EDITORIAL

Clostridium difficile infection and inflammatory bowel disease: Understanding the evolving relationship

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Abstract

Clostridium difficile (*C. difficile*) infection (CDI) is the leading identifiable cause of antibiotic-associated diarrhea. While there is an alarming trend of increasing incidence and severity of CDI in the United States and Europe, superimposed CDI in patients with inflammatory bowel disease (IBD) has drawn considerable attention in the gastrointestinal community. The majority of IBD patients appear to contract CDI as outpatients. *C. difficile* affects disease course of IBD in several ways, including triggering disease flares, sustaining activity, and in some cases, acting as an "innocent" bystander. Despite its wide spectrum of presentations, CDI has been reported to be associated with a longer duration of hospitalization and a higher mortality in IBD patients. IBD patients with restorative proctocolectomy or with diverting ileostomy are not immune to CDI of the small bowel or ileal pouch. Whether immunomodulator or corticosteroid therapy for IBD should be continued in patients with superimposed CDI is controversial. It appears that more adverse outcomes was observed among patients treated by a combination of immunomodulators and antibiotics than those treated by antibiotics alone. The use of biologic agents does not appear to increase the risk of acquisition of CDI. For CDI in the setting of underlying IBD, vancomycin appears to be more efficacious than metronidazole. Randomized controlled trials are required to clearly define the appropriate management for CDI in patients with IBD.

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INTRODUCTION

Clostridium difficile (*C. difficile*) infection (CDI) is the leading identifiable etiology for antibiotic-associated diarrhea and is associated with substantial morbidity and mortality. Since the initial report of this bacterium as a cause of antibiotic-associated pseudomembranous colitis in 1978[1], the incidence of CDI has increased over the years. Although knowledge in epidemiology, pathogenesis, risk factors, diagnosis and management of CDI has tremendously increased, the frequency and severity of CDI continue to increase at an alarming rate[2-4]. A hypervirulent strain of *C. difficile*, BI/NAP1/027 was reported from North America

and Europe which was associated with a more severe and complicated disease and a higher mortality[3,4]. In addition to its effect on morbidity and mortality, CDI is also associated with increasing duration of hospitalization and costs. The expected health care costs due to CDI alone are estimated as being up to 3.2 billion dollars per year in the US[5]. Clearly the impact of CDI on the health care system continues to grow with emergence of community-acquired CDI[6,7].

Inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory conditions. IBD patients frequently require corticosteroids, antibiotics (in CD), immunomodulators, and biological therapy. Some of these agents can increase the risk of acquisition of CDI. In a large population-based cohort study, the use of biologic agents does not appear to increase the risk for CDI[8]. Recently published single-center studies and national inpatient database studies reported rising rates of CDI among IBD patients and their contributions to an increased rate of hospitalizations and mortality[9-12]. The risk of CDI in IBD patients appears to persist even after colectomy. CDI can involve the small bowel[13]. CDI has also been reported in UC patients with restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA)[14,15] .However, the exact pathogenic role of *C. difficile* in these clinical settings are unclear. *C. difficile* may cause an isolated infectious colitis superimposed on IBD, or in some patients, may precipitate an IBD flare leading to two separate but simultaneous inflammatory processes. The other possibility is that *C. difficile* may be just a colonizer and that IBD flare probably occurs independently. When patients with IBD develop worsening symptoms and *C. difficile* is isolated in their stool, there are no clear guidelines to suggest whether to withhold or continue IBD-related medications, including antibiotics, corticosteroids, immunosuppressants, or biologics, while instituting appropriate therapy for CDI. In a retrospective study, patients treated by combination therapy of antibiotics and immunomodulators had a trend towards increased mortality when compared with those treated by antibiotics alone[16]. Lastly, there is no consensus on which antibiotic regimen should be considered as the first-line agent for the treatment of CDI complicating IBD.

Given the uncertainty in the pathogenesis and controversy on management of patients with concurrent CDI and IBD, we embarked on this project to clarify some issues on evolving CDI in IBD. The main goals of this article are to provide information on the pathogenesis and impact of CDI on disease course of IBD, to discuss diagnosis and treatment modalities of CDI in IBD, and to compare the clinical, laboratory, macroscopic and microscopic features between isolated CDI and superimposed CDI on IBD.

REVIEW CRITERIA

In February 2010, we searched MEDLINE from 1970 to the present using the Medical Subject Headings terms "Clostridium difficile, inflammatory bowel disease, Ul-

cerative colitis, Crohn's disease, Clostridium difficile and inflammatory bowel disease, Clostridium difficile and diagnosis, Clostridium difficile and treatment". Full papers and abstracts without language restrictions were considered. Important developments in research, reports from centers of excellence, and our own research developments form the basis of this article.

EPIDEMIOLOGY

CDI occurs predominantly in hospitalized patients and the incidence is increasing across the US with 3 million new cases of CDI occurring each year and as many as 10% of patients being affected within 2 d of hospitalization[17]. The prevalence of carrier state of the bacterium ranges from 0% to 3% in healthy individuals to 20% in hospitalized patients[17]. Interestingly, only one third of all infected patients developed diarrhea, while the remaining two thirds were asymptomatic carriers. Antibiotic exposure is the major risk factor for CDI.

The Center for Disease Control (CDC) reports of community acquired *C. difficile* colitis in the US has made the picture more concerning[6,7]. The traditional risk factors, such as recent hospitalization, being elderly, or having an underlying health condition were often absent. Close to 25% of patients who developed community acquired *C. difficile* colitis were young, healthy patients with no recent hospitalization in the past year.

