SUPPLEMENT ARTICLE

Clinical Recognition and Diagnosis of *Clostridium difficile* Infection

John G. Bartlett[1] and Dale N. Gerding[2,3]

[1]Johns Hopkins University School of Medicine, Baltimore, Maryland; and [2]Hines Veterans Affairs Hospital, Hines, and [3]Loyola University Chicago Stritch School of Medicine, Chicago, Illinois

Prompt and precise diagnosis is an important aspect of effective management of *Clostridium difficile* infection (CDI). CDI causes 15%–25% of all cases of antibiotic-associated diarrhea, the severity of which ranges from mild diarrhea to fulminant pseudomembranous colitis. Several factors, especially advanced age and hospitalization, should be considered in the diagnosis of CDI. In particular, nosocomial diarrhea arising >72 hours after admission among patients receiving antibiotics is highly likely to have resulted from CDI. Testing of stool for the presence of *C. difficile* toxin confirms the diagnosis of CDI. However, performance of an enzyme immunoassay is the usual method by which CDI is confirmed, but this test appears to be relatively insensitive, compared with the cell cytotoxicity assay and stool culture for toxigenic *C. difficile* on selective medium. Endoscopy and computed tomography are less sensitive than stool toxin assays but may be useful when immediate results are important or other confounding conditions rank high in the differential diagnosis. Often overlooked aspects of this diagnosis are high white blood cell counts (which are sometimes in the leukemoid range) and hypoalbuminemia.

Infection with toxin-producing *Clostridium difficile* strains is a common cause of diarrhea. The severity of *C. difficile* infection (CDI) ranges from mild diarrhea to pseudomembranous colitis (PMC) and can result in death [1]. A total of 15%–25% of all cases of antibiotic-associated diarrhea (AAD) result from CDI. The likelihood that *C. difficile* is the cause of AAD increases with the severity of disease, reaching 95%–100% among patients with documented antibiotic-associated PMC [2]. Accurate diagnosis early in the disease course is important to the successful management of CDI.

RISK FACTORS FOR CDI

The major risk factors for colonic CDI are antibiotic exposure, hospitalization, and advanced age. Previous antibiotic use is the predominant risk factor for *C. difficile* acquisition, with relative risks (RRs) of 5.9 (95% CI, 4.0–8.5) for *C. difficile* diarrhea and 4.2 (95% CI, 3.1–5.9) for *C. difficile* carriage [3]. Some antibiotics, particularly clindamycin (RR, 9.0), cephalosporins (RRs range from 7.8 for cefaclor to 36.2 for cefotaxime), and β-lactams (RRs range from 2.0 for penicillin to 22.1 for ampicillin and amoxicillin–clavulanic acid), are associated with a relatively high risk of *C. difficile* acquisition [1, 3, 4]. Fluoroquinolones have been in use since 1988, but they have only recently been implicated as common causes of CDI [5–8]. Use of combination antibiotic therapy and long-term receipt of antibiotic therapy are also risk factors. However, CDI can occur even in patients exposed to short-term prophylactic antibiotic courses [4].

The prevalence of *C. difficile* spores in the environment is relatively high among hospitals and long-term care facilities [9]. Thus, it is not surprising that patients in these facilities have higher rates of *C. difficile* colonization (colonization rate, 10%–25% among hospitalized patients and 4%–20% among residents of long-term care facilities) than healthy adults in the general population (colonization rate, 2%–3%) [2, 10]. Resi-

Reprints or correspondence: Dr. John G. Bartlett, Johns Hopkins University School of Medicine, 1830 Monument St. #437, Baltimore, MD 21205 (jb@jhmi.edu).

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Table 1. Clinical differences between diarrhea due to *Clostridium difficile* infection (CDI) and antibiotic-associated diarrhea (AAD) due to other causes.

