ORIGINAL PAPERS

Characteristics of *Clostridium difficile* infection in patients with discordant diagnostic test results

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ABSTRACT

Background: Clinical features of *Clostridium difficile* infection (CDI) cases diagnosed by detection of polymerase chain reaction (PCR), with negative toxin enzyme immunoassay results (EIA) have not been fully elucidated. The purpose of this study was to determine the magnitude of CDI patients who had negative EIA toxin determinations but positive PCR tests, and their differences in clinical presentation.

Methods: We performed a retrospective study comparing the clinical features of CDI cases detected by EIA (toxins A + B) with cases detected by PCR (toxin negative, PCR positive) over a 16-month period. Only patients with an initial *Clostridium difficile* infection episode that fulfilled a standardized definition were included.

Results: During the study period, 107 episodes of CDI were detected. Seventy-four patients (69%) had positive glutamate dehydrogenase (GDH) antigen and EIA determinations (EIA positive patients). Thirty-three patients (31%) had GDH positive, negative toxin EIA and positive PCR determination (PCR positive patients). PCR positive patients were younger, 57 (27) years (mean [SD]), than EIA positive patients, 71 (16) years, (p < 0.001). Fewer PCR positive patients were receiving proton pump inhibitors (21 patients, 64%) than EIA positive patients (61 patients, 82%, p = 0.034). The clinical presentation was similar in both groups. In the multivariate analysis, lower age was identified as the only independent variable associated with PCR positive patients.

Conclusions: One third of *Clostridium difficile* infection patients present negative toxin EIA and PCR positive tests. Performing PCR determination after the negative EIA test is more relevant in younger patients.

Key words: Clostridium difficile. Antibiotic-associated diarrhea. Aged. Proton pump inhibitors. Polymerase chain reaction. Enzyme immunoassay.

INTRODUCTION

Clostridium difficile infection (CDI) has become the most common nosocomial infection of the gastrointestinal tract in both immunocompetent and immunocompromised patients (1). Various laboratory tests can be used to diagnose CDI and, in fact, the tests available in each institution may be very different (2-4). There are differences in terms of sensitivity, specificity, cost and time requirements, and there is considerable persistent debate with respect to the optimal method of detection (5). Toxigenic culture is a very sensitive method and is considered as the gold standard for CDI diagnosis. However it is not widely used owing to time and technical requirements (3,6). In contrast, enzyme immunoassay (EIA)-based methods to detect glutamate dehydrogenase (GDH) can serve as a screening tool in combination with more specific tests (7). Despite their lower sensitivity, EIA for toxins A and B are widely used due to their rapid turnaround time and ease of use (3,5,6). More recently, real-time polymerase chain reaction (PCR) assays targeting the ToxB gene tcdB have been commercialized and appear to be both quick and highly sensitive (2,4,8). However, the reported percentage of patients that may be diagnosed by PCR after performing EIA is quite different (2,4).

The clinical implications of using a more sensitive assay, such as PCR, to diagnose CDI have not been fully elucidated. Various studies that aimed to compare the clinical features of cases detected by different techniques have yielded conflicting results (9,10). If the additional cases detected by PCR, but not by less sensitive methods, were clinically similar to cases detected by all these methods, their detection would be clinically relevant as this could have an impact on patient outcome. However, if the additional cases detected by PCR were clinically less severe and had a more favorable outcome, the importance of detecting these cases would be more questionable.

The purpose of this study was to determine the proportion of CDI patients diagnosed by GDH and PCR and to

compare their clinical features with those of patients who were diagnosed by GDH and EIA.

METHODS

Study design and setting

A retrospective, observational case study of CDI was performed. The study included a cohort of CDI patients diagnosed between January 2013 and April 2014 in a tertiary care referral hospital in Madrid (Spain) with approximately 20,000 patient admissions annually.

Inclusion and exclusion criteria

CDI cases were defined as patients who had diarrhea with three or more daily bowel movements for a period of 48 hours and were found to be positive for toxigenic *C. difficile* in stool samples by EIA or PCR testing (11,12). Patients with diarrhea who did not meet the inclusion criteria were excluded. In the case of patients suffering recurrent cases of CDI (defined as a second CDI episode occurring within 8 weeks of a previous episode), only the first incidence was considered to avoid overrepresentation of relapsing cases and the possible effect of treatment on diagnostic tests. In any event, no patient was included more than once.

