Detection of Free Toxin B in the Stool of Asymptomatic Clostridioides difficile Carriers by the Cell Cytotoxicity Neutralization Assay

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Cell cytotoxicity neutralization assay (CCNA) is considered to be a gold standard to diagnose Clostridioides difficile infections. We performed CCNA on 77 consecutive admission screening rectal swabs from asymptomatic toxigenic C. difficile carriers. Thirty-nine percent of specimens from asymptomatic carriers were positive. Thus, CCNA specificity may be lower than previously thought.

Keywords. C. difficile; colonization; diagnosis; epidemiology; laboratory diagnosis.

Clostridioides (formerly Clostridium) difficile is a gram-positive, anaerobic, spore-forming bacillus that is responsible for ongoing health care–associated and, more recently, community outbreaks of C. difficile infections (CDIs) worldwide. Despite much improvement in controlling its spread, it is still estimated to cause 224,000 health care–associated infections and 12,800 deaths per year in the United States [1]. The diagnosis of CDI usually requires the presence clinical criteria (such as diarrhea or toxic megacolon) combined with a laboratory confirmation assay [2]. There are 2 main types of diagnostic assays for laboratory confirmation: (1) assays that detect the presence of toxigenic C. difficile in a stool sample by culture or by nucleic acid amplification tests (NAATs) and (2) assays that detect free toxin A and/or B in the stool by cell culture neutralization assay (CCNA) or enzyme immunoassay (EIA). NAAT and EIA are the most frequently used clinical assays, while culture and CCNA are mostly confined to research settings.

Laboratory confirmation of CDI has been complicated by the phenomenon of asymptomatic carriage of toxigenic C. difficile, leading to concerns of overdiagnosis of CDI. Detection of asymptomatic colonization rather than true CDI is believed to mainly affect assays that detect toxigenic C. difficile (ie, toxigenic culture and NAATs) in individuals who have diarrhea for a reason other than CDI [3]. On the other hand, the detection of free toxin in a stool sample is thought to strongly suggest the presence of CDI in a patient with compatible symptoms. Hence, CCNA is considered one of the clinical gold-standard assays for the diagnosis of CDI [4]. It is also one of the reference assays (along with toxigenic culture) against which new assays are compared to determine their sensitivity and specificity [2, 5].

However, few studies have rigorously evaluated the specificity of CCNA. Estimating the specificity of an established gold standard is a complex endeavor, as there is a lack of alternative diagnostic approaches against which it can be compared. One strategy that could be used to indirectly estimate the potential for overdiagnosis associated with CCNA would be to determine the positivity rate of the assay on the stool of individuals who clearly do not have CDI, for example, among asymptomatic carriers of toxigenic C. difficile. As these patients are by definition asymptomatic, detection of Toxin B by CCNA in a large proportion of these patients would call into question its ability to differentiate actual CDI from diarrhea unrelated to CDI among asymptomatic carriers. Previous studies published decades ago demonstrated toxin excretion by CCNA in 50%–80% of culture-confirmed asymptotically colonized individuals, however, to our knowledge, this phenomenon has not been studied in the NAAT era [6–8]. We thus performed the CCNA on rectal swabs collected from known asymptomatic C. difficile carriers in order to determine the proportion of asymptomatic carriers who harbor detectable Toxin B by this method.

METHODS

The study was performed between April 13 and September 2, 2015, at the Quebec Heart and Lung Institute. In November 2013, the institute implemented an infection prevention and control policy of detection and isolation of asymptomatic C. difficile carriers on hospital admission using a commercial polymerase chain reaction (PCR) detecting the tcdB gene (BD Max Cdiff assay, Franklin Lake, NJ, USA) performed on rectal swabs [9]. An asymptomatic carrier was defined as any patient with a positive admission screening and without diarrhea on the day of admission [9]. Successive rectal swabs that were positive for the tcdB gene by PCR were kept frozen at −80°C until being used for...
The assay uses Vero cell line to detect the presence of a cytopathic effect neutralized by *C. difficile* antitoxin B (Bartels immunodiagnostic Supply, Bellevue, WA, USA). A sample is considered positive if a cytopathic effect is observed within 72 hours with neutralization by the antitoxin. In the case of multiple admission screening swabs from the same patient during the study period, only the first one was included in the study. Also, as the potential detection of Toxin B in the stool of an asymptomatic carrier could, in theory, represent early, presymptomatic CDI, we also performed a retrospective chart review of the hospital Infection Prevention and Control database to determine whether any of these patients subsequently developed health care–associated CDI as defined by the Quebec CDI surveillance program [9]. Ethics approval was obtained from the institutional review board.

