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C.difficile (including epidemiology)

Is *Clostridium difficile* infection a risk factor for subsequent bloodstream infection?

Robert J. Ulrich [a, d], Kavitha Santhosh [a, b], Jill A. Mogle [a, b], Vincent B. Young [a, b, c], Krishna Rao [a, b, d, *]

[a] *Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109, USA*
[b] *Division of Infectious Diseases, University of Michigan School of Medicine, Ann Arbor, MI 48109, USA*
[c] *Department of Microbiology and Immunology, University of Michigan School of Medicine, Ann Arbor, MI 48109, USA*
[d] *Veterans Affairs Ann Arbor Healthcare System, Ann Arbor, MI 48105, USA*

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A B S T R A C T

Background: *Clostridium difficile* infection (CDI) is a common nosocomial diarrheal illness increasingly associated with mortality in United States. The underlying factors and mechanisms behind the recent increases in morbidity from CDI have not been fully elucidated. Murine models suggest a mucosal barrier breakdown leads to bacterial translocation and subsequent bloodstream infection (BSI). This study tests the hypothesis that CDI is associated with subsequent BSI in humans.
Methods: We conducted a retrospective cohort study on 1132 inpatients hospitalized >72 h with available stool test results for toxigenic *C. difficile*. The primary outcome was BSI following CDI. Secondary outcomes included 30-day mortality, colectomy, readmission, and ICU admission. Unadjusted and adjusted logistic regression models were developed.
Results: CDI occurred in 570 of 1132 patients (50.4%). BSI occurred in 86 (7.6%) patients. *Enterococcus* (14%) and *Klebsiella* (14%) species were the most common organisms. Patients with BSI had higher co-morbidity scores and were more likely to be male, on immunosuppression, critically ill, and have a central venous catheter in place. Of the patients with BSI, 36 (42%) had CDI. CDI was not associated with subsequent BSI (OR 0.69; 95% CI 0.44–1.08; $P = 0.103$) in unadjusted analysis. In multivariable modeling, CDI appeared protective against subsequent BSI (OR 0.57; 95% CI 0.34–0.96; $P = 0.036$). Interaction modeling suggests a complicated relationship among CDI, BSI, antibiotic exposure, and central venous catheter use.
Conclusions: In this cohort of inpatients that underwent testing for CDI, CDI was not a risk factor for developing subsequent BSI.

1. Introduction

Clostridium difficile infection (CDI) causes a colitis that has become the leading source of nosocomial diarrhea worldwide [1]. In the United States, it is estimated to cause 453,000 infections per year, 107,600 of which are hospital-onset [2]. The clinical presentation varies from mild diarrhea to debilitating disease with high fever, severe abdominal pain, and a paralytic ileus (megacolon) or perforation [3]. The burden of disease, despite best efforts, is not decreasing—CDI mortality has dramatically increased in the past decade, and is now the leading cause of gastroenteritis-associated death in the United States [4,5]. The cause of mortality in CDI is likely multifactorial, but as discussed below evidence from murine models posits a role for bacterial translocation in the pathogenesis of complicated CDI.

It has been established that *C. difficile* cytopathic toxins cause mucosal injury and a pseudomembranous colitis [6]. These toxins, identified as *C. difficile* toxins A and B (TcdA and TcdB), inactivate members of mucosal Rho GTP-ases, and this is proposed to result in colonocyte death, neutrophilic colitis, and loss of intestinal barrier function [7]. Recent murine models suggest that loss of barrier function during CDI predisposes to bacterial translocation and systemic dissemination, with ensuing septic shock and death [8,9].

* Corresponding author. 1150 West Medical Center Drive MSRB 1, Room 1510B, Ann Arbor, MI 48109-5534, USA.
E-mail addresses: rulrich@med.umich.edu (R.J. Ulrich), krirao@med.umich.edu (K. Rao).

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This dissemination is proposed to be a primary cause of mortality; mice treated with antibiotics targeted at gut bacteria had significantly improved survival [9].

In humans, there are only two studies in the literature thus far examining if CDI and BSI are related. CDI was determined to be a risk factor for VRE bacteremia in a small cohort (N = 59) of VRE-colonized acute leukemia patients [10]. A more recent retrospective analysis of CDI + patients found no difference in the incidence of non-Staphylococcal BSI relative to CDI, but BSI occurring after CDI was more likely to be without an obvious source, suggesting colonic translocation [11]. In addition to the above studies regarding gut bacterial translocation, *C. difficile* bacteremia itself is a rare clinical entity [12].

In non-CDI colitis, there is some evidence that translocation can be clinically significant. Inflammatory bowel disease (IBD) was found to be a risk factor for anaerobic bacteremia in a recent large population surveillance study [13]. Also, antibiotic induced diarrhea was associated with *Klebsiella oxytoca* bacteremia, suggesting translocation [14]. Additionally, there are reports of BSI attributed to colonic infections with CMV, *Strongyloides stercoralis*, and *Entamoeba histolytica* [15–18].

