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Clostridioides (Clostridium) difficile (including epidemiology)

Prediction of poor outcome in *Clostridioides difficile* infection: A multicentre external validation of the toxin B amplification cycle

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ABSTRACT

Classification of patients according to their risk of poor outcomes in *Clostridioides difficile* infection (CDI) would enable implementation of costly new treatment options in a subset of patients at higher risk of poor outcome. In a previous study, we found that low toxin B amplification cycle thresholds (C_t) were independently associated with poor outcome CDI. Our objective was to perform a multicentre external validation of a PCR-toxin B C_t as a marker of poor outcome CDI. We carried out a multicentre study (14 hospitals) in which the characteristics and outcome of patients with CDI were evaluated. A subanalysis of the results of the amplification curve of real-time PCR gene toxin B (XpertTM *C. difficile*) was performed. A total of 223 patients were included. The median age was 73.0 years, 50.2% were female, and the median Charlson index was 3.0. The comparison of poor outcome and non−poor outcome CDI episodes revealed, respectively, the following results: median age (years), 77.0 vs 72.0 ($p = 0.009$); patients from nursing homes, 24.4% vs 10.8% ($p = 0.039$); median leukocytes (cells/μl), 10,740.0 vs 8795.0 ($p = 0.026$); and median PCR-toxin B C_t, 23.3 vs 25.4 ($p = 0.004$). Multivariate analysis showed that a PCR-toxin B C_t cut-off <23.5 was significantly and independently associated with poor outcome CDI ($p = 0.002$; OR, 3.371; 95%CI, 1.565−7.264). This variable correctly classified 68.5% of patients. The use of this microbiological marker could facilitate early selection of patients who are at higher risk of poor outcome and are more likely to benefit from newer and more costly therapeutic options.

1. Introduction

Clostridioides difficile infection (CDI) causes increased morbidity and mortality and is the leading cause of hospital-acquired diarrhea [1–4]. As new treatment options emerge[5, 6], classification of patients according to the risk of poor outcome (PO) in CDI (recurrence, treatment failures, and/or progression to severe complicated forms) would enable the implementation of costly new treatment options in the patients deemed at higher risk for poor outcome CDI.

Even though patient risk factors have been associated with recurrence or severity of CDI, clinical data are not sufficiently accurate for prediction of poor outcome at diagnosis [7]. Several characteristics of the microorganism, such as sporulation, germination, presence of binary toxin, and different ribotypes, have been studied as risk factors for poor outcome, although data are limited, contradictory, and not readily available at diagnosis[8–13].

Few studies have evaluated the toxin B amplification cycle threshold (C_t) as a marker for the severity of CDI or poor outcome of CDI [14–16]. In a previous study, we prospectively analyzed a derivation and validation cohort of CDI patients at our institution and found that low toxin B amplification C_t was independently associated with poor outcome CDI [17]. There are no multicentre studies or studies that have performed an external validation of this

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microbiological marker as a predictor of poor outcome CDI. Our objective was to perform a multicentre external validation of a PCR-toxin B amplification C_t as a marker of poor outcome CDI.

2. Material and methods

2.1. Design and study population

We conducted a multicentre study, involving 14 hospitals from various geographic areas that were representative of Spain as a whole. Only centres that performed Xpert PCR assay on all samples and not as part of a diagnostic algorithm were included in order to avoid bias resulting from selecting only CDI episodes with low toxin production.

Each participating hospital included patients with toxigenic *Clostridioides difficile* detected by XpertTM *C. difficile* PCR Assay (GeneXpert, Cepheid). Patients under 18 years of age, patients who did not meet the criteria for diarrhea (with <3 unformed stools in 24 h), and those with a previous episode of CDI in the previous 2 months were excluded. Patients were randomly selected from among those who met the inclusion criteria during the study period (2016–2017). Patient data were recorded for at least 2 months after completion of treatment for the CDI episode.

2.2. Definitions

A CDI episode was defined as the presence of a positive test result for toxigenic *C. difficile* and 1 of the following: diarrhea (≥3 unformed stools in 24 h) or colonoscopic evidence of pseudomembranous colitis.

Severity of CDI was defined according to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines [19].

Recurrence (R-CDI) was defined as the return of symptoms and a positive stool sample result for toxigenic *C. difficile* separated from the former by between 15 and 60 days after recovery from a previous episode (at least 3 days without diarrhea and clinical improvement) [20]. Episodes occurring more than 60 days after the previous one were not considered recurrences but new episodes that were not linked to the previous one.