Characteristic	Diarrhea due to CDI	AAD due to other causes
Symptoms	Diarrhea; often evidence of colitis (i.e., cramps, fever, and fecal leukocytes)	Diarrhea, usually mild-to-moderate in severity; no evidence of colitis
CT or endoscopy findings	Often evidence of colitis; no evidence of ileitis	Usually normal
Results of stool toxin assay	Positive	Negative
Epidemiologic pattern	May be epidemic or endemic	Sporadic
Treatment		
Withdrawal of implicated antibiotic	May resolve but often persists or progresses	Usually resolves
Oral metronidazole or vancomycin therapy	Often associated with a prompt response	Not indicated

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dence in an intensive care unit, prolonged hospital stay, and, possibly, physical proximity to an infected individual have also been reported as risk factors for CDI [3, 11]. In addition, a variety of other factors affect the patient's vulnerability to CDI. Elderly patients are at noticeably higher risk, with disease rates for patients ⩾65 years of age as much as 20-fold higher than those for younger patients [7, 8, 12, 13]. Other factors that increase vulnerability include underlying disease severity, nonsurgical gastrointestinal procedures, and, possibly, the use of antiulcer medications [3]. Patients who have a suppressed immune system or a poor immune response to *C. difficile* toxins are also at increased risk [14].

The presence or absence of these risk factors, especially antibiotic use in conjunction with a recent hospital stay, should be considered in the differential diagnosis of diarrhea. In particular, patients with diarrhea arising >72 h after hospital admission who are receiving antibiotics are much more likely to have CDI than infection with an alternative enteric pathogen [15]. Because *C. difficile* may cause diarrhea in outpatients (albeit at a much lower rate than it causes diarrhea in inpatients), patients may have *C. difficile*–positive stool specimens on admission. Risk factors for CDI are discussed further elsewhere in this supplement [16].

CLINICAL PRESENTATION OF CDI

AAD has several possible causes. Etiologic infectious agents include *Staphylococcus aureus,* enterotoxin-producing strains of *Clostridium perfringens, Salmonella* species, and *Klebsiella oxytoca* [17]. These organisms are rare causes, however, and 70%–80% of AAD cases have no established microbial pathogen. Many cases are probably episodes of osmotic diarrhea resulting from the failure of the fecal flora to catabolize carbohydrates [18]. Most laboratories report that only 10%–25% of stool specimens submitted for *C. difficile* toxin testing have positive results, so most cases of AAD have another cause. However, *C. difficile* accounts for most of the cases characterized by colitis and nearly all cases that show PMC. A definitive diagnosis of CDI requires laboratory identification of *C. difficile* toxin in a stool sample and/or visualization of PMC, in addition to clinical symptoms (usually diarrhea) consistent with CDI.

Although laboratory confirmation of the presence of *C. difficile* toxin is usually required for a definitive diagnosis, several clinical factors can help focus the diagnosis and aid in differentiating between diarrhea due to CDI and AAD due to other causes (table 1) [4]. The most common clinical presentation of CDI is diarrhea associated with a history of antibiotic use. The onset of diarrhea is typically during or shortly after receipt of a course of antibiotic therapy but may occur from a few days after the initiation of antibiotic therapy to as long as 8 weeks after the termination of therapy [19]. For mild-to-moderate disease, diarrhea is usually the only symptom, with patients experiencing up to but usually considerably less than 10 bowel movements per day [20]. Stools are usually watery, with a characteristic foul odor, although mucoid or soft stools also occur. Gross blood in the stool is rare [19].

Other clinical features consistent with CDI include abdominal cramps, fever, leukocytosis, and hypoalbuminemia (table 1). Systemic symptoms are usually absent in mild disease but are common in moderate or severe disease [5]. Overall, fever occurs in ~28% of cases, leukocytosis in ~50%, and abdominal pain in ~22% [20]. Fever and leukocytosis may be severe in many patients, with temperatures occasionally reaching 40°C and WBC counts sometimes approaching 50,000 cells/mm^3 [19]. Abdominal pain, when it occurs, is usually localized in the lower quadrants. Hypoalbuminemia is the result of large protein losses attributable to leakage of albumin and may occur early in the course of disease [21].