Patient identification and clinical variables

Patients were identified through daily contact with the Microbiology Department. Data were extracted from the patients' electronic medical history. Variables for the analysis included age, gender, hospital admission during the previous month, administration of omeprazole (or other proton pump inhibitors), antibiotics or chemotherapy during the previous 3 months, antibiotics with activity against *C. difficile* during the previous month, community origin of CDI, clinical presentation, peripheral blood cell count and general biochemistry, mortality and recurrence of infection (according to the criteria used in previously defined cases of CDI) during the 4 months following the episode studied. Most patients were treated with metronidazole (500 mg/8 h PO or IV) according to current guideline recommendations (13). The decision to use oral vancomycin (125 mg/6 h), fidaxomicin (200 mg/12 h) or combined treatment was taken by the attending physician. Hospital-acquired cases had *C. difficile* toxin-positive fecal specimens obtained > 48 hours after hospital admission or, in those patients who had a documented hospital admission, in the 4 weeks prior to the positive test result. *C. difficile* toxin-positive fecal specimens were obtained from community-acquired cases within 48 hours of hospital admission or in an outpatient setting. No outbreaks of diarrheal pathogens were recorded by the hospital infection control unit during the study period. Patients were divided into two groups: one included all patients who tested positive by both GHD antigen and toxins A + B by EIA, in whom PCR testing was not carried out (EIA positive patients), and the other included patients with positive PCR and EIA GDH antigen tests, but with negative EIA toxin determination (PCR positive patients).

Microbiological methods

Microbiological diagnosis of CDI was performed by detection of the GHD and toxins A and B through EIA (C. DiffQuikChek Complete Techlab®, Blacksburg, Virginia, US). A PCR was performed using the GenomEra CDX *C. difficile* assay (Abacus Diagnostica, Finland) to search for the presence of the toxin B gene exclusively in cases where the GDH antigen test was positive and the EIA toxins test was negative. Therefore, no additional diagnostic tests (PCR) were performed in cases that were positive for both GDH antigen and EIA toxins. The diagnostic strategy used has been described as the most appropriate when considering all the diagnostic tests available today (14). Patients suffering from concomitant infection due to other enteropathogens were not included.

Statistical methods

Data are shown as mean and standard deviation (SD). For the analysis of risk factors, Student's t-test or, in the case of not normally distributed data, the Mann-Whitney U test was used to compare quantitative variables. The Chi-square test, with Yates' correction or Fisher's exact test (when necessary), was used to compare qualitative variables. A multivariate analysis was performed in order to identify independent risk factors for CDI. A final model was developed by stepwise regression from a maximum model which included all the associated variables with a p-value ≤ 0.2 in the univariate analysis. Odds ratios and 95% confidence intervals were computed. Statistical significance was established at $p \leq 0.05$ (two-tailed).

Ethical aspects

The study was approved by the hospital ethics committee. This investigation did not receive any funding.

RESULTS

During the study period, 2,030 fecal samples from patients presenting diarrhea underwent *C. difficile* diagnostic tests in our hospital. GDH antigen determination was positive in 210 cases (11%) and negative in 1,820 (89%). Of these GDH positive cases, 80 (38%) presented negative EIA toxin and PCR test determinations. Twenty-three episodes (11%) corresponded to recurrences, and one hundred and seven cases (51%) corresponded to initial CDI episodes. Seventy-four patients (69%) presented GDH antigen and toxin (A + B) detection. Thirty-three patients (31%) presented GDH positive, toxin negative and PCR positive. Figure 1 is a flowchart showing all included and excluded patients.