Recent papers have highlighted a hypervirulent form of *C. difficile* strain, BI/NAP1/027 that was shown to be associated with a more severe and complicated disease course and a higher mortality. This strain appears to spread across the US. In a recent CDC report with regard to the BI/NAP1/027 strain of *C. difficile*, 38 states were reported to have the hypervirulent strain of the bacterium in their population[6,7]. This particular strain of *C. difficile*, toxinotype III, North American PFGE type 1, and PCR ribotype 027 (NAP1/027) carries the binary toxin gene cdtB (cytolethal distending toxin B gene) and an 18-base pair deletion in tcdC; it produces 16-23 times more toxin A and B than the routine strain[3,5]. In addition, this hypervirulent strain was reported to be associated with increased disease severity[18] and possibly transmissibility and to cause outbreaks in Europe and the US[3,4]. The increasing use of fluoroquinolones may be one of the reasons for selecting the hypervirulent BI/NAP1/027 *C. difficile* strain since it is resistant to this class of antibiotics and possibly less responsive to other antibiotics.

PATHOGENESIS OF *C. DIFFICILE*-INDUCED DIARRHEA

Pathogenic strains of *C. difficile* produce two potent toxins, toxin A, an enterotoxin, and toxin B, a cytotoxin. The genes encoding toxin A and B are encoded in the *C. difficile* pathogenicity locus (*tcdA* and *tcdB*) which also encode two additional regulatory genes (*tcdC* and *tcdD*)[19]. The *tcdD* gene product up-regulates toxin transcription, while *tcdC* prob-

ably encodes a toxin gene repressor[19]. The fifth gene of the pathogenicity locus, *tcdE* is postulated to release both toxins A and B into the colonic lumen by lysing the cell walls[20]. Both toxins A and B have a 49% amino acid homology and possess a N-terminal domain that possesses cytotoxic activity, a transmembrane domain that facilitates toxin entry into the cytoplasm and a C-terminal domain that favors toxin binding to the epithelial cells[19]. Both toxins A and B are UDP-glucose hydrolases and glucosyltransferases and contribute to infectious and inflammatory diarrhea; however toxin B may be the major inflammatory toxin[21]. The toxins initially attach to non-proteinaceous disaccharide Gal beta 1-4GlcNac residues in the colon. Both toxins play a role in the initial binding to the colonic epithelial cells. After adhesion, the toxin enters the cell through receptor-mediated endocytosis and catalyzes the transfer of a glucose residue from UDP-glucose to guanosine triphosphate-binding rho proteins[19], the intracellular signaling molecules regulating cytoskeletal organization and gene expression. Glucosylation of rho proteins in turn leads to disruption of protein synthesis, and cell death. This leads to the inflammatory diarrhea seen in patients with CDI[22].

INFLAMMATORY BOWEL DISEASE AND CDI

Almost three decades before, LaMont *et al*[23] postulated that *C. difficile* toxin complicates chronic IBD and contribute to relapse in some patients. Since then, isolated case series of CDI contributing to symptomatic relapse in patients with IBD have been reported[24-27].

Incidence and prevalence

Paralleling the rising burden of CDI in the general population, recent years have witnessed a dramatic increase in CDI in patients with IBD. Recently, two single-center studies and two national inpatient database studies have reported a rising rate of CDI among IBD patients and their contributions of increased rates of hospitalization and mortality[9-12]. In a retrospective study of all confirmed CDI patients from a tertiary care center over a 7 years period, there was a doubling in the CDI rate in CD patients (9.5 to 22.3/1000 admissions) and tripling in UC patients (18.4 to 57.6/1000 admissions)[9]. A similar increase in the rate of CDI in IBD patients from 1.8% in 2004 to 4.6% in 2005 was observed in a subsequent study from a different tertiary-care center[10]. Furthermore, both studies identified that IBD patients, in particular those with UC, were at a disproportionately higher risk for acquiring CDI than non-IBD patients. In a large study utilizing the Healthcare Cost and Utilization Project Nationwide Inpatient Sample inpatient care database in the US, hospitalized patients with concurrent CDI and IBD had a 4 times greater mortality than those admitted to hospital for IBD or CDI alone[11]. In a subsequent study utilizing the same National Inpatient Sample database to study the temporal pattern of CDI, the prevalence of CDI among UC patients (37.3 per 1000) was higher than that among CD patients (10.9

per 1000), non-IBD gastrointestinal (GI) patients (4.8 per 1000), and general medicine patients (4.5 per 1000). In addition the incidence of CDI among UC patients almost doubled (26.6 per 1000 to 51.2 per 1000) over the 7-year period. CDI was independently associated with a greater mortality among patients with UC, but not CD[12].

Superimposed infections of pathogenic bacteria or viruses may contribute to exacerbation of IBD. Concurrent CDI is one of them. In a Scandinavian study in 1983, only 5% of patients admitted for a flare had CDI which would make routine screening not cost-effective[28]. However recent studies reported that approximately 5%-19% of newly-admitted patients for relapsing IBD tested positive for *C. difficile* toxins[29,30]. Similar to the adult population, pediatric IBD patients also seem to be susceptible to CDI as a recent Italian study identified *C. difficile* toxins in 24.7% of patients with diarrhea or abdominal pain[31]. *C. difficile* carriage status was studied with stool culture and molecular microbiological methods in IBD patients in clinical remission with no recent hospitalization or antibiotic exposure[32]. Toxigenic *C. difficile* was demonstrated more frequently in IBD patients (8.2%) than in healthy volunteers (1.0%). However, none of these patients developed CDI after a 6 mo follow-up and all the ribotypes identified were community-acquired, highlighting the acquisition of *C. difficile* in IBD patients even in remission from a wide variety of community sources[32]. The clinical relevance and significance of this community-acquired *C. difficile* carriage is interesting and its effect on the outcome of IBD has not been studied.

Risk factors

Environmental exposure continues to be the most common route of acquisition of CDI. Recent hospitalization increases the risk for nosocomial acquisition of CDI, the most common setting for the infection. Antibiotic-resistant *C. difficile* spores survive in hospital environment and can be isolated on toilets, bedrails, floors, telephones, call buttons, stethoscopes, and the hands of healthcare workers[19-21]. Sharing a room with an infected patient also increases the risk of infection[19].

