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ORIGINAL ARTICLE

Molecular-based diagnosis of *Clostridium difficile* infection is associated with reduced mortality

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Abstract

Polymerase chain reaction (PCR) for the diagnosis of *Clostridium difficile* infection (CDI) might result in overdiagnosis. The clinical outcomes of symptomatic CDI patients diagnosed by PCR remain uncertain. We aimed to determine whether patients whose diagnosis of CDI was based on PCR had different characteristics and clinical outcomes than those diagnosed by toxin immunoassay. Consecutive CDI patients, hospitalized at Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel, between January 2013 and January 2016, were identified retrospectively and included in the study. Diagnosis of CDI was based on PCR or diagnosis by immunoassay for *C. difficile* toxin. The main outcome was 30- and 90-day all-cause mortality. The PCR group included 165 patients and the immunoassay group included 157 patients. In comparison to the immunoassay group, patients in the PCR group were more likely to be younger, to be independent, to undergo previous abdominal surgery, and to use laxatives. The 30-day mortality rate in the PCR group was significantly lower than that in the immunoassay group, 29/165 (18%) vs 49/157 (31%), respectively; $p = 0.028$. On multivariate analysis, PCR diagnosis was associated with reduced mortality, OR 0.48 (95% CI 0.26–0.88). PCR-based diagnosis of CDI is associated with reduced all-cause mortality rates. Further studies are needed to determine the management of patients with discrepant immunoassay and PCR diagnosis of CDI.

Keywords *Clostridium difficile* · PCR · Mortality · Immunoassay · Diarrhea

Background

Due to concern of underdiagnosis of *Clostridium difficile* infection (CDI), highly sensitive molecular methods as polymerase chain reaction (PCR) were developed [1] that may account for a part of the increased incidence of CDI reported in the recent years [2, 3]. One of the proposed methods for diagnosis of CDI by stool samples involves a two-step approach by the use of PCR for discordant results by the C.DIFF QUIK CHEK COMPLETE assay (TechLab, Blacksburg, VA) [4], using the Xpert *C. difficile* PCR assay (Cepheid, Sunnyvale, CA) [5]. Previous studies have examined the clinical outcomes of patients with CDI according to the *C. difficile* toxins' detection method [6–10], and some had showed better clinical outcomes of patients with PCR-based diagnosis of toxin alone [11, 12]. We conducted a retrospective analysis in order to detect differences in the clinical outcomes of patients with PCR-based diagnosis of CDI vs antigen detection-based methods.

Methods

Study design

We performed a retrospective data analysis of consecutive hospitalized patients who were diagnosed with CDI. The

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cohort included hospitalized patients in internal medicine wards, at Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel (900 bed tertiary care, university-affiliated hospital), from January 2013 to January 2016. We included patients with clinical suspicion of CDI, defined by diarrhea not attributed to any other cause and associated with either a positive C.DIFF QUIK CHEK COMPLETE assay for antigen/toxins for *C. difficile* (immunoassay group) or patients with C. DIFF QUIK CHEK COMPLETE assay positive for glutamate dehydrogenase (GDH) antigen/negative for toxin, but Xpert *C. difficile* PCR-positive assay (PCR group). Thus, PCR was performed only on samples from patients with discordant results. The patients were treated for CDI either by metronidazole or oral vancomycin. Diarrhea was defined as passage of three or more unformed stools for at least 2 consecutive days. The decision to test for *C. difficile* and to treat CDI was made by physicians uninvolved in the study. Patients were included only once in the study, for the first episode fulfilling inclusion criteria. We included only the first sample for each patient. The study was approved by the hospital's ethics committee.

Data collection

The index point was defined as the day of stool sample received in the laboratory. Data collection was performed by using the electronic patient file. Data on the diarrheal episode, management, and outcomes were collected. A CDI severity index was calculated for each patient based on the presence of each of the following factors: acute kidney injury, hypoalbuminemia, leukocytosis, and active malignancy [13].

Outcomes

Data on the primary and secondary outcomes was extracted from the nationwide electronic medical records. The primary outcome was 30-day all-cause mortality. Secondary outcomes included 90-day all-cause mortality rates, number of patients with recurrent CDI within 90 days of the index point, length of hospital stay (LOS), length of clinical illness, complication of CDI (including need for urgent colectomy, toxic mega-colon, need for ICU transfer), and severe adverse events related to the antibiotic therapy (i.e., severe allergic response, need for drug discontinuation, neuropathy).

Statistical methods

We compared results between the control and PCR group. Dichotomous outcomes were compared using the Pearson Chi-square test. Continuous variables were compared using the student *T* test or the Mann-Whitney *U* test, as appropriate. Risk factors for mortality were assessed through univariate analysis ($p < 0.05$) and then entered into a logistic multivariate analysis using the backward stepwise method. Variables showing high correlation (Spearman's correlation coefficient > 0.5) were omitted. Odds ratios (OR) were calculated with 95% confidence intervals (CI). Analyses were conducted using IBM SPSS Statistics 20 (IBM, Armonk, NY).

