first identified as the cause of antibiotic associated pseudomembranous colitis in 1978 (Bartlett et al., 1978). Since this time, its impact on the healthcare community has grown substantially. In 2011, it was reported to have surpassed methicillin-resistant *Staphylococcus aureus* as the most common healthcare associated infection in the US (Miller et al., 2011). Traditionally a nosocomial infection, CDI has been increasingly identified as a cause of community acquired diarrhea, frequently in patients without known identifiable risk factors (Gupta and Khanna, 2014). Along with the increased prevalence of CDI has been an increase in morbidity and mortality associated with the disease (Depestel and Aronoff, 2013). Until recent years, diagnosis of CDI was based on enzyme immunoassay (EIA) testing for toxin A and B. The low sensitivity of these tests has prompted the clinician practice of ordering repeat testing in symptomatic patients with a negative result in hopes of improving diagnostic yield. Studies have shown that this practice may not achieve this goal and may be economically wasteful (Cardona and Rand, 2008; Mohan et al., 2006).

PCR testing for *C. difficile* toxins A and/or B has gained favor due to superior sensitivity and specificity compared to EIA (Peterson et al., 2007). The increased sensitivity of PCR has drastically decreased the need for repeat testing and multiple studies have shown that repeat testing within 7 days of negative PCR yields positive results in only 1–3% of cases (Aichinger et al., 2008; Green et al., 2014; Luo and Banaei, 2010). In addition to low diagnostic yield, the practice of repeat testing proximal to a negative result adds unnecessarily to the cost of care through expensive molecular diagnostic test reagents. Despite these disadvantages, it remains a relatively common practice to order repeat PCR testing shortly after a negative result. This practice may be potentiated by the rare cases of conversion from negative to positive test. Establishment of specific risk factors positively correlated with a negative to positive test conversion within a 7 day period could aid in guiding clinicians who are considering a repeat test for *C. difficile* and would also reduce the burden of repeat test volume and associated cost to the clinical laboratory and payor.

We conducted a single center, retrospective study to determine the proportion of *C. difficile* tests that were ordered within 7 days of a previously positive or negative PCR result. Our primary goal was to determine the utility (diagnostic yield) of repeat testing for *C. difficile* using molecular methods within 7 days of a negative test. A secondary goal was to identify specific factors associated with conversion from negative to positive test result within a 7-day period to aid in selective test utilization.

* Corresponding author. Tel.: +1-414-805-6988.
E-mail address: bbuchan@mcw.edu (B.W. Buchan).

https://doi.org/10.1016/j.diagmicrobio.2017.11.021
0732-8893/

Table 1
Summary of *C. difficile* PCR tests performed.

Test category	Number of tests	%
Total tests	20,526	100.0
Repeat tests[a]	1637/20,526	8.0
Initial test positive	554/1637	33.8
Repeat test positive[b]	541/554	97.7
Repeat test negative	13/554	2.3
Initial test negative	970/1637	59.3
Repeat test positive	44/970	4.5
Repeat test negative	926/970	95.5

[a] Tests repeated within 7 days of a previous valid test result.
[b] Repeat test refers to the initial repeated test result. There were 113 additional tests that were repeated more than twice within 7 days of initial test result.

2. Materials and methods

2.1. Study enrollment

This study was a single-center, retrospective chart review conducted at the Medical College of Wisconsin and Wisconsin Diagnostic Laboratories (Milwaukee, WI). The review was conducted in accordance with an institutional review board (IRB) approved protocol.

All orders for "*C. difficile* NAAT" (Xpert *C. difficile* Assay, Cepheid, Sunnyvale, CA) between October 28, 2013 and October 28, 2015 were reviewed. This included a total of 20,866 individual tests ordered on both inpatient and outpatient subjects. Among these, 340 (1.6%) were canceled or gave invalid test results and were excluded from our analysis. The test result, date of test order, and total number of tests per patient were recorded for the remaining 20,526 test orders. Patients with multiple or "repeat" *C. difficile* NAAT test orders within a 7-day period were identified. The medical record was available for 24 patients with an initial negative test followed by a positive test within the subsequent 7 days. Clinicopathologic features including patient age, gender, length of hospital stay, presence of diarrhea, leukocytosis, fever, history of antibiotic use (including empirical treatment for *C. difficile*, i.e. metronidazole or oral vancomycin), laxative usage, and history of positive *C. difficile* test within the prior 60 days were recorded. These data were compared to a subset of patients ($n = 248$) with initial and repeat negative *C. difficile* NAAT results to identify specific factors associated with conversion from positive to negative test result within 7 days.