Interestingly in a majority of IBD patients, CDI seems to often be contracted outside of the hospital. In a recent study, the median time to development of CDI in non-IBD patients was 4 d in contrast to less than a day with CDI in IBD patients[9]. In another study, 76%-79% of patients acquired CDI from the community[10]. Toxigenic *C. difficile* was demonstrated more frequently in IBD patients in complete remission with no recent hospitalization or antibiotic exposure (8.2%) than in healthy volunteers (1.0%) and the ribotypes identified were community-acquired, highlighting the acquisition of *C. difficile* in IBD patients[32]. In a case-control study from our institution, we also observed that 47.2% of patients had acquired the infection outside the hospital[33].

Almost any antibiotic has been associated with the development of CDI. The risk of CDI varies depending on the type of antibiotic, frequency, duration, route of antibiotic use, and the use of concurrent medications[34-37]. How-

ever, even a short-term use of prophylactic antibiotics can cause CDI[38]. The most common implicated antibiotics associated with CDI till recently were ampicillin, amoxicillin, cephalosporins, and clindamycin[34-37]. However, with widespread use, fluoroquinolones have become one of the common predisposing factors for CDI[38-41]. The exact mechanism of antibiotic-associated CDI in IBD is unclear. In addition, frequency of antibiotic exposure in relation to CDI risk in IBD has been highly variable. Antibiotic use prior to 3 mo before the development of CDI was seen in only 40% of IBD patients compared with 69% in the non-IBD population in a study[42]. A separate study showed that 61% of IBD patients with antibiotic exposure developed *C. difficile*[10]. In a cohort study, 57.2% of patients acquiring a CDI received antibiotics in the previous 6 mo[8]. In our recent study, antibiotic exposure within 30 d prior to *C. difficile* testing was found to be associated with an increased risk for CDI with odds ratio of 12.0 [95% confidence interval (95% CI): 1.2-124.2][33].

Immunosuppression is also proposed as another risk factor for the development of *C. difficile* infection. Cancer chemotherapy, particularly methotrexate[43] or patients with organ transplantation on immunosuppression appear to be at risk. The role of immunomodulators in the development of CDI is controversial. Previously studies have reported the association of immunomodulators with CDI in IBD[9,10]. However, in the studies from our institution and others suggest that immunosuppressive treatment was not associated with the risk of CDI[33,44,45].

The relationship of the use of corticosteroids to the risk of CDI has been studied in IBD patients. In a large cohort study of IBD patients, corticosteroid use with or without simultaneous use of other immunomodulating drugs was associated with a 3-fold increase in the risk of CDI (relative risk = 3.38, 95% CI: 1.88-6.10)[8]. Even in the absence of other immunomodulating drugs, the risk increased 2.5 fold in patients using corticosteroids[8]. Corticosteroids have also been shown to increase the risk of CDI relapse in solid organ transplant patients[44]; however corticosteroids and their risk of association to CDI have not been studied in IBD patients.

The use of biologics, specifically infliximab to the risk of CDI was studied in IBD patients and there appears to be no association of the use of biologics on the risk of development of CDI[8].

The normal bacterial flora in the bowel is an important natural defense and inhibits the growth of *C. difficile*[46]. In addition, the gastric acid barrier is a host mechanism to protect against ingested microorganisms[47]. The use of proton pump inhibitors and the risk for CDI is a subject of controversy, as published results of studies have been conflicting. Some initial studies demonstrated a higher risk of development of CDI with proton pump inhibitor therapy but this finding has not been consistently demonstrated[42,48].

Similar to the non-IBD populations, increasing age has been proposed as a risk factor for CDI in the IBD population[49-51]. IBD patients with CDI, however, were younger than the corresponding non-IBD population who developed CDI. In addition to immunosuppressive medication, host immunity, particularly the humoral arm, may play a role in determining susceptibility to CDI[52]. Thus serum and intestinal secretory antitoxin antibodies may afford protection and may be associated with mild colitis or carriage, while patients with deficient response develop severe or recurrent CDI[52]. Recurrent CDI is also suggested to be because of alterations in the fecal microbiota with markedly decreased diversity as demonstrated by phylogenetic analysis of 16S rRNA-encoding gene sequences[53].

IBD itself has been shown to be a specific risk factor for the development of CDI, particularly in those with colonic involvement[9,11,54]. Patients with UC appear to be at a higher risk for the development of CDI than CD and the presence of colonic disease conferred 3-fold greater risk (odds ratio = 3.12, 95% CI: 1.28-5.12) for CDI[11]. Similarly CD patients with colonic involvement seem to be at a greater risk for CDI than those with isolated small bowel disease[11]. The risk of CDI in relation to disease activity is unclear. A recent study suggested that patients with a greater disease activity may be at a higher risk for CDI[31]. However, in the population based study by Nguyen *et al*[12], the inverse association between CDI and colectomy rate led to the suggestion that IBD patients with CDI have lesser disease activity, although there was no information on the disease activity. Similarly, in our recent study we did not find any difference in the endoscopic disease activity in UC patients with and without CDI[33].

OUTCOME AFTER TREATMENT AND NATURAL HISTORY OF CDI

Short-term outcome
Patients with CDI are at risk for complications including toxic megacolon, colonic perforation, and peritonitis with sepsis. Patients with IBD are similarly at risk for these complications. Single-center and nationwide studies have studied the outcome of CDI in patients with IBD. The results have been highly variable with some studies reporting shorter stay in patients with CDI in IBD than those in non-IBD patients[42], and some studies showing similar lengths of stay[55], while other studies highlighting increased hospitalization duration and costs[10].

