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Clinical, immunological and microbiological predictors of poor outcome in *Clostridium difficile* infection

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ABSTRACT

Background: Clostridium difficile infection (CDI) causes increased morbidity and mortality. Clinical data cannot clearly predict poor CDI outcome. Data on the value of microbiological predictors is scarce.
Objective: To identify early predictors of poor outcome of CDI.
Methods: We prospectively included patients with CDI aged >2 years. Clinical, immunological (Toxin B IgG/Ig A and Toxin A IgG/Ig A), microbiological factors (bacterial load, toxin quantification, sporulation, germination, and metronidazole susceptibility) were evaluated to identify early independent predictors of poor outcome.
Results: We identified 204 cases of CDI; outcome was poor in 22.1%. Advanced age, presence of comorbidities, leukocytosis and high toxigenic *C. difficile* load were independently associated with poor outcome. We could not demonstrate this correlation for antitoxin antibodies.
Conclusion: We identified high bacterial load as a microbiological predictor of poor outcome. We propose this factor to be included in combined clinical and microbiological prediction rules of poor outcome in CDI.

1. Introduction

Clostridium difficile infection (CDI) is the leading cause of hospital-acquired diarrhea and is associated with a considerable health and cost burden (Dubberke & Olsen, 2012; Wiegand et al., 2012; Asensio et al., 2013; Miller et al., 2002). Between 12% and 18% of patients progress to severe disease (Henrich et al., 2009; Hensgens et al., 2013; Lungulescu et al., 2011) and approximately 20% have 1 or more recurrences (McFarland et al., 1999) (poor outcome). Prediction of poor outcome is necessary in order to plan appropriate management of CDI, because current guidelines recommend different therapeutic approaches depending on disease severity and high risk for recurrence (Cohen et al., 2010; Bauer et al., 2009).

To our knowledge, clinical criteria obtained at the diagnosis of the CDI episode are not sufficiently accurate to predict poor outcome (Crook et al., 2012). A wide variety of risk factors have been reported for disease severity, mortality, and recurrence, although none seems to be widely accepted. Few prospective and validated studies have been conducted to establish a robust prediction score (Abou Chakra et al., 2012). Moreover, complementary microbiological data that can improve prediction of poor outcome are limited and contradictory (Merrigan et al., 2010; Oka et al., 2012; Burns et al., 2011).

Our objectives were to identify early clinical and microbiological factors that can predict poor outcome of CDI.

2. Material and methods

2.1. Setting

Our institution is a large teaching hospital with 1550 beds. The clinical microbiology laboratory receives samples from patients hospitalized at our center and from all the outpatient institutions in our catchment area.

2.2. Design and study population

During a 6-month period (Jan 2013–June 2013), we prospectively included patients (inpatients and outpatients) with CDI. Children under the age of 2 years or patients who had a recurrence of an episode that had occurred before the study period were not included. Patients were followed up throughout the study period and for at least 2 months after their last CDI episode or recurrence (last patient follow-up, October 22, 2013).

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2.3. Definitions

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

The type of CDI episode according to the potential site of acquisition was defined according to the criteria of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *Clostridium difficile* (Kuijper et al., 2006).

Severity of CDI was defined according to the Society of Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) guidelines for mild to moderate and severe CDI (Cohen et al., 2010). The definition was modified slightly for severe complicated CDI, which was defined as CDI with septic shock or colectomy or megacolon or CDI-related admission to the intensive care unit (ICU) within 7 days of the positive sample or CDI-associated mortality.

2.3.1. Recurrent (R-CDI)

R-CDI was defined as the return of symptoms and a positive stool sample 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).(Leslie et al., 2012) 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.

2.3.2. Treatment failure

Treatment was considered to have failed when the patient did not recover from a CDI episode and had a positive stool sample separated from a previous sample by less than 15 days.

2.3.3. Poor outcome

Poor outcome was defined as R-CDI, treatment failure, or progression to severe complicated CDI.

2.3.4. CDI-associated mortality

Mortality was considered to be associated with CDI when death was not clearly attributable to other unrelated causes, occurred within 10 days of the CDI diagnosis, and/or was due to well-known complications of CDI.