**RESULTS**

A total of 77 NAAT-positive rectal swabs were included. Of these samples, 30 (39%) demonstrated *C. difficile* toxin production by CCNA, 43 (56%) did not demonstrate toxin production, and 4 (5%) were contaminated with an unidentified toxin. Of these patients, 2 (3%) subsequently developed symptomatic health care–associated CDI, both of whom were cytoxin-positive on admission. The delay between admission screening and the CDI episodes was 2 and 15 days, respectively. Presence of cytoxin on admission was not predictive of subsequent CDI in our cohort of patients (2/30 [7%] vs 0/43 [0%], respectively; *P* = .17 by Fisher exact test).

**DISCUSSION**

There is considerable uncertainty regarding the optimal method to diagnose CDI [2, 5]. Some assays such as toxigenic culture and PCR are often perceived as having poor specificity because they can detect the presence of *C. difficile* in individuals who have diarrhea for reasons other than CDI such as laxative use or chemotherapy. On the other hand, the detection of free toxin by CCNA is considered highly specific for CDI. In their most recent guidelines, the Infectious Diseases Society of America (IDSA) considers CCNA to be the most specific diagnostic assay for CDI, implying that most patients with compatible symptoms truly have CDI [2]. However, our study suggests otherwise, as free toxin at concentrations detectable by the CCNA was detectable in the stool of 39% of asymptptomatically colonized *C. difficile* carriers. Considering that asymptomatic carriage can affect between 5% and 11% of hospitalized patients, this indicates that many individuals have detectable free toxin in their stool even in the absence of CDI [11]. This suggests that the specificity of CCNA may be lower than generally believed. Furthermore, detectable toxin was not predictive of the subsequent development of symptomatic CDI. Risk factors for progression to CDI in persons asymptomatically colonized on admission to the hospital have been recently described and remain an area of active investigation [12].

Few recent studies have assessed the presence of *C. difficile* Toxin B in the stool of asymptomatic carriers. In a cohort of 44 NAAT-positive asymptomatic *C. difficile* carriers in the United States, 6 patients (14%) had detectable toxin A and/or B in their stool as measured by EIA [13]. Similarly, in 2 recent studies using quantitative EIA, the concentration of free toxin in stool was not predictive of symptomatic CDI [14, 15]. New diagnostic tools may be needed in the future to improve the diagnosis of CDI. For example, serum and stool inflammatory markers may show promise in this regard and are an area of active research [16].

Our study, along with the data cited above, suggests that detection of Toxin B by CCNA may sometimes lead to unnecessary treatment of asymptomatic carriers or to failure to consider alternative causes of diarrhea in patients with a positive test result. Our results are strengthened by the use of a commercially validated NAAT assay, which is representative of real-world practice for the diagnosis of CDI, as well as the use of a standardized definition for CDI and asymptomatic carriage in order to ensure proper case ascertainment.

Our study has limitations. Chart review was retrospective, and the sample size was small and limited to a single center. Furthermore, while our study focused on CCNA, we were unable to perform the more commonly used EIA in parallel due to the paucity of fecal material on rectal swabs. The positivity rate would presumably have been lower with EIA due to its higher limit of detection (LOD). In addition, while the use of rectal swabs has previously been studied for toxin detection using EIA, it has not been validated for use with CCNA [17]. However, we would expect rectal swabs to be less sensitive than stool samples to detect Toxin B. Thus, the true proportion of asymptomatic toxin excretion as determined by CCNA in our study population may be even higher than what was measured in our study.

In conclusion, free Toxin B was detectable by CCNA in the stool of many asymptomatic carriers of *C. difficile*. This study emphasizes that the diagnosis of CDI cannot be based on laboratory results alone. The interpretation of any *C. difficile* laboratory result must include clinical symptoms to help distinguish asymptomatic colonization from true CDI. Further studies are required to better ascertain the clinical specificity of this assay for the diagnosis of true CDI.

**Acknowledgments**

**Financial support.** This work was supported by the Fondation de l’Institut Universitaire de Cardiologie et Pneumologie de Quebec. The funder had no role in the study design, data collection and analysis, writing of the report, or the decision to submit for publication.

**Potential conflicts of interest.** Dr. Longtin reported receiving research grants from Merck, BD, and Gojo outside of this work. All other authors reported no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that

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**Patient consent.** The design of this work has been approved by the Institut Universitaire de Cardiologie et Pneumologie de Québec ethics committee with a waiver of patient consent.

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