Treatment of CDI was considered to have failed when the patient did not recover from a CDI episode after appropriate *anti*-CDI therapy, thus requiring a change of treatment.

Poor outcome was defined as R-CDI, treatment failure, or progression to severe complicated CDI. Mortality was considered to be associated with CDI when death was not clearly attributable to other, unrelated causes and occurred within 10 days of the CDI diagnosis, and/or was due to well-known complications of CDI.

2.3. Data collection

The data collected included age, sex, and patient origin (nursing home, hospital department, outpatient clinic) at the time of the CDI diagnosis, and date of previous hospital discharge, if applicable. Data regarding the underlying condition were recorded using the McCabe and Jackson score, and comorbidity factors were scored according to Charlson's comorbidity index [21,22].

Microbiological parameters of the CDI diagnosis included the following: glutamate dehydrogenase (GDH) and toxin A/B enzyme immunoassay (EIA) result, and, from the Xpert™ *C. difficile* assay (Cepheid; California, USA), PCR amplification C_t for toxin B (*tcdB*), binary toxin (*cdt*), and base pair deletion at nucleotide 117 in *tcdC*.

The severity of the CDI episode was classified according to ESCMID criteria, and antibiotic treatment for CDI was recorded. Outcomes were also recorded, as follows: progression to more severe disease, need for ICU admission, need for surgery for the CDI episode, recurrence, mortality, and CDI-associated mortality.

2.4. Data analysis

All analyses were performed using SPSS 18.0 (SPSS Inc, Chicago, Illinois, USA). Qualitative variables are expressed with their frequency distribution. Quantitative variables are expressed as the median and interquartile range (IQR). Groups were compared using the Fisher exact test for categorical variables and the *t*-test or Mann-Whitney test for continuous variables. For the outcome analysis, we excluded patients who were lost to follow-up, patients who did not receive treatment for CDI, and patients who died from unrelated causes before the end of the follow-up period. Proportions were calculated with a 95% confidence interval (CI) following a binomial distribution. A multivariate logistic regression model was used to assess predictors of poor outcome of CDI. The odds ratio (OR) and 95% CI were calculated. A p value < 0.05 was considered significant.

2.5. Ethical issues

This study was approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón Ethics Committee and the Spanish Agency for Medicines and Health Care Products.

3. Results

We enrolled 223 patients, whose demographic and clinical characteristics of patients with CDI are shown in Table 1. Median age was 73.0 years, and 50.2% of patients were female. Overall, 96.4% of the patients were hospitalized at the time of the diagnosis of the CDI episode, and 12.1% came from nursing homes. Most cases (65.9%) involved a non-fatal underlying disease. The median Charlson comorbidity index was 3.0. Of the 223 CDI episodes, 66.8% were mild to moderate, 31.4% were severe, and 1.8% severe-complicated. Overall mortality was 16.1%.

Data on 171 patients were available for the complete outcome analysis (23 patients died from unrelated causes before the end of the follow-up period, 15 were not treated for CDI, 2 entered a clinical trial, and 12 were lost to follow-up with incomplete data). Outcome was considered poor in 41 patients (24.0%), of whom 24 (14.0%) were R-CDI, 11 (6.4%) had experienced treatment failures, and 9 (5.3%) progressed to severe-complicated disease. Mortality attributable to CDI was 5.3%.

The comparison of poor outcome CDI and non–poor outcome CDI episodes revealed that poor outcome CDI patients were older (median age, 77.0 vs 72.0 years; $p = 0.009$), resided more frequently in nursing homes (10.8% vs 4.4%; $p = 0.039$), presented higher leukocyte counts (cells//μl) on the day of CDI diagnosis (10,740.0 vs 8795.0; $p = 0.026$), and presented lower PCR toxin B amplification C_t (median PCR toxin B amplification C_t, 23.3 vs 25.4; $p = 0.004$).

After adjustment for age and sex, the multivariate analysis showed that, in this multicentre validation cohort, the variables independently associated with poor outcome of CDI were PCR toxin B amplification C_t ($p = 0.008$; OR, 0.857; 95% CI, 0.764–0.961) and age ($p = 0.038$; OR, 1.029; 95% CI, 1.002–1.058).

We classified patients according to toxin B amplification C_t as high-risk prediction of poor outcome CDI (cycles<23.5), medium-risk prediction of poor outcome CDI (cycles 23.5–27.9), and low-risk prediction of poor outcome CDI (cycles≥28.0) (Fig. 1).

When we applied our proposed cut-off (<23.5) to the multicentre validation cohort for prediction of prediction of poor outcome CDI, we found an independent association between the

Table 1
Demographic and clinical characteristics of patients with *Clostridioides difficile* infection.