Two PCR positive patients (6%) had previously received metronidazole to treat other infections *versus* 4 patients (5%) among EIA positive patients (p = 0.603). PCR positive patients were younger 57 (27) years (mean [SD]) than toxin positive patients 71 (16) years, p < 0.001 (Table I). In the first group there were fewer patients treated with proton
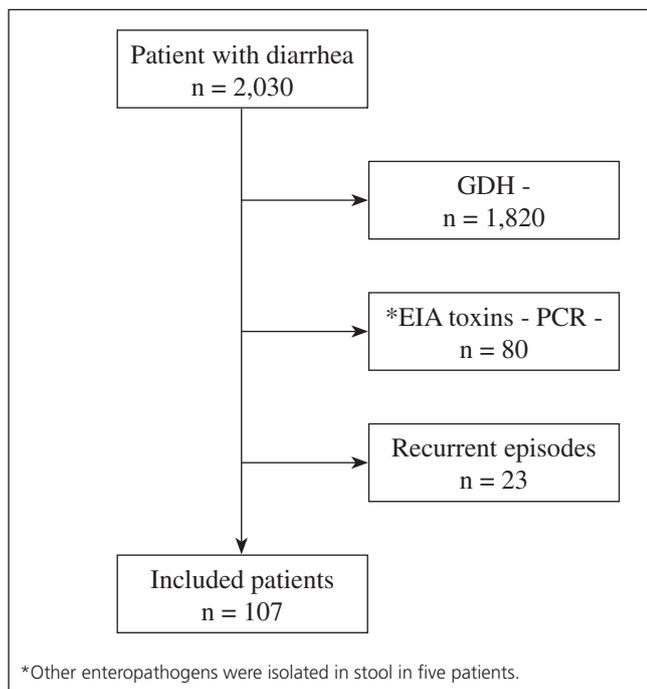
Fig. 1. Flowchart showing excluded and included patients.

pump inhibitors (21 patients, 64%) than in EIA positive patients (61 patients, 82%, p = 0.034). In the first group there were also more patients treated with chemotherapy (4 patients, 12%) than among EIA positive patients (1 patient, 1%, p = 0.031).

Nine patients (27%) had fever in the PCR positive group and 27 patients (37%) in the toxin positive group. The number of bowel movements during the day prior to the diagnosis was 5.5 (4.2) in PCR positive patients and 4.9 (3) in those who were EIA toxin positive (Table I).

Plasmatic albumin levels in EIA positive patients were 3.37 (0.56) mg/dl and 3.16 (0.59) md/dl in the PCR positive patients (p = 0.084). Two PCR positive patients (6%) and 14 EIA positive patients (18%) presented at least one recurrence after the initial episode (p = 0.071). The two PCR+ patients presented one recurrence each, whereas the 14 EIA positive patients had a total of 20 recurrences. Three patients died but not as a result of CDI; two were in the PCR positive group (one from bacteremia and the other due to disseminated cancer) and one patient in the toxin positive group (due to abdominal sepsis).

Age was the only quantitative variable maintaining statistical significance after conversion into a qualitative variable (age ≤ 65 years). Other quantitative variables such as temperature or plasma albumin concentration (which approached but did not achieve statistical significance) showed p > 0.2 when converted to qualitative variables.

Table I. Clinical and laboratory characteristics of CDI patients diagnosed by toxin or PCR determination

	PCR (n = 33)	Toxin (n = 74)	OR (% CI)	p
Age (years, mean)	55.6 (SD = 26.7)	70.1 (SD = 16.1)		< 0.001
Male gender	19 (58)	35 (47)		0.327
Proton pump inhibitor, 90 previous days	21 (64)	61 (82)	0.37 (0.25-0.94)	0.034
Chemotherapy, 90 previous days	4 (12)	1 (1)	11.11 (1.11-100)	0.031
Antibiotics, 90 previous days	22 (67)	58 (78)		0.197
Antibiotic with *C. difficile* activity[1]	2 (6)	4 (5)		0.603
Recurrence	2 (6)	14 (18)		0.071
CDI community acquisition	16 (49)	28 (38)		0.301
Outpatient treatment	4 (12)	10 (14)		0.557
Fever (> 38 °C)	9 (27)	27 (37)		0.351
Number of bowel movements per day	5.5 (SD = 4.2)	4.9 (SD = 3.0)		0.917
Ileus	0 (0)	1 (1)		0.691
Albumin (g/dl, mean ± SD)	3.37 (SD = 0.56)	3.16 (SD = 0.59)		0.084
Leukocyte count (cells/mm³, mean ± SD)	10,587 (SD = 7,580)	12,062 (SD = 7,724)		0.361
Creatinine (mg/dl, mean ± SD)	1.12 (SD = 0.84)	1.26 (SD = 1.04)		0.500
ICU admission	2 (6)	7 (9)		0.434
Mortality	2 (6)	4 (5)		0.603

Figures in brackets are qualitative variables expressed as percentages. [1]Antibiotic with activity against *C. difficile* administered during previous 30 days. CDI: *Clostridium difficile* infection; PCR: Polymerase chain reaction; SD: Standard deviation; ICU: Intensive care unit.