2.2. Statistical analysis

For comparison of categorical and continuous independent variables with the categorical binary outcome variable (repeat test positive or negative) univariate and multivariate logistic regression were used (Microsoft Excel 2010, Redmond, WA, USA).

3. Results

3.1. Summary of repeat C. difficile test results

During the two-year period reviewed for this study, a total of 20,526 *C. difficile* PCR tests were reported as positive or negative (Table 1). Among these, 1637 (8.0%) were tests repeated within 7 days of previously valid test result. In considering only single repeat test orders, 970 (59.3%) followed an initial negative and 554 (33.8%) followed an initial positive test result. An additional 113 (6.9%) tests were repeated more than once within 7 days of the original test. Among the 554 repeated tests with initial positive result, 541 (97.7%) remained positive while only 13 (2.3%) converted to negative. Among the 970 repeated tests with an initial negative result, 926 (95.5%) remained negative while 44 (4.5%) converted to positive.

3.2. Univariate analysis of factors contributing to discordant initial and repeat test results

Full medical records were available for 24/44 (54.5%) patients with a positive repeat test following an initial negative result. Clinicopathologic characteristics of these 24 patients were compared to a control group (negative repeat test, $n = 248$) using a univariate logistic regression (Table 2). The variables that reached statistical significance between the groups were history of *C. difficile* infection in the past 60 days (3.2% vs. 41.7%, $P > 0.001$) and history of any antibiotic therapy in the last 14 days (86.7% vs. 62.5%, $P = 0.003$). A third factor, duration of hospital stay also reached statistical significance (8.67 days vs. 4.14 days, $P = 0.007$). Interestingly, history of targeted anti-*C. difficile* antibiotic therapy was not statistically different between the groups (13.7% vs. 12.5%, $P = 0.85$).

Patients with a history of *C. difficile* confirmed by PCR within the 60 days prior to initial test were 19 times more likely to have a repeat positive result within 7 days of a negative result ([95% confidence interval (CI), 6.64–54.17], $P < 0.001$). Conversely, patients with history of any antibiotic therapy within 14 days prior to initial test were 3.9 times more likely to have a repeat negative result ([95% CI, 1.6–10.0], $P = 0.003$). Longer duration of hospital stay demonstrated a negative correlation with conversion to positive test following an initial negative result, with each additional day in hospital conferring a 0.86 odds of having a positive repeat result ([95% CI, 0.78–0.96], $P = 0.007$).

3.3. Multivariate analysis of factors contributing to discordant initial and repeat test results

The two groups were also compared in multivariate model including the variables that reached statistical significance in the univariate analysis, in addition to average length of stay and history of empiric

Table 2
Univariate logistic regression analysis of factors contributing to discordant initial and repeat test results.

Clinicopathologic feature	Initial negative Repeat negative	Initial negative Repeat positive	*P*	Odds ratio for repeat positive test (95% CI)
Number	248	24	NA	NA
Age, average	59	55	0.37	0.99 (0.96–1.01)
Gender, Male	130/248 (52.4%)	12/24 (50%)	0.82	0.91 (0.39–2.1)
History of *C. difficile* (PCR confirmed) in 60 days preceding test	8/248 (3.2%)	10/24 (41.7%)	<0.001	18.97 (6.64–54.17)
Presence of diarrhea at time of test	220/248 (88.7%)	22/24 (91.7%)	0.66	1.40 (0.31–6.27)
Fever (>38 °C) at time of test	39/248 (15.7%)	5/24 (20.8%)	0.35	1.65 (0.57–4.77)
Leukocytosis (>11,000 leukocytes/μL) at time of test	106/248 (42.7%)	9/24 (37.5%)	0.80	1.12 (0.46–2.69)
Received any antibiotic therapy in 14 days preceding test	215/248 (86.7%)	15/24 (62.5%)	0.003	0.255 (0.10–0.63)
Received empiric therapy[a] for *C. difficile* in 7 days preceding test	34/248 (13.7%)	3/24 (12.5%)	0.85	0.88 (0.25–3.12)
History of laxative use within the last week (%)	100/248 (40.3%)	9/24 (37.5%)	0.76	0.88 (0.37–2.08)
Average length of stay in days (range)	8.67 (0–67)	4.14 (0–13) (unknown for 2 patients)	0.007	0.86 (0.78–0.96)

[a] Metronidazole or oral vancomycin.

Please cite this article as: Mostafa ME, et al, Effective utilization of *C. difficile* PCR and identification of clinicopathologic factors associated with conversion to a positive..., Diagn Microbiol Infect Dis (2017), https://doi.org/10.1016/j.diagmicrobio.2017.11.021