Results

A total of 322 patients, 165 patients in the PCR group and 157 in the immunoassay group, were included. The characteristics of these patients are presented in Table 1. Compared to the immunoassay group, patients in the PCR group were younger (median age 69 vs 75 years, $p = 0.007$) and independent in their ADLs (46 vs 35%, $p = 0.044$). The PCR group surpassed the immunoassay group in regard to history of laxative and corticosteroid use, solid organ transplantation, and previous abdominal surgery (22.4 vs 7%, $p < 0.01$). At presentation, the PCR group patients had lower leukocyte count and higher albumin levels than the immunoassay group patients. As well as, the CDI score was lower for the PCR group (median 1, IQR 0–1 vs 1, IQR 1–2, $p = 0.001$), difference derived by more patients with a CDI score of 0 (29.7 vs 14.6%, $p = 0.007$). The management of the patients was similar as evidenced by the similar number of treatment days with metronidazole and vancomycin, ICU admissions, and the need for emergent colectomy.

Primary outcome—30-day all-cause mortality

Thirty-day all-cause mortality rates and other clinical outcomes are presented in Table 2. The 30-day crude mortality rate was 78/322 (24.2%). Unadjusted, the mortality rate in the PCR group (29/165; 17.6%) was significantly lower than that in the immunoassay group (49/157; 31.2%), $p = 0.028$. Mortality was significantly higher for patients with increased CDI severity score. On multivariable analysis, PCR-based diagnosis of CDI was associated with half the odds for mortality, OR 0.48 (95% CI 0.26–0.88), while age, residence in long-term care facility (LTCF), Charlson comorbidity index, need for ICU admission, and CDI severity score of 3/4 were associated with increased 30-day mortality rates (Table 3).

Secondary outcomes—90-day all-cause mortality, LOS, length of diarrheal illness, complications, recurrence, and adverse events

Ninety-day all-cause mortality rates and other clinical outcomes are presented in Table 2. The 90-day crude all-cause mortality rate was 118/322 (36.8%). Unadjusted, the mortality rate in the PCR group (29.7%) was lower than that in the immunoassay group (44.2%), 49/165 vs 69/157, respectively; $p = 0.007$. On multivariable analysis, PCR-based diagnosis of CDI was associated with reduced risk for mortality, OR 0.55

Table 1 Patients' characteristics (normally distributed continuous variables are shown as mean with SD or median with IQR for not normally distributed variables)

	PCR group ($N = 165$)	Immunoassay group ($N = 157$)	Entire cohort ($N = 322$)	p value
Age (median, IQR)	69 (55–83)	75 (65–85)	74 (60–84)	0.007
Female	91 (55.2%)	78 (49.7%)	169 (52.5%)	0.326
ADL status—dependent	89 (53.9%)	102 (65%)	191 (59.3%)	0.044
LTCF residency	43 (26.1%)	38 (24.2%)	81 (25.2%)	0.701
Previous admission within 3 months	118 (71.5%)	118 (75.2%)	236 (73.3%)	0.46
Previous antibiotics within 3 months	106 (64.2%)	106 (67.5%)	212 (65.8%)	0.536
Steroids	57 (34.5%)	27 (17.2%)	84 (26.1%)	< 0.01
Insulin treatment	38 (23%)	25 (15.9%)	63 (19.6%)	0.108
Immunosuppression	54 (32.7%)	37 (23.6%)	91 (28.3%)	0.068
Prior laxatives	23 (13.9%)	11 (7%)	34 (10.6%)	0.043
Statins	49 (29.7%)	58 (36.9%)	107 (33.2%)	0.168
PPI	77 (46.7%)	68 (43.3%)	145 (45%)	0.545
Charlson score	6 (3–8)	5 (2–7)	5 (3–7)	0.209
Solid organ tumor	29 (17.6%)	31 (19.7%)	60 (18.6%)	0.617
Hemato-oncology	23 (13.9%)	15 (9.6%)	38 (11.8%)	0.223
Chemotherapy	36 (21.8%)	24 (15.3%)	60 (18.6%)	0.132
Diabetes	55 (33.3%)	47 (29.9%)	102 (31.7%)	0.513
Hemodialysis	3 (1.8%)	4 (2.5%)	7 (2.2%)	0.654
Presence of PEG/NGT	36 (21.8%)	33 (21%)	69 (21.4%)	0.861
Previous abdominal surgery	37 (22.4%)	11 (7%)	48 (14.9%)	< 0.01
Clinical presentation				
Albumin	3.1 (2.7–3.5)	2.85 (2.3–3.2)	3 (2.5–3.4)	< 0.01
WBC	9.69 (6.27–15.61)	12.68 (8.11–18.61)	11.35 (7–17.1)	0.003
Creatinine	0.95 (0.57–1.76)	1.03 (0.67–1.71)	0.975 (0.617–1.75)	0.411
PLT	245 (166.5–339.5)	244 (169–347.5)	244.5 (168–344)	0.546
HB	9.8 (8.7–11.55)	10.5 (9.05–11.4)	10.1 (8.8–11.5)	0.144
Heart rate	83.66 ± 16.06	92.11 ± 17.37	86.93 ± 17.33	0.002
Temperature	83 (73–98)	82 (75–90)	82 (74–93)	0.357
Sepsis syndrome	46 (27.9%)	55 (35%)	101 (31.4%)	0.167
CDI severity score, categorical				0.007
0	49 (29.7%)	23 (14.6%)	72 (22.4%)	
1	68 (41.2%)	68 (43.3%)	136 (42.2%)	
2	34 (20.6%)	46 (29.3%)	80 (24.8%)	
3/4	14 (8.5%)	20 (12.7%)	34 (10.6%)	
CDI severity score (median, IQR)	1 (0–2)	1 (1–2)	1 (1–2)	0.001
Hypoalbuminemia ≤ 3	77 (47%)	102 (65.4%)	179 (55.9%)	0.001
Acute kidney injury	29 (17.7%)	40 (25.5%)	69 (21.5%)	0.089
WBCs ≥ 20,000	22 (13.3%)	35 (22.3%)	57 (17.7%)	0.035
Any active malignancy	52 (31.5%)	45 (28.7%)	97 (30.1%)	0.577
Clinical management				
Metronidazole—days of treatment	10 (7–14)	10 (6–14)	10 (6–14)	0.729
Vancomycin—days of treatment	13 (10–15)	14 (7–15)	14 (8–15)	0.7