Based on the available evidence, it is not yet established whether CDI is a risk factor for subsequent BSI in humans. As CDI is becoming a more frequent and virulent nosocomial pathogen, determining if it predisposes to potentially lethal BSI could inform clinical decision-making. The objective of this study was to determine if CDI is associated with BSI in humans.

2. Methods

2.1. Study design and population

This study was approved by the University of Michigan Institutional Review Board. We conducted a retrospective cohort study on inpatients at the University of Michigan Hospital who were tested for possible healthcare associated *C. difficile* infection (>48 h after admission) between October 2010 and January 2013. All laboratory testing of inpatients was performed at the discretion of the inpatient care team, which ordered *C. difficile* testing per institutional guidelines that mirror national guidelines recommending testing only symptomatic patients with suspected CDI [19,20]. Patients hospitalized <72 h, those with a prior positive and redundant blood culture or a positive CDI within the prior 8 weeks were excluded.

2.2. Microbiology

Testing was performed on stools in the clinical microbiology laboratory via an algorithm (Fig. 1) using the C. DIFF QUIK CHEK COMPLETE® test for *C. difficile* glutamate dehydrogenase (GDH) and toxins A or B (Techlab, Inc., Blacksburg, VA) by EIA. All GDH^{+}/$toxin^{-}$ stool tests were subjected to analysis for the *tcdB* gene by real-time PCR using the GeneOhm™ Cdiff Assay (BD, Franklin Lakes, NJ) run on a Cepheid SmartCycler® System (Cepheid, Sunnyvale, CA). Confirmation of all positive *C. difficile* tests was attempted by anaerobic culture on taurocholate-cycloserine-cefoxitin-fructose agar at 37 °C. Attempts were made to ribotype samples using high-throughput, fluorescent PCR-ribotyping as described elsewhere [21,22]. Blood culture collection and detection of positives was performed using the aerobic and anaerobic BacT/Alert system (BioMerieux, Durham, NC) and handled per the clinical microbiology laboratory protocol [23]. Briefly, all culture bottles are read by the automated system and positives reported to the laboratory staff every 15 min, followed by an initial Gram stain and subculturing/identification as appropriate.

2.3. Data extraction

The electronic medical record of each subject with a positive blood culture subsequent to CDI onset was reviewed by a physician to determine if it represented a true BSI, using the algorithm outlined in Fig. 2, and not a contaminant. If the blood culture was determined to be a contaminant, this patient was treated as a non-BSI case in our analysis. Concurrent presence/absence of a central venous catheter and the type was also obtained. Other variables were also extracted: demographics, medications, laboratory results including *C. difficile* test results, and 30 day outcomes including mortality, colectomy, ICU admission, and readmission.

2.4. Study definitions

In our study, subsequent BSI was defined as positive blood culture results obtained <48 h prior to, or any time after, CDI stool assay collection that was determined to be a true (non-contaminant) BSI using Fig. 2 algorithm. The <48 h prior criterion for BSI was selected as there is often a delay between CDI symptom onset and test result availability. In this algorithm, suspected pathogen was defined as a bacterial species documented to be overwhelmingly pathogenic (>88% clinical significance) [24]. Standard criteria (to determine positivity of Coagulase-negative *Staphylococcus*) was defined using a previously determined algorithm (sensitivity 62%, specificity 91%) combining white blood cell count, presence or absence of fever and/or hypotension [25]. Charlson comorbidity index (weighted and non-weighted) are previously validated comorbidity indices based off ICD-9 codes, shown to predict patient mortality [26,27], and these were calculated. Ventilator status was defined by evidence in the electronic medical record of endotracheal intubation during the index hospitalization. Immunosuppression was defined as prednisone use of ≥5 mg per day or any steroid-sparing agent. Prior fluoroquinolone use and concurrent non-CDI antibiotic use are in relation to CDI stool assay testing, during the index hospitalization. Prior CDI was defined as a positive CDI test >8 weeks prior to the index CDI stool assay. Modified SIRS criteria was positive if any two of the following criteria were present: leukocytosis >12,000 cells/mm^3 or leukopenia <4000 cells/mm^3; tachypnea (respiratory rate >20); fever (>38 °C) or hypothermia (<36 °C). The primary outcome was presence of subsequent bloodstream infection. Secondary outcomes included 30-day mortality, colectomy, readmission, and ICU admission.

2.5. Statistical analysis

After data cleaning, descriptive statistics were prepared using proportions for categorical variables and measures of central tendency/spread for continuous variables. For all analytical statistics, P values < 0.05 were considered statistically significant. To analyze relationships between predictor variables and the primary/secondary outcomes, simple, unconditional logistic regression was employed. Multiple logistic regression was employed for adjusted analysis. Multivariable models were constructed through stepwise addition, incorporating variables significant on unadjusted analysis. To assess for possible confounding or collinearity, the point estimates for coefficients and the significance testing results were scrutinized as each new variable was added to the model. Likelihood ratio testing was used to exclude variables from the models with $P > 0.05$. As noted below, given the complex relationship between two predictors and the primary outcome, interaction terms from the