Characteristics	N = 223
Demographic data	
Female gender	112 (50.2%)
Age, median years (IQR)	73.0 (58.0–84.0)
McCabe and Jackson	
Non-fatal	147 (65.9%)
Ultimately fatal	53 (23.8%)
Rapidly fatal	24 (10.8%)
Hospitalized	215 (96.4%)
Hospitalization unit (n = 215)	
Intensive-care unit	4 (1.9%)
Medical unit	154 (71.6%)
Surgical unit	27 (12.6%)
Onco-hematology	30 (14.0%)
Underlying condition	
None	8 (3.6%)
Transplant recipient	21 (9.4%)
Cardiovascular	81 (36.3%)
Malignancy	62 (27.8%)
Respiratory	48 (21.5%)
Neurologic	37 (16.6%)
Gastrointestinal	57 (25.6%)
Liver disease	24 (10.8%)
Hematologic	33 (14.8%)
Endocrine	42 (18.8%)
Metabolic	42 (18.8%)
Infectious disease	17 (7.6%)
Allergic	8 (3.6%)
Rheumatologic	13 (5.8%)
Psychiatric	10 (4.5%)
Ocular	7 (3.1%)
Cutaneous	14 (6.3%)
Nephro-urologic	51 (22.9%)
Charlson score, median (IQR)	3 (1-5)
Type of CDI episode	
H-CDI	181 (81.2%)
C-CDI	35 (15.7%)
I-CDI	8 (3.6%)
Severity of CDI episode	
Mild-moderate	149 (66.8%)
Severe	70 (31.4%)
Severe-complicated	4 (1.8%)

IQR, interquartile range; CDI, *C. difficile* infection; H-CDI, healthcare-associated CDI; C-CDI, community-associated CDI; I-CDI, indeterminate CDI.

high-risk category (<23.5) and poor outcome (p = 0.003; OR, 3.298; 95% CI, 1.508–7.215) as shown in Table 2. We successfully stratified 68.5% (95% CI, 60.8%–75.4%) of patients with prediction of poor outcome CDI when our proposed cut-off was applied.

3. Discussion

In this multicentre validation study, PCR toxin B amplification cycle performed on the day of CDI diagnosis was independently associated with a prediction of poor outcome CDI. The proposed microbiological marker cut-off (toxin B amplification $C_t < 23.5$) correlated well with all unfavorable outcomes. The use of this microbiological marker of poor outcome could facilitate early selection of patients who are more likely to benefit from newer and more costly therapeutic options.

Several studies have shown a correlation between *C. difficile* bacterial load and PCR C_t [20,23] and between PCR C_t and toxin positivity determined by cell culture cytotoxicity neutralization assay or enzyme immunoassay (direct toxin assays) [16,24,25]. Consistent with these studies, we also found that C_t at diagnosis possibly acted as a marker of the amount of toxin produced and correlated with the enzyme immunoassay results.

Only a few studies have addressed the potential of PCR C_t for predicting presence of free toxin as a marker of CDI and severity of CDI, with discordant results [14,15,17,26,27]. Rao et al. found no correlation between PCR C_t and severity of CDI or overall mortality [14]. In this study, it is notable that the median C_t obtained was higher than that reported elsewhere (34.3). Kim et al. [26] stratified 282 patients into three categories (positive for toxigenic *C. difficile* without CDI clinical criteria, mild CDI, and severe CDI) and found that the median C_t values (27.5, 28.2 and 26.1) were not sufficiently statistically significant to confirm the correlation with the clinical spectrum of CDI.

However, a few more recent studies do report a correlation with severity of CDI, although most were single-centre studies [15,16]. The retrospective study by Jazmati et al. [15] revealed that samples from patients with severe disease showed significantly lower C_t values than those of patients in the other groups (26.5 ± 4.8 [n = 9] vs 31.2 ± 4.8 [n = 45]; p = 0.02). The study was based on a low number of patients with severe CDI [n = 10]). Garvey et al. observed