2.4. Data collection

Patient information was collected directly at the bedside by one of the investigators. Data were also obtained by reviewing hospital medical records, accessing the local electronic medical information system, and telephoning patients directly. The data collected included age, sex, hospital department or outpatient clinic diagnosis of CDI, and history of hospital admissions up to 3 months before diagnosis (to determine the source of CDI). Data regarding the underlying condition were recorded using the McCabe and Jackson prognosis of underlying diseases, and comorbidity factors according to the Charlson index. Data on risk factors for CDI present in the month prior to the diagnosis of CDI were collected (previous antibiotics, proton-pump inhibitors, use of a nasogastric tube, mechanical ventilation, and surgery). Data were also recorded for history of previous CDI episodes, chemotherapy, previous admission to the gastroenterology department, dialysis, inflammatory bowel disease, and infection or colonization by methicillin-resistant *S. aureus*.

The clinical data recorded for the CDI episode were as follows: days of diarrhea, presence of abdominal pain, abdominal distention, fever, hypotension, toxic megacolon, pseudomembranous colitis, and severity of the CDI episode according to ESCMID criteria. Analytical data on the day of diagnosis were recorded. Antibiotic treatment for the CDI episode and outcomes were also recorded (need for ICU admission, need for surgery for CDI episode, recurrence, mortality, and CDI-associated mortality).

2.5. Laboratory procedure

2.5.1. Processing of samples for diagnosis of CDI

Unformed stool samples received in the laboratory were processed for diagnosis of CDI. Stool samples transported in formaldehyde and formed samples were excluded. Rectal exudates or colon biopsy specimens were taken in the case of patients with paralytic ileus or megacolon.

Toxigenic culture in *Clostridium* selective agar medium (bioMeriéux) was performed for all samples, and plates were incubated under anaerobic conditions at 35–37 °C for 48 h. Following incubation, colony morphotypes compatible with *C. difficile* were selected using a binocular magnifying glass. Colonies suspected of being toxigenic *Clostridium difficile* were identified using an immunochromatographic system (C Diff Quik-Chek Complete assay; TechLab, Blacksburg, VA, USA) and the MRC-5 cell line cytotoxicity test.

The rapid detection test consisted of a diagnostic algorithm based on immunochromatographic glutamate deshidrogenase (GDH) and toxin detection (C Diff Quik-Chek Complete assay) and on real-time polymerase chain reaction assay of the B toxin gene (XpertTM *C. difficile*). In the case of direct cytotoxicity testing on the sample, the MRC-5 cell line was used as described previously (Alcala et al., 2008).

A positive result for toxigenic *C. difficile* was regarded as a positive result with either of the reference techniques (toxigenic culture or direct cytotoxicity in stools).

Serum levels of IgA and IgG versus toxin A and B (on days 3 and 12 of the CDI episode) were determined using enzyme-linked immunosorbent assay (ELISA) (TgcBiomics, Bingen, Germany) to detect antitoxin antibody.

To determine the concentration of toxigenic *C. difficile* (colony-forming units [cfu] per gram of stool), the sample was homogenized and subjected to 10-fold serial dilutions. An aliquot of 100 μL of each sample was inoculated quantitatively in ChromID *C. difficile* medium (BioMérieux, Marcy l'Etoile, France). After 48 hours of incubation, cfu were counted (expressed as cfu per gram of stool). This test was performed directly from the stool sample that confirmed the diagnosis.

Toxin quantification was defined as the amount of toxin per gram of stool and was calculated as the product of the concentration of toxigenic *C. difficile* cfu per gram of stool and the quantity of toxins A and B, which was measured using a modified semi-automated ELISA (bioMérieux, Marcy l'Etoile, France).

The sporulation rate was determined from *C. difficile* colonies after 5 days' incubation at 37 °C and a further 24 hours' incubation at 4 °C in Brucella agar. A 0.5 McFarland suspension of the colonies was observed microscopically using a counting chamber. The sporulation rate was calculated as the number of spores per milliliter divided by the total number of cells (spores and vegetative cells) per milliliter.

The germination rate was determined by heating a 0.5 McFarland suspension of *C. difficile* cells at 80 °C for 10 minutes to kill all the vegetative cells. The suspension underwent 4 serial dilutions (10-fold). A 100-μl aliquot from each of the 4 dilutions and from the primary suspension was inoculated into Brucella agar, and the colonies were counted after 48 hours of incubation. The germination rate was calculated as the number of cfu per milliliter divided by the microscopic counts of spores per milliliter.

Susceptibility of metronidazole was determined on fresh isolates using E-test strips (AB Biodisk, Solna, Sweden) and by disc diffusion (5 μg discs, Oxoid Basingstoke, United kingdom) in Brucella agar. Readings were performed after incubation for 48 hours at 37 °C under anaerobic conditions.

2.6. Data analysis

All analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Qualitative variables appear with their frequency distribution. Quantitative variables are expressed as the median and interquartile