Evidence of colitis includes fever, cramps, leukocytosis, presence of leukocytes in feces, and colonic inflammation visualized by endoscopy (for pseudomembranes) or CT (for colonic-wall

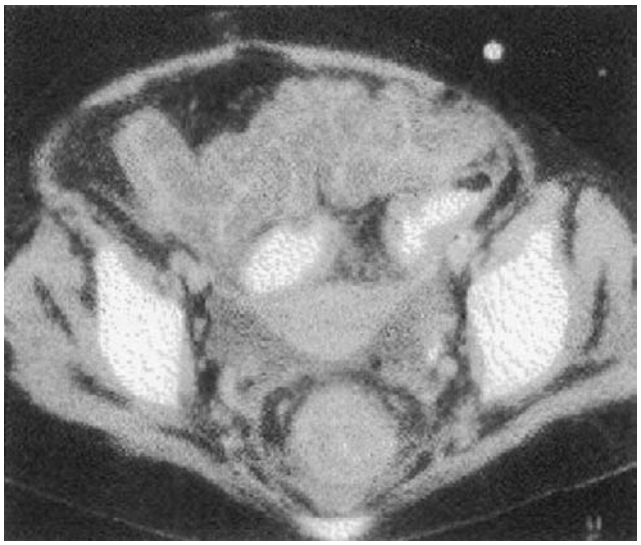

Figure 1. CT scan showing the "accordion sign." Note the thickened, low attenuation of the colon wall suggestive of submucosal edema. This finding is seen in patients with pseudomembranous colitis but is also observed in patients with other inflammatory conditions involving the colon. Reproduced with permission from the following article published by the Radiological Society of North America: Kawamoto S, Horton KM, Fishman EK. Pseudomembranous colitis: spectrum of imaging findings with clinical and pathologic correlation. Radiographics 1999; 19:887–97.

thickening) [5, 19]. Severe disease may cause paralytic ileus that can evolve into toxic megacolon, with nausea, vomiting, dehydration, lethargy, or tachycardia in addition to fever and abdominal pain [5, 19].

It should be noted that on rare occasions diarrhea may be absent in patients with severe CDI. This presentation occurs when the infection causes paralytic ileus, preventing the passage of stool. This is perhaps most common in postoperative patients who are receiving narcotics for pain. Therefore, symptoms such as otherwise unexplained fever, leukocytosis, and abdominal pain in a patient with recent antibiotic exposure should raise suspicion of CDI, even in the absence of diarrhea. Patients with advanced disease may have a leukemoid reaction involving WBC counts of >100,000 cells/mm^3, shock, and renal failure, or they may have severe hypoalbuminemia resulting in anasarca [22].

None of these clinical features are specific to CDI, and a variety of disorders may cause similar clinical presentations. These include diarrhea caused by other enteric pathogens, intra-abdominal sepsis, ischemic colitis, idiopathic inflammatory bowel disease, tube feeding, and/or use of medications, such as lactulose.

DIAGNOSIS OF CDI

Imaging studies. Imaging techniques, such as radiography, CT, and endoscopy, have largely been superseded by laboratory testing for *C. difficile,* because they are expensive, unpleasant to the patient, relatively insensitive, usually not specific, and unnecessary given the availability of a toxin assay [4]. Nevertheless, these procedures are often done for other reasons.

Detection of PMC by means of endoscopic visualization is diagnostic of CDI (although there are many other causes of PMC, they are exceedingly rare) [23]. Colonoscopy is the preferred procedure because PMC in up to one-third of patients will involve the right colon only and will consequently escape detection by sigmoidoscopy [24, 25]. PMC is often not present, making endoscopy relatively insensitive (51%). This procedure also risks perforation in cases of fulminant colitis [1, 23]. CT imaging can be valuable in the diagnosis of PMC or fulminant CDI. Characteristic features include colonic-wall thickening, pericolonic stranding, the "accordion sign," the "double-halo sign" (also known as the "target sign"), and ascites, which suggest hypoalbuminemia [23]. The accordion sign shows oral contrast material with high attenuation in the colonic lumen alternating with an inflamed mucosa with low attenuation (figure 1). The image is similar to an accordion and suggests PMC. The double-halo sign is seen with intravenous contrast material and shows varying degrees of attenuation attributable to mucosal hyperemia and submucosal inflammation (figure 2). There should be no small bowel involvement, because *C. difficile* is typically restricted to the colon. These findings are highly suggestive of advanced PMC [23]. In our practices, the presence of low-attenuation colonic-wall thickening, ascites, and the ac-