The colectomy rate in CDI is an important measurement of short-term outcome in CDI. Colectomy has been shown to be independently associated with a greater than 2-fold increase in inpatient mortality (incidence rate ratio = 2.4, 95% CI: 1.8-3.2)[56]. However studies have reported varying rates of colectomy for CDI in the setting of IBD. In a large study utilizing the Health Care inpatient care database, the development of CDI was inversely related to the risk of colectomy[11]. Similarly a subsequent study reported low rates for colectomy after CDI (1 of 15 patients)[42]. In our recent study with colectomy at 3 mo following CDI infection being the end point, we did not find CDI as a risk factor[33]. In a single-center case-control study, the rate of emergent colectomy in their CDI-UC population was 23% with the indication being toxic complications (4 of 11) or
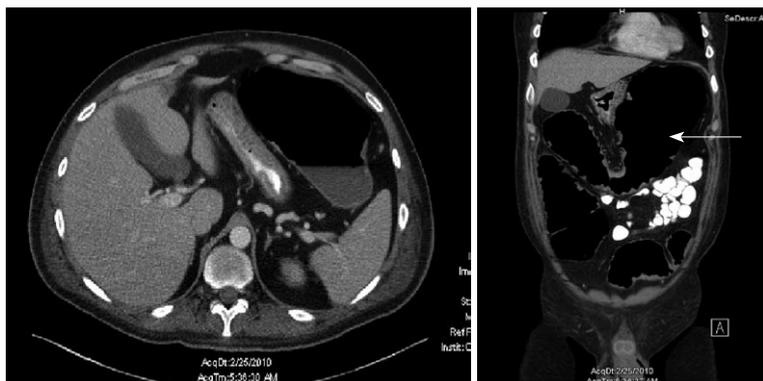
Figure 1 Toxic megacolon in a 27-year-old patient with *Clostridium difficile* infection who had underlying ulcerative colitis, resulting in emergent subtotal colectomy. Arrow indicate dilated colon.

medically refractory disease (7 of 11) compared to 13.4% in the *C. difficile*-negative IBD population[55]. However, it is interesting that 7/11 patients had medically refractory disease and may be *C. difficile* was a colonizer. Also there was no statistically significant difference in the short-term risk of colectomy at 1 mo[55]. In another study, underlying IBD was associated with 6 fold greater risk of bowel surgery compared with patients with CDI without underlying IBD[10]. We believe that the lower risk of colectomy with UC-CDI in most studies may be due to the fact that patients with UC exacerbation resulting from CDI are much more likely to improve with proper pathogen-directed medical therapy. Therefore, treating CDI in UC patients may actually prevent the need for colectomy in the short term (Figure 1).

Long-term outcome

There are limited studies available investigating the long-term outcome of CDI in patients with IBD. In a recent retrospective case control study, UC-CDI patients had worse clinical outcome than UC patients without CDI, with a follow-up of up to a year after CDI[55]. On the other hand, the study did not discriminate between recurrent CDI *vs* worse IBD disease activity because of the retrospective nature and study design. However, *C. difficile*-positive patients had significantly more UC-related hospitalizations (58 hospitalizations *vs* 27 hospitalizations) and emergency room visits in the year following initial admission (8 visits *vs* 1 visit). Also, up to a year following the index admission, patients with CDI had significantly higher rates of colectomy compared to *C. difficile*-negative patients (44.6% *vs* 25%). In a case-control study comparing the disease course for 1 year before and 1 year after the initial infection in 87 patients with IBD with *C. difficile*, colectomy occurred in only 10.3% of patients (9/87) following CDI[57]. While 8% had fewer hospitalizations in the year following infection, 41.3% of patients (36/87) followed for a year after CDI had no difference in the number of hospitalizations. However, 46% of patients (40/87) had more hospitalizations in the year following CDI (range 1-9 hospitalizations)[57]. Also 53% (46/87) of IBD patients with CDI required an escalation in their IBD medical therapy including initiation of biologic therapy (26%; 23/87), dose escalation of current biologic (8%; 7/87), escalation or initiation of azathioprine/6-MP (11.5%; 10/87) or methotrexate (7%; 6/87)[57]. Both these studies are limited by their retrospective nature and it is

Table 1 Short and long-term outcomes with *Clostridium difficile* infection and inflammatory bowel disease

Short-term outcomes
  Toxic megacolon
  Colonic perforation
  Peritonitis with sepsis
  ? Increased hospitalization duration and costs
  Colectomy rates highly variable
Long-term outcomes
  Increased UC related hospitalization and emergency room visits
  ? Escalation of medical treatment
  Increased rate of colectomy

UC: Ulcerative colitis.

unclear whether underlying IBD severity was responsible for this outcome or whether *C. difficile* produces certain immunological changes that leads to a worse long term clinical outcome. Table 1 summarized both the short and long term outcome of CDI in IBD.

CLINICAL, RADIOGRAPHIC, ENDOSOPIC, AND HISTOLOGIC FEATURES

Patients with CDI can present with a wide variety of clinical manifestations ranging from an asymptomatic carrier state to fulminant colitis with megacolon. The most common clinical presentation of CDI is diarrhea and abdominal pain. The diarrhea is usually watery in patients with CDI; however in patients with underlying IBD, it may be bloody or mucous[49,58]. There are associated systemic symptoms and low-grade fever with a polymorphonuclear leukocytosis.

Although 0% and 3% of healthy adults may carry *C. difficile*, the frequency of asymptomatic carriage of *C. difficile* in patients with IBD is not exactly known. *C. difficile* carriage status in 122 IBD patients in clinical remission in the outpatient setting with no recent hospitalization or antibiotic exposure was studied with stool culture and molecular DNA-based microbiological methods. The strains were characterized by toxin typing, ribotyping, and pulsed-field gel electrophoresis. Toxigenic *C. difficile* was demonstrated more frequently in IBD patients (8.2%) than in healthy volunteers (1.0%). However, none of these patients developed CDI after a 6 mo follow-up and all the ribotypes identified were community acquired highlighting

the acquisition of *C. difficile* in IBD patients even in remission from a wide variety of community sources[32].

Some patients may present with severe disease causing paralytic ileus, which may evolve into toxic megacolon characterized by a dilated colon (> 7 cm in its greatest diameter), and signs and symptoms of severe toxicity (fever, chills, dehydration, high white count). There is associated dilatation of the small intestine in patients with megacolon mimicking an intestinal obstruction. Bowel perforation may also occur[59-61]. Diarrhea may be absent because of paralytic ileus, particularly in postoperative patients who receive narcotics for pain control. Patients may also have anasarca due to severe hypoalbuminemia[62]. Patients may present without diarrhea but only with abdominal pain, fever and leukocytosis (a leukemoid reaction with a white blood cell count up to 100 000 cells/cu.mm.)[62]. A high degree of suspicion is required to diagnose CDI in these settings.