Table 1 (continued)

	PCR group ($N = 165$)	Immunoassay group ($N = 157$)	Entire cohort ($N = 322$)	p value
ICU admission	1 (0.7%)	6 (3.8%)	7 (2.2%)	0.762
Urgent colectomy	3 (2.1%)	4 (2.5%)	7 (2.2%)	0.654
Any complication	4 (2.4%)	10 (6.3%)	14 (4.3%)	0.103

ADL activities of daily life, *LTCF* long-term care facility, *PPI* proton pump inhibitors, *PEG/NGT* percutaneous endoscopic gastrostomy/nasogastric tube, *WBC* white blood cells, *PLT* platelets, *HB* hemoglobin, *CDI Clostridium difficile* infection, *ICU* intensive care unit

(95% CI 0.31–0.97, Table 3). Other factors associated with increased risk of 90-day all-cause mortality were age, residence in LTCF, Charlson comorbidity index, need for ICU admission, and CDI severity score above 2.

LOS, length of diarrheal illness (for all patients and for patients who were discharged alive), and rehospitalization for all causes in the following 3 months were similar between the groups. Severe complication of CDI were noted in 4/165 patients in the PCR group and in 10/157 patients in the immunoassay group, $p = 0.10$. We observed a trend towards less hospitalization for recurrent CDI (12.7 vs 19.1%, $p = 0.117$) for patients in the PCR group which were discharged alive (Table 2). No severe adverse events related to the use of metronidazole or vancomycin were noted during the follow-up time.

Discussion

In this study, we demonstrated lower 30- and 90-day all-cause mortality rates in patients with PCR-based diagnosis of CDI, in comparison to patients with antigen-/toxin-positive stool immunoassay. We also demonstrated increased mortality with several "traditional" CDI risk factors as increased age, Charlson comorbidity index, LTCF residency or being independent in ADLs, and higher CDI severity score. We noted a trend for less recurrences of CDI in the PCR group (12.7 vs 19.1%).

Our findings suggest that PCR-based diagnosis of CDI is associated with either a non-CDI-related diarrheal illness or less severe disease and hence the reduced mortality. Patients with PCR-based diagnosis of CDI might represent a carrier state of *C. difficile*; thus, only a minute amount of toxin is produced which are undetected by the toxin assay. In those patients, several other etiologies for diarrhea might exist, often simultaneously [14]. Our patients in the PCR group were more likely to be under immunosuppression by steroids, chemotherapy, and organ transplantation; they were more likely to use laxatives and undergone previous abdominal surgery (that might affect the intestinal motility). Alternatively, patients with PCR-based diagnosis of CDI might have less severe disease due to small amount of toxin production. This is supported by significantly more patients with CDI severity score of 0/1 in our cohort (70 vs 55%).