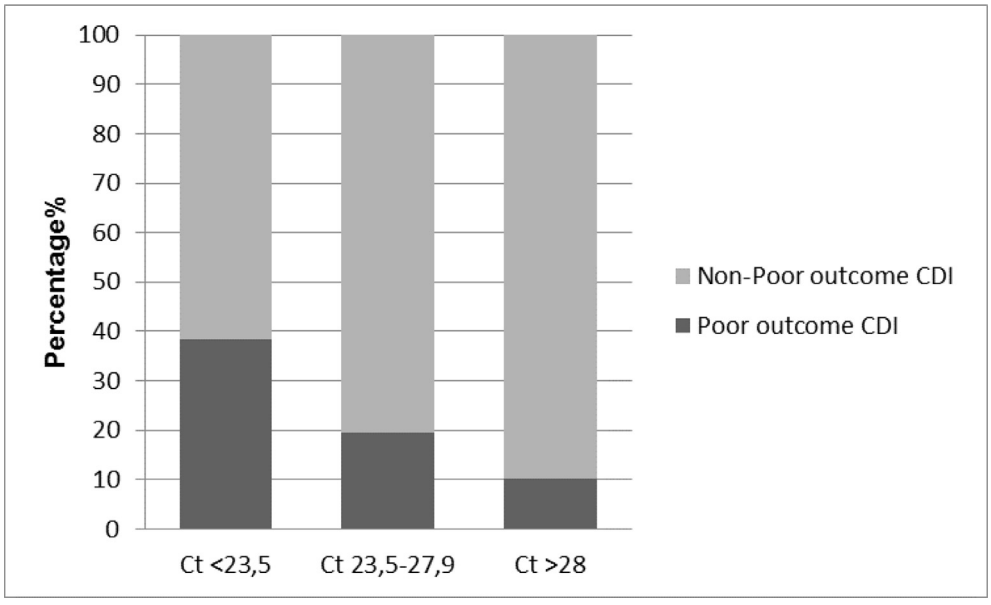

Fig. 1. Risk category of prediction of poor outcome CDI according to PCR toxin B amplification cycle threshold.

Table 2
Multivariate analysis for the multicentre validation cohort.

Variables	Odds Ratio	95% CI	P Value
Sex	0.719	0.332–1.559	0.404
Age	1.031	1.003–1.060	**0.030**
Leukocytes (cells/l)	1.000	1.000–1.000	0.138
Nursing home	1.792	0.669–4.800	0.246
Toxin B PCR amplification cycle threshold <23.5	3.298	1.508–7.215	**0.003**

CI, confidence interval.

that a $C_t \leq 26$ indicated more severe CDI and was associated with higher mortality [25].

Kamboj et al. [16] observed that the median C_t value was 28.0 for non-severe CDI, 24.5 for severe CDI, and 22.5 for complicated CDI ($p = 0.005$). While the study also addressed R-CDI, the authors found no correlation between the C_t value and cytotoxicity in patients whose disease recurred and those whose disease did not; only 19 patients had R-CDI. They established a cut-off at 28, which revealed all but one case of severe CDI. The study population comprised only cancer patients.

Our group previously demonstrated that C_t may be valuable for determining not only the severity of infection, but also the risk of recurrence and mortality, which is precisely the focus of this study [17]. An objective marker such as C_t could help to establish the prognosis of patients who are at risk of a poor outcome and could be the target for new, more expensive therapeutic options, such as bezlotoxumab and fidaxomicin, or options that are more difficult to access, such as fecal transplantation.

No multicentre studies have demonstrated the value of C_t as a predictor of poor outcome including recurrent CDI. In the only multicentre study performed they found no significant association between recurrence and low C_t values, however their original study was not designed to capture recurrence data systematically[28]. We validate this microbiological marker for prognosis of poor outcome. While prediction rules are often based on subjective clinical judgment or on radiological findings that are not readily available [29–32], C_t is a simple objective marker that is available at diagnosis and for which correlations have been established in different settings and conditions. In our previous study [18], samples (weighed to an exact amount) were processed and homogenized. However, in the present validation study, no standard process or special procedure was undertaken: samples were processed according to the manufacturers' instructions and routinely at each centre.

Our study is limited by the fact that it was performed in a single country and our data only relate to one PCR assay, so confirmation with respect to other assays is required. Culture was not performed in all cases and we were not able to obtain data on the different ribotypes. The PCR assay was part of the microbiological confirmation for toxigenic *C. difficile*. Also, we defined as a CDI episode a positive result for toxigenic *C. difficile* and the presence of diarrhea, we could not rule out if the diarrhea was explained by other causes not related to the presence of toxigenic *C. difficile*. However, to our knowledge, this is the first multicentre validation study to demonstrate the utility of this widely used microbiological test in the prognosis of poor outcome CDI including recurrent CDI.

In conclusion, the results from this multicentre cohort confirm that the proposed C_t cut-off (<23.5) correlated well with unfavorable outcomes and may therefore serve as a universal tool for prediction of prediction of poor outcome CDI.

Transparency declarations/Potential conflicts of interest

The authors declare that they have no conflicts of interest.

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