Abdominal imaging

Plain radiography is usually normal in patients with CDI, unless they have complications like ileus or toxic megacolon or perforation. CT imaging is useful in the diagnosis of severe or fulminant CDI and the characteristic features include colonic-wall thickening, pericolonic stranding, the "accordion sign", and the "double-halo sign"[63]. The accordion sign is seen with oral contrast and shows the high attenuation in the colonic lumen alternating with a low attenuation inflamed mucosa, while the double-halo sign is seen with intravenous contrast[63]. The presence of these signs in the right clinical setting may suggest a diagnosis of CDI.

Endoscopy

Lower endoscopic visualization forms an important part in the evaluation of patients with CDI in IBD. Isolated CDI produces the classic endoscopic appearance of pseudomembrane formation which is described in 50% of patients[64,65]. However in patients with underlying IBD, classic endoscopic or histologic features of pseudomembranes are conspicuously absent, making it hard to diagnose CDI in patients with worsening diarrhea[10,42]. In fact, recently published studies from Milwaukee and Belgium did not identify pseudomembranes in any of the IBD patients with CDI who underwent endoscopic evaluation[10,42]. However endoscopy may be useful to assess disease activity of IBD and also to rule out other secondary causes of diarrhea including concurrent cytomegalovirus infection[66].

Histology

The classic histologic picture in CDI is the presence of pseudomembranes. Pseudomembrane formation is caused by sloughing and necrosis of the mucosa with ulceration secondary to inflammation. Pseudomembranes are actually characteristic "volcano" lesions with focal ulceration with inflammation composed of polymorphonuclear leukocytes, fibrin, chronic inflammatory cells, and epithelial debris[66]. In patients with IBD, pseudomembranes are not commonly present, CDI tends to produce a nonspecific mucopus; erythema and friability are commonly encountered endoscopic findings[10].

LABORATORY DIAGNOSIS

Although a variety of laboratory tests are used for the diagnosis of CDI, enzyme linked immunoassay (ELISA) is the most commonly used test to detect the toxin.

Enzyme linked immunoassay

These assays are based on the detection of toxins A and/or B using either a monoclonal antibody or a polyclonal antiserum that recognizes the specific toxin. The ELISA test is inexpensive and the results are available within 2-6 h. The most widely used ELISAs for detection of both toxins A and B in stool are somewhat less sensitive (70%-90%) than the cell cytotoxicity assay (see below). Up to 30% of tests may be falsely negative in comparison to the cell cytotoxicity assay or culture[67,68]. They do, however, demonstrate excellent specificity (99%)[68,69]. The lower sensitivity of these tests can be improved by performing ELISAs on 2 or 3 specimens rather than on 1 specimen, which increases the diagnostic yield by 5%-10%[70]. In IBD patients, the diagnostic yield of ELISA testing may be much lower. Four sequential stool samples were shown to increase the diagnostic yield to 92%[42].

Latex agglutination assay

Latex agglutination assay is based on the glutamate dehydrogenase (GDH) enzyme produced by *C. difficile*. The sensitivity of these tests approached almost 96%-100% in a recent study[71]. However, certain other organisms can also produce GDH and also the positivity indicates only the presence of the organism, rather than *in vivo* production of *C. difficile* toxins. It is not recommended for routine clinical use.

Cell cytotoxicity assay

Cell cytotoxicity assay is the gold standard test for diagnosis of CDI. It detects as little as 10 picograms of toxin and it is the most sensitive available test for detection of toxin B[72-75]. It is based on the principle that the toxins in the stool exert a cytopathic effect characterized by cell rounding which can be demonstrated in tissue culture. The high sensitivity (94%-100%) and specificity (99%) of the cytotoxicity assay is its major advantage. Disadvantages are its relatively high technical expertise and the 24-48 h needed to complete the assay[76].

C. difficile culture

Stool culture is seldom used for routine diagnosis because of labor intensiveness, long turnaround time (24-48 h) and a low specificity. The *in vivo* production of toxins can be seen in hospitalized patients who are asymptomatic carriers. It fails to differentiate toxin-producing from nontoxigenic strains. However, because culture permits molecular typing of the organisms, it is essential for monitoring molecular epidemiology and antibiotic susceptibility[72].

We do not recommend its routine use in the diagnosis of CDI in clinical practice.

Polymerase chain reaction for toxin gene detection
Polymerase chain reaction (PCR) based primers for the detection of genes for toxins A and is highly sensitive and specific for the diagnosis of CDI[77,78]. Culture of the organisms may be required for PCR, which makes the process more technically demanding and challenging. A study based on the nested PCR assay reported a 99% concordance with the cytotoxicity assay and a sensitivity of 96.3% and a specificity of 100%[78].

TREATMENT OF CDI
The Society for Healthcare Epidemiology of America recommends initiating empiric therapy for CDI immediately after stool procurement for patients with severe symptoms consistent with CDI[34]. Empiric treatment is warranted if the clinical suspicion is high without waiting for the results as early initiation of treatment is critical in improving the outcome. Agents that decrease intestinal motility, such as narcotics and loperamide, should be avoided because of the risk of decreasing toxin clearance and the risk for ileus and/or megacolon[79].