Finally, age (≤ 65 years), previous proton pump inhibitor treatment and previous chemotherapy were included in the multivariate analysis. The multivariate analysis showed that age (≤ 65 years) was the only variable related to CDI with positive PCR and negative EIA toxin determination (OR 4.3; CI 95% 1.8-11.1). Treatment with proton pump inhibitors (OR 0.82; CI 95% 0.32-2.03) and chemotherapy during the 90 previous days (OR 4.93; CI 95% 0.51-48.17) was not independently associated with PCR positive/EIA negative CDI.

DISCUSSION

The main result of this study was that almost one third of the patients could only be diagnosed through PCR testing, which exhibited greater sensitivity than EIA toxin determinations. Given that PCR was more useful in younger patients, performing *C. difficile* PCR in stool from patients with negative toxin determinations may be more decisive in patients of a less advanced age.

Our findings are comparable with those previously reported that also advocated consideration of PCR determination as the preferred diagnostic test for CDI in most institutions (9,10). According to these results, EIA toxin determination should be used as part of a detection algorithm that involves 2 or more detection methods (15). PCR determination proved more useful in younger patients, which has previously been related to lower intestinal bacterial load and less severe clinical presentation (10). However, there was no difference regarding the effectiveness of the distinct techniques in relation to acquisition site, i.e. community- or hospital-acquired, despite the fact that community-acquired CDI infections arise more frequently in younger patients (16,17).

According to one recent study, previous oral vancomycin treatment was associated with positive PCR and negative EIA tests in CDI patients (10). However, in our study, toxin negative EIA determinations could not be attributed to this fact because there was no difference between the two groups with respect to antibiotic treatment active against *C. difficile* prior to the diagnosis of CDI.

One finding in this study was the relationship in the univariate analysis between the administration of proton pump inhibitors and the CDI diagnosis by PCR. In our series, proton pump inhibitors may have been a surrogate marker of aged patients when taking into consideration that younger age was the only independent variable related to CDI diagnosis by PCR. Nevertheless, the use of these drugs may be questioned in many cases, and especially in those with doubtful indications (18-20). Chemotherapy was similarly associated with PCR positive patients, probably because this treatment had been received by younger individuals.

It has been supposed that CDI cases detected only by PCR may be less severe and may develop fewer complica-

tions than cases detected by toxin determination (10). In our patients, most CDI episodes were of moderate severity, which may be related to the very low incidence of infection with the hypervirulent strain (ribotype 027) found in Spain (21,22). The frequency of abdominal pain and the similar number of bowel movements in both groups do not suggest a less severe presentation in PCR positive patients. Leukocytosis is one of the clues which could lead to a suspicion of CDI in appropriate cases (10,16,23). A higher leukocyte count in patients diagnosed by EIA has been reported previously (10), however this difference was not statistically significant in our study. In addition, there were no relevant differences between the two groups with respect to mortality.

A previous study had shown a lower rate of recurrence and lower *C. difficile* fecal bacterial load in PCR positive patients (10). In our study, a higher proportion of EIA positive patients presented recurrences (18%) but did not reach statistical significance.

Among the limitations of this study are its retrospective nature and the small number of patients. The latter may have influenced the lack of power to detect other potential clinical differences between those diagnosed by PCR or by EIA toxin detection, such as, for example, the role of risk factors including antibiotic or proton pump inhibitor treatment, presence of fever, white blood cell count, plasmatic creatinine level or a trend towards recurrence.

In summary, PCR testing is significantly more sensitive than the EIA technique for the diagnosis of CDI, and this difference is more marked in younger patients. A prospective, multicenter study with greater statistical power would be necessary to further assess risk factors in this group. Larger studies are also required to demonstrate whether PCR determination should preferably be used in younger patients or in those presenting clinical characteristics associated with negative EIA results (20,23).

ACKNOWLEDGEMENTS

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