Our findings are in concordance with some studies that examined the laboratory confirmation of CDI by PCR and clinical outcomes. Polage et al. [11] examined a similar cohort of patients, discovering that positive PCR patients had less complication and less CDI-related mortality. The toxin-positive assay group had longer duration of diarrhea. Similar to our findings, patients in the toxin-negative/PCR-positive group had been more likely to have several other explanations for the diarrhea. Baker et al. examined the clinical outcomes of patients suspected of CDI by the two-step methods [12]. Mortality rates for that cohort were significantly lower among the toxin-negative/PCR-positive group as well as days of diarrhea and CDI recurrences. Better clinical outcomes for patients with PCR-based diagnosis of CDI alone, however, were

Table 2 Outcomes

	PCR group ($N = 165$)	Immunoassay group ($N = 157$)	p value
30-day all-cause mortality	29 (17.6%)	49 (31.2%)	0.004
90-day all-cause mortality	49 (29.7%)	69 (44.2%)	0.007
*Duration of hospitalization—all patients	13 (5–22.5)*	12 (7–20)*	0.958
– For patients discharged alive	13 (5–22)	11 (7–19)	0.732
Duration of diarrhea—all patients	4 (2–7)*	4 (2–9.5)*	0.422
– For patients discharged alive	4 (2–7)	4 (2–8)	0.977
Rehospitalization for any cause	65 (39.4%)	64 (40.8%)	0.802
Rehospitalization for CDI recurrence	21 (12.7%)	30 (19.1%)	0.117

CDI *Clostridium difficile* infection

*Median/interquartile range

Table 3 Risk factors for 30-/90-day mortality and univariate and multivariate analysis

Risk factor	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)
30-day all-cause mortality		
Testing method—PCR	0.47 (0.28–0.79)	0.48 (0.26–0.88)
Charlson score	1.21 (1.11–1.31)	1.17 (1.002–1.24)
LTCF residency	2.36 (1.36–4.1)	2.42 (1.31–4.47)
Age	1.04 (1.02–1.06)	1.033 (1.009–1.057)
ICU admission	4.11 (1.07–15.7)	5.44 (1.17–25.37)
CDI severity score—0	1	1
CDI severity score—1	2.36 (1.02–4.46)	1.73 (0.7–4.27)
CDI severity score—2	2.85 (1.17–6.92)	1.54 (0.58–4.09)
CDI severity score—3/4	9 (3.32–24.39)	5.56 (1.82–16.97)
90-day all-cause mortality		
Testing method—PCR	0.53 (0.34–0.84)	0.54 (0.30–0.95)
Charlson score	1.29 (1.2–1.4)	1.2 (1.08–1.33)
LTCF—residency	2.4 (1.43–4.02)	2.45 (1.35–4.44)
Age	1.04 (1.03–1.06)	1.03 (1.01–1.05)
ICU admission	14.69 (1.81–118.98)	19.57 (1.96–202.2)
CDI Severity score—0	1	1
CDI Severity score—1	2.92 (1.41–6.09)	2.2 (0.98–4.93)
CDI Severity score—2	2.92 (1.41–6.09)	2.2 (0.98–4.93)
CDI Severity score—3/4	10.2 (3.92–26.35)	5.75 (1.96–16.87)*

OR odds ratio, *PCR* polymerase chain reaction, *LTCF* long-term care facility, *ICU* intensive care unit, *CDI Clostridium difficile* infection

not observed in all studies. In a retrospective study of cancer patients with CDI, discordant results between PCR and cytotoxin assay were not associated with increased mortality [10]. Similar results were observed in studies by Guerrero et al. [9], Longtin et al. [6], and Berry et al. [8], in all of which the small number of patients with discordant PCR and toxin assay precluded statistically significant results.

Our study has some limitations. First, the design of the study is retrospective and subjected to selection bias. However, our center management policy is the same for patients with PCR or antigen/toxin assay-based diagnosis of CDI; therefore, both groups were managed similarly by antibiotics and supportive therapy. Second, this is a single-center experience and other laboratories might encounter different results. Third, our results might be confounded by other unaccounted explanations for the reduced mortality observed in the PCR group. However, our univariate and multivariate analysis of risk factors for mortality is in concordance with other studies of CDI as age, comorbidity, and severity score, thus increasing the validity of our results. Finally, the PCR assay was only performed on samples with discordant GDH/toxin results. Thus, different results might have been encountered if PCR assay was performed systematically and compared with the results obtained from the immunoassay.

In conclusion, PCR-based diagnosis of CDI is associated with reduced all-cause mortality at 30 and 90 days in comparison to patients with antigen-/toxin-positive assay. Future studies should focus on the management of patients with positive PCR and low CDI severity score, since this group might represent patients with diarrhea caused by other conditions. We suggest managing those patients by cessation of the "culprit" antibiotic alone, and withholding specific anti *C. difficile* therapy until other etiologies for the diarrhea had been excluded.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the institutional research ethics committee of Rabin Medical Center.

Informed consent The committee waived the need for informed consent.

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