Specific antibiotic therapy should be initiated as soon as possible. Oral metronidazole in a dose of 250-500 mg four times a day for 10-14 d or oral vancomycin at 125-500 mg four times a day for 10-14 d is the treatment of choice in patients with CDI. Metronidazole can be administered intravenously (in doses of 500 mg four times daily) in patients who are unable to take oral agents[66]. Bacitracin, teicoplanin and fusidic acid have been used in the treatment of CDI, but their efficacy has not been proved superior to vancomycin/metronidazole in large systematic meta-analysis[80,81]. A large meta-analysis of 1157 patients from 12 randomized trials assessed the efficacy of eight antibiotics for the treatment of CDI. None of the antibiotics are superior to others for symptomatic cure and/or reduction in complications[82]. Thus metronidazole is the initial drug of choice because of similar efficacy, lower cost and lesser risk of selecting vancomycin resistant *enterococci* in mild to moderate disease. However in patients with severe disease, multiple studies have shown a failure rate of 22%-38% with metronidazole[83]. Studies have shown similar cure rates in patients with mild disease with either use of metronidazole or vancomycin, while in severe disease the eradication rate with metronidazole is 76%, as compared with vancomycin, which gives a cure rate of 97%[84]. These data support the use of vancomycin as the first line treatment for severe CDI, also in patients with mild to moderate CDI who do not improve within 72 h of initiation of treatment with metronidazole should be switched to vancomycin. Severe CDI requires aggressive treatment and doses up to 2 g/d of vancomycin may be required in patients with severe disease. A recent phase 3 trial compared the efficacy and safety of OPT-80, fidaxomicin that is bactericidal *via* inhibition of RNA polymerase and oral vancomycin in treating CDI. The

clinical cure rates after OPT-80 (fidaxomicin) or vancomycin treatment were comparable[85]. However, OPT-80 was associated with a highly significant lower recurrence rate than vancomycin[85]. Further evidence of its efficacy needs to be studied. Anion-binding resins, such as cholestyramine and colestipol have also been used along with antibiotics[86]. These are proposed to bind to the *C. difficile* toxins and may have adjunctive benefit. However these agents have not been studied in IBD patients.

The efficacy of metronidazole or vancomycin specifically in the IBD population with CDI is unknown, but one study reported that just less than one quarter of the IBD patients with CDI required to be initiated on oral vancomycin because of lack of sufficient response with metronidazole[42]. Neither vancomycin nor rifaximin have been studied in randomized controlled trials for CDI in IBD patients.

There are no guidelines or evidence to suggest that one particular antibiotic regimen is better than the other in IBD patients who develop CDI. However colectomy rates in hospitalized patients with IBD was reportedly less from 45.5% in 2004, to 3.5% in 2006 in a single center study where vancomycin was adopted as the first line therapy in IBD patients with CDI after 2005[87,88].

Patients with fulminant colitis require initiation of treatment with oral vancomycin at a high dose of 500 mg every 6 h which may be administered with a nasogastric tube because of paralytic ileus. We also tend to use intravenous metronidazole along with vancomycin in these cases in our clinical practice. Emergent surgery is required for patients who do not respond to the above medical management and in patients with impending perforation and toxic megacolon. Patients usually undergo a subtotal colectomy and a temporary ileostomy and are associated with a high perioperative mortality rate approaching close to 40%[89].

There is no consensus on whether IBD-related medications, particularly immunomodulators and corticosteroids should be discontinued during the anti-CDI therapy. In a retrospective study of 155 patients from Europe with CDI complicating IBD, 104 (67%) were cotreated with antibiotics and immunomodulators (defined as the use of prednisone, azathioprine/6-mercaptopurine, methotrexate, biologics, cyclosporine, tacrolimus) for their *C. difficile*-associated IBD exacerbation, while the remaining 51 (33%) were treated with antibiotics alone[32]. The primary outcome of the study was colon perforation or toxic megacolon, shock, colectomy, and mortality. Patients treated by combination therapy had a trend towards a worse outcome when compared to those treated by antibiotics alone (likelihood ratio = 11.9; 95% CI: 0.9-157)[32]. Thus in most patients with CDI, it may be inappropriate to escalate immunosuppressive therapy during the acute CDI episode. However, the question of whether to add immunomodulator therapy in patients who are not on it before the CDI episode remains unanswered. In a recent survey of 169 North American gastroenterologists, there was significant disagreement on whether combination antibiotics and immunomodulators or antibiotics alone should be given

Table 2 Differentiating *Clostridium difficile* infection and inflammatory bowel disease

Features	Isolated CDI	CDI and IBD
Setting	Often hospital acquired	Often community-acquired
Risk factors	Antibiotic exposure prior to infection common	Many patients lacking of history of antibiotic exposure
	Immunomodulator and corticosteroid use	Immunomodulator and corticosteroid use playing even a greater role
	Increasing age	Increasing age
		Risk greater with ulcerative colitis than Crohn's disease, more with colonic involvement than small bowel disease
Clinical features	Usually watery diarrhea	May be bloody or mucous diarrhea
Outcome	Short term complications including toxic megacolon, colonic perforation, and peritonitis with sepsis	Short term complications including toxic megacolon, colonic perforation, and peritonitis with sepsis similar to patients without IBD
		Hospitalization costs and length of stay variable in studies
		Increased mortality in some studies
		Risk of colectomy unclear
		Long term outcome unclear, increased hospitalizations and escalation in medication use and colectomy rates reported with retrospective data
Diagnosis	ELISA testing for toxins	ELISA testing may be less sensitive
Endoscopy and histology	Pseudomembranes common	Pseudomembranes rare
Treatment	Metronidazole for mild to moderate severity	? Vancomycin for any hospitalized IBD patient
	Vancomycin for severe disease	
Recurrence	20% after the first episode of CDI	Rates highly variable 10%-58%, may be higher
Extra-colonic gastrointestinal manifestations	Small bowel can be affected	Most cases of small bowel involvement in IBD patients
		Pouchitis can also be seen

IBD: Inflammatory bowel disease; ELISA: Enzyme linked immunoassay; CDI: *Clostridium difficile* infection.

to flaring IBD patients with CDI. Overall, 77/169 (46%) of the respondents elected to add on corticosteroids as a combined treatment with antibiotics, whereas 82/169 (54%) treated the flare with antibiotics alone. When maintenance azathioprine was regularly taken, only 11% of respondents withdrew it upon the diagnosis of CDI[90].

RECURRENT CDI

Recurrence of CDI is common, affecting approximately 20% of patients. Recurrence typically occurs 1 to 2 wk after stopping metronidazole or vancomycin, but it can be delayed for up to 12 wk[49,90]. Risk factors for recurrent CDI include a prior history of recurrence, increasing age, use of additional antimicrobials, and an inadequate protective immune response to *C. difficile* toxins[49,91].