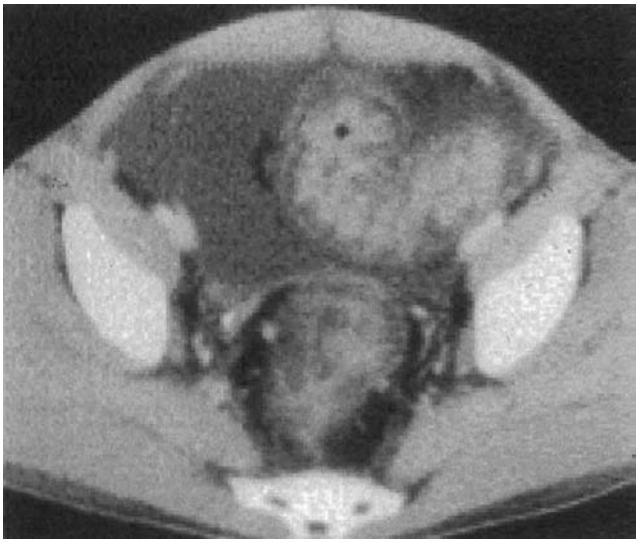

Figure 2. CT scan showing the "double-halo sign" suggestive of pseudomembranous colitis. Typical findings are 2 or 3 concentric rings with different attenuation, indicating submucosal hyperemia and submucosal edema. Reproduced with permission from the following article published by the Radiological Society of North America: Kawamoto S, Horton KM, Fishman EK. Pseudomembranous colitis: spectrum of imaging findings with clinical and pathologic correlation. Radiographics 1999; 19:887–97.

cordion sign or double-halo sign with no small bowel involvement justifies a probable diagnosis of *C. difficile*–associated colitis. Findings of plain radiography are usually normal in patients with CDI, unless they have ileus or toxic megacolon.

Laboratory testing. Laboratory analysis of stool samples is the standard diagnostic test for CDI. A variety of laboratory tests have been developed [26–34]. Laboratory testing for *C. difficile* is recommended for adults and for children ⩾1 year of age who have otherwise unexplained diarrhea associated with antibiotic use. The "3-day rule" states that a variety of community-acquired pathogens can cause diarrhea ⩽3 days after hospital admission, but after 3 days *C. difficile* is by far the most common enteric pathogen recovered [31]. Consequently, if stool samples are obtained after hospital day 3, the only enteric pathogen some laboratories will test for is *C. difficile* [15]. There are many exceptions to the 3-day rule [35].

Although no gold standard exists for diagnosis of CDI, the cell cytotoxicity assay is the best available test. It detects *C. difficile* toxins at picogram levels, it was the first test described [36], and it is still the most sensitive available test for detection of toxin B [37]. In one trial, the cell cytotoxicity assay had a sensitivity and specificity of 98% and 99%, respectively, compared with diagnosis on the basis of both clinical and laboratory criteria, but this assay was not compared with stool culture for toxigenic *C. difficile* on selective, prereduced, cycloserine-cefoxitin-fructose agar (hereafter, "stool culture") [38]. The major disadvantages of the cell cytotoxicity assay is that it is technically demanding and has a relatively long turnaround time (typically 24–48 h) [31, 33]. Because of the high sensitivity of the cell cytotoxicity assay, testing of additional samples provides little or no new information and is not usually warranted [39].

The cell cytotoxicity assay is not as sensitive as stool culture [40]. Current EIAs for detection of toxin A only or both toxins A and B in stool are relatively insensitive, missing ~40% of diagnoses, compared with stool culture [26] and the cell cytotoxicity assay [41]. Even the widely used EIAs for toxin A and B are only 70%–80% as sensitive as the cell cytotoxicity assay, which in turn is much less sensitive than stool culture [26, 38]. Stool culture is seldom used for routine diagnosis in the United States because test turnaround takes 24–48 h and because it is not specific for in vivo production of toxins. However, because culture permits molecular typing of the organisms, it is essential for monitoring molecular epidemiology and antibiotic susceptibility [37].

Although stool culture has high sensitivity, the specificity for CDI is low, because the rate of asymptomatic carriage of *C. difficile* among hospitalized patients is so high. To increase the specificity, culture broth can be further evaluated by means of a cell cytotoxicity assay or EIA [31]. A positive result of this so-called toxigenic culture indicates the presence of a toxin-producing *C. difficile* strain in stool, which, in the presence of diarrhea, is considered to be evidence of CDI. Because toxin is much more labile in stool than in *C. difficile* spores, a toxin test that does not detect *C. difficile* toxin in stool recovered from a person with diarrhea is presumed to have yielded a false-negative result. Although an alternative interpretation of this result is that the patient was colonized asymptomatically with a toxigenic strain of *C. difficile* and had diarrhea from an unrelated cause, most clinicians would opt to treat such a patient for CDI rather than conclude that the diarrhea was not associated with *C. difficile*.

Several rapid commercial EIAs give results within hours rather than days but have a lower sensitivity than a stool culture or cell cytotoxicity assay. The common-antigen test (also known as the glutamate dehydrogenase [GDH] test) is an EIA for the GDH enzyme. *C. difficile* constitutively produces GDH in easily detectable levels, so tests based on GDH detection have good sensitivity, reaching 96%–100% in a recent study [33]. This is equivalent to a positive culture result, because it only indicates the presence of the organism, rather than in vivo production of *C. difficile* toxin. In addition, other organisms occasionally produce GDH. The test is rapid, with a turnaround time of 15–45 min, and relatively inexpensive, costing approximately $8 [33, 37]. It is optimally used as a relatively sensitive screening test to detect GDH-positive stool specimens that require further testing by cell cytotoxicity assay, EIA for toxins, or toxigenic culture.

Commercially available tests for *C. difficile* toxins include ELISAs for toxins A or toxins A and B and immunochromatography for toxin A. The toxin A ELISA and immunochromatography assays detect toxin A exclusively and therefore miss the ordinarily small but clinically important fraction of *C. difficile* strains that express only toxin B (i.e., A^-B^+ strains) [41]. However, because such strains have caused hospital-based epidemics, the inability to detect them can result in misdiagnosis and failure to detect outbreaks. Rapid turnaround time (~2 h for ELISAs and <1 h for immunochromatography) with high specificity is the primary advantage of these methods [5]. Performing EIAs on 2 or 3 specimens rather than on 1 specimen not only increases the diagnostic yield by 5%–10% [42] but also increases the cost, because each assay costs approximately $40 (which includes the price of reagents, technician salaries, and overhead) [4]. Detection limits for these methods range from 100 to 1000 pg of toxin [43, 44]. One study reported a detection limit of 10,000 pg [27]. These limits are much higher than the lower limit of detection for the cell cytotoxicity assay (<10 pg) and presumably account for the relatively poor sensitivity of EIAs. The reported sensitivities vary over a wide range in different reports: early studies reported sensitivities of 85%–95%, whereas recent evaluations have reported sensitivities ranging from 33.3% to 59.4% for toxin A and as low as 38% for toxins A and B [27, 28, 31, 33, 38, 44, 45].

At present, no single commercially available test offers good sensitivity and specificity in combination with a rapid turnaround time and low cost. In the United States, >90% of laboratories use EIA because it is inexpensive, fast, and technically easy to perform. Laboratories in Australia and many European countries use culture to screen for *C. difficile,* followed by cell cytotoxicity assay or EIA to detect toxin [46]. The Triage *C. difficile* Panel (Biosite) performs simultaneous EIAs for GDH and toxin A and has a reported sensitivity and specificity of 59.4% and 89.7%, respectively [45]. The 2-step protocol uses the common-antigen assay as a screening test to exclude *C. difficile* in the 75%–90% of stool specimens that do not contain *C. difficile.* Specimens that are GDH positive are further analyzed by a cell cytotoxicity assay to improve the specificity of the nonspecific GDH test result [33]. In the 2-step approach, the test for GDH determines whether *C. difficile* is absent or likely present; if the latter, the cell cytotoxicity assay is performed for confirmation [27, 30, 33]. This approach reduces costs by decreasing the number of cell cytotoxicity assays required while maintaining good sensitivity and specificity. The disadvantage is the delay in obtaining test results and the increase in technical expertise required for the cell cytotoxicity assay. The speed and sensitivity of the 2-step approach could be increased by doing an EIA for toxins A and B on GDH-positive stool specimens (rapid turnaround but not highly sensitive) and performing stool culture (slow turnaround but highly sensitive). This modified approach is also useful because *C. difficile* isolates from each positive stool specimen can be recovered for molecular typing and susceptibility testing. A controlled comparison of the modified 2-step method with alternative testing methods is needed to determine its sensitivity, specificity, relative diagnostic rapidity, and relative cost.