There are limited data available on the risk of CDI recurrence in IBD patients. In a study published in abstract form from Milwaukee in 2005, recurrent CDI was reported in 27/46 (58%) of patients[92]. In a subsequent study from the same center, recurrent *C. difficile* occurred in (10/87) 11.5% of patients[57]. Thus the risk appears to be highly variable and prospective studies need to be undertaken to clearly clarify the risk of CDI recurrence in IBD patients.

Management of a first recurrence of CDI is identical to a primary episode. Long tapering courses of vancomycin or pulsed treatment reduce recurrence and are suggested for treating second episode of recurrence[93,94]. Because of the risk of often-irreversible neuropathy with long-term use of metronidazole, it is not used for treatment of second relapse. Recently, several small series reported the efficacy of rifaximin in treating recurrent CDI[95,96]. Similarly reconstitution of the fecal flora by administration of stool is effective in small series[97,98] as previous studies have shown loss of diversity of fecal flora[53].

Other treatments including the use of active and passive immunization by administration of immunoglobulins or oral administration of antibodies from colostrum of cows immunized against toxins are under investigation for future use[91].

The treatment of recurrent disease in IBD patients is unclear in the absence of evidence based studies. In a study from Milwaukee of 14 IBD patients, rifaximin at a dose of 200 mg three times a day for 2 wk, followed by 200 mg once daily for 2 wk and 200 mg every other day for the final 2 wk of the taper resulted in resolution of infection in all the patients[92]. In the absence of data, we recommend treating patients in a similar way to the non-IBD population as far as recurrence is concerned (Table 2).

CDI IN SPECIAL SITUATIONS

C. difficile enteritis

Small intestinal *C. difficile* has increasingly been reported. The spectrum of CDI has definitely expanded with small bowel involvement (Figure 2)[99]. They are more frequently reported in patients with IBD who have undergone total colectomy or some form of gastrointestinal surgery[13]. The most common presentation is increased ileostomy output with associated dehydration. In patients with small bowel CDI, the risk factors seem to be slightly different. Antibiotic use and IBD predispose to small bowel CDI similar to CDI of the colon. Prior surgeries of the colon/colectomy, and host factors including advanced age, immunocompromised state are proposed as additional risk factors for small bowel CDI[100]. More than 90% of patients reported in the literature had gastrointestinal surgery of the colon.

The reason for the predisposition of patients who undergo colonic surgery to small bowel CDI is not clear al-
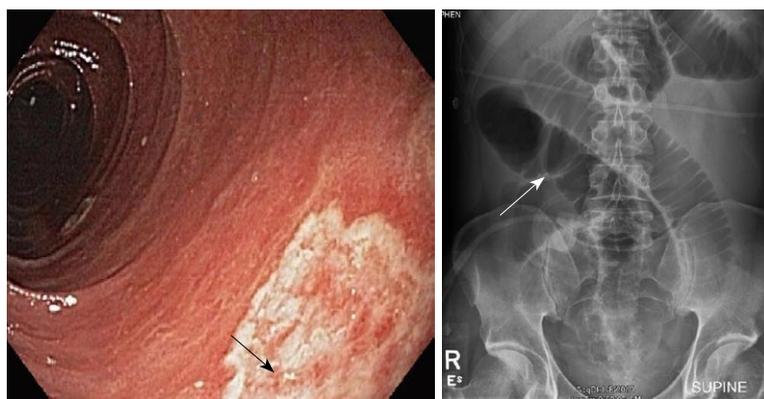
Figure 2 Recurrent *Clostridium difficile* enteritis in neoterminal ileum in a 36-year-old patient with diverting ileostomy for ileal pouch who had a preoperative diagnosis ulcerative colitis. Arrows: Enteritis due to *Clostridium difficile* infection and dilated loops of small bowel.

though multiple hypotheses are proposed. Firstly, changes occur in the small-bowel bacterial flora resembling colonic flora and after colectomy this may make it susceptible to overgrowth with *C. difficile,* particularly with concomitant antibiotic use[101]. This is based on the fact that the neoterminal ileum is colonized by colonic-type bacterial flora after ileocolonic resection[102]. Prolonged exposures to fecal stream may make the small bowel mucosa undergo metaplastic changes, as seen in patients with IPAA[103]. This notion was further supported by the fact that similar changes may occur in patients with end ileostomy and the long latent period between the surgery and the infection supports this. Changes occur in the ileostomy flora resembling the fecal flora[104]. In patients who develop infection in the immediate post operative period, a majority of patients had CDI of the colon prior to surgery which leads to the hypothesis that in those patients CDI of the small bowel may be secondary to migration of *C. difficile* into the small bowel after surgery. CDI is a toxin-mediated disease process. Although receptors for *C. difficile* toxins are typically on colonic epithelium, the receptors for toxin B is ubiquitous and may be present on small bowel epithelium which could mediate diarrhea in the immediate post operative period in the absence of colonic phenotype changes[105]. Secondly, colonization of the small bowel occurs because the protective mechanisms are compromised by colonic resection surgeries. The mechanical action of the ileocecal valve may be lost because of surgery[106]. In addition, continued peristalsis in the small bowel also inhibits colonization of the small bowel with *C. difficile*[106]. Therefore, surgeries involving only the left side of the colon with preservation of the ileocecal valve do not seem to increase the risk of CDI of the small bowel, highlighting the importance of the ileocecal valve in preventing colonization.

Initial studies highlighted that infection of the small bowel with *C. difficile* was associated with an increased mortality[13]. The increased permeability of the small intestinal mucosa was hypothesized to be due to result in profound sepsis[107]. However, recent studies showed a favorable prognosis. In fact, two large recent case series reported no mortality[100,108]. This may be probably secondary to increased awareness of the problem and early intervention.