The limitations of the available *C. difficile* testing methods can have a considerable impact on treatment. Early initiation of therapy may be critical, because some tests have long turnaround times and others have poor sensitivity. Untreated patients with negative results of stool tests but clinical presentations compatible with CDI are subject to the "tyranny of the test result" if physicians believe the false-negative results and fail to initiate treatment [47]. The Society for Healthcare Epidemiology of America recommends initiating empirical therapy for *C. difficile* immediately after specimen procurement for patients with severe symptoms consistent with CDI [1]. Given the potentially increased virulence of the epidemic BI/NAP1 strain (restriction-endonuclease analysis group BI/North American PFGE type 1), early treatment may be critical to the outcome of the patient and may potentially reduce spread of the organism by stopping diarrhea sooner.

CONCLUSIONS AND RECOMMENDATIONS

CDI should be suspected in patients with otherwise unexplained diarrhea who received antibiotics ⩽1 week (but sometimes up to 2 months) before onset. Diarrhea is the primary clinical symptom of CDI. Many patients with CDI have clinical features of colitis, including fever, leukocytosis, and cramps. The severity of abdominal and systemic symptoms increases with the severity of CDI. Often forgotten clues to this diagnosis are high WBC counts (which are sometimes in the leukemoid range) and hypoalbuminemia. Because diarrhea may be absent in patients with severe CDI resulting from paralytic ileus, other signs and symptoms of CDI (especially unexplained leukocytosis) should cause suspicion of CDI, even in the absence of diarrhea. Testing of stool for the presence of *C. difficile* toxin is used most often to confirm the diagnosis of CDI; however, these assays are relatively insensitive, compared with stool culture. Endoscopy and CT imaging are less sensitive than stool toxin tests but may be useful when immediate results are needed or other conditions rank high in the differential diagnosis.

Laboratory testing is recommended for patients ⩾1 year of age who have symptoms consistent with CDI and a recent history of antibiotic use. The type of test to be used depends on what is available in a given laboratory, which is often determined by turnaround time, the required technical skill, and cost. Most laboratories use the EIA to detect toxin A or toxins A and B. Although the EIA has good specificity, it is only 70%–80% sensitive, requiring repeat testing, use of alternative tests, or initiation of empirical treatment for some patients. If an EIA is used, the assays for toxins A and B are preferred, because some cases of CDI involve strains that produce only toxin B. The cell cytotoxicity assay is the most sensitive and specific stool toxin assay for detection of *C. difficile,* but it has a 24–48-h turnaround time, is more expensive than the EIA, and is technically demanding. Stool culture is the most sensitive method but requires 48 h and demonstration that the *C. difficile* isolate is toxigenic. The common-antigen assay lacks specificity but is sensitive and rapid and can be done as a screening test for a subsequent cell cytotoxicity assay. Recent reports indicate that this 2-step method has reasonably good sensitivity, specificity, and cost, although there is a 24–48-h delay in results [33]. Stool culture for *C. difficile* has also been recommended as the second step of the 2-step method, because of its high sensitivity and potential use in molecular typing for epidemiologic correlations [48]. Given these issues, it may be necessary to treat seriously ill patients empirically for CDI if they have clinical findings compatible with this diagnosis and if the test method is either insensitive (as with the EIA) or has a long turnaround time (as with the cell cytotoxicity assay or culture).

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