The treatment of small bowel CDI is controversial and stratification of the disease severity as CDI of the colon could be used to initiate appropriate management plan. In a series of 11 patients, more than 50% responded to metronidazole alone[108]. In another series, all six patients were treated with a combination of metronidazole and vancomycin[100]. Thus similar to colonic CDI, oral vancomycin may be a first-line agent for severe CDI, while in mild to moderate disease, metronidazole may be used. However in patients who do not improve within 72 h of initiation of treatment with metronidazole, vancomycin needs to substituted instead of metronidazole.

C. difficile pouchitis

CDI has been reported in patients with IPAA[14,15,109,110]. CDI in IPAA can either present with asymptomatic colonization or with chronic antibiotic-refractory pouchitis or occasionally with fatal outcome. As the majority of patients have a history of short- or long- term exposure to antibiotics, CDI should be excluded in pouch patients with persistent symptoms with or without endoscopic findings of pouchitis or other pouch disorders.

In patients with IPAA, the epithelium of pelvic pouches undergoes morphologic changes facilitating fecal flora establishment[109]. These histologic adaptive changes include villus atrophy, Paneth cell hyperplasia, and a partial transition to colonic mucin phenotype without complete metaplasia[103]. In a recent study of 115 patients with IPAA, 21 (18.3%) were tested positive for *C. difficile* toxin A or B[14]. Three of those patients had chronic antibiotic-refractory pouchitis and all 3 patients had clinical remission and disappearance of *C. difficile* toxin from the stool with anti-*C. difficile* treatment with rifaximin or tinidazole. Three additional patients with other pouch-associated disorders also symptomatically improved with treatment of CDI. We also recently reported a patient who developed CDI of the pouch and neoterminal ileum immediately after ileostomy closure with a fatal outcome[111]. Fulminant outcomes of CDI of the pouch have also been described recently in a case report[112]. Similar to IBD patients with CDI who do not have the classic endoscopic or histologic features of pseudomembranes[10,42], superimposed CDI in pouch patients hardly have endoscopic or histologic features of pseudomembranes which makes the diagnosis challenging.

The treatment of CDI in IPAA is empiric at this point. There are no published prospective trials. The traditional

drugs used in the management of CDI are metronidazole and vancomycin. Previous studies suggest that metronidazole may be not completely protective against CDI of the pouch, as the bacterial infection can develop while the patients had been still on metronidazole[109,110]. Therefore, in patients with *C. difficile*-associated pouchitis, metronidazole may not be considered as the first-line agent. Based on our own experience and limited published literature, rifaximin, tinidazole, or vancomycin have been used with satisfactory results[14,110].

C. difficle infection in diverted bowel

Diversion colitis is common in segments of the colorectum after surgical diversion of the fecal stream, which may persist indefinitely unless the excluded segment is reanastomosed[113]. Patients with diverted bowel appear not immune to the development of CDI in the excluded downstream bowel segment. There has been a case report in which, following subtotal colectomy and end-ileostomy for medically refractory disease, a UC patient subsequently developed severe CDI in the rectal remnant (Hartmann pouch) and the patient responded to metronidazole suppositories[110].

RECOMMENDATIONS

In patients with IBD who present with worsening symptoms, CDI needs to be thought off and ruled out. In patients with a suspected diagnosis of CDI in IBD, stool studies for CDI are sent and empiric treatment is started. ELISA is the most commonly used method of diagnosis of CDI. We do not usually wait for the stool studies to return back to start treatment. We start all our IBD patients with suspected CDI on vancomycin 125 mg orally every 6 h and continue their previous immunosuppressive therapy. We do not add any new immunomodulators or escalate immunosuppressive medications in patients with suspected CDI in IBD unless CDI is ruled out with serial stool studies (at least 3-4). The duration of antibiotic use is 14 d. Routine endoscopy is not performed in these patients as the yield of pseudomembranes is very low unless an alternative diagnosis such as cytomegalovirus infection is being entertained. We also follow these patients serially to study the impact of CDI on the short term and long term outcome of IBD.

FUTURE DIRECTIONS

The pathogenesis and natural history of CDI in IBD patients is not entirely clear. The role of CDI in IBD exacerbation needs to be further investigated. It is unclear how to distinguish whether CDI is precipitating an IBD flare or whether it is an innocent bystander, as medical treatment targeted CDI does not necessarily induce IBD into remission. There is need for research to study the role of asymptomatic carriage of *C. difficile* and its impact on the longer-term outcomes of CDI in IBD. Although some retrospective studies have suggested worse long-term outcome of CDI in IBD patients, it needs to be

prospectively studied. Management of these patients can be challenging. Future studies to ascertain the appropriate management of CDI in IBD is required in particular, as there is little consensus on whether antibiotics and immunomodulators or antibiotics alone should be administered to these patients. Randomized controlled trials comparing metronidazole and vancomycin are also required to clearly understand the best management of *C. difficile* flares in IBD patients. A multidisciplinary approach involving gastroenterologists and colorectal surgeons, together with a team of GI pathologists and GI radiologists is necessary to successfully manage and treat patients with these disorders. Development of animal models with concurrent CDI and IBD would help us to understand the pathogenesis and manage these patients better.

CONCLUSION

CDI has continuously evolved over the years rising from a relative "benign" disease entity due to antibiotic exposure to a significant public health problem. CDI poses substantial challenge to epidemiologists, infection control practitioners, infectious disease specialists, gastroenterologists, gastrointestinal surgeons and hospital administration. The rising incidence, with increasing hospitalization rate, length of hospital stay, morbidity and mortality is of great concern. There has been a tremendous increase in the burden of CDI over the past few years with higher rates of surgery and mortality in the IBD population compared with the non-IBD cohort. The increase in the risk of community-acquired CDI in IBD population highlights that a high index of suspicion should be maintained even in the absence of conventional risk factors, such as antibiotic use or health care exposure. Patients with IBD even after colectomy are not immune to CDI. Pseudomembranes on endoscopy and histology appear to be uncommon in CDI superimposed on IBD. Randomized controlled trials are required to define the appropriate strategy for risk stratification and management for CDI in patients with IBD. In addition, preventive measures are the key and require concerted effort from all quarters from epidemiologists to hospital administration and clinicians.

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