Clostridioides difficile Infection in Inflammatory Bowel Diseases Patients

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Clostridioides difficile (*C. difficile*) represents a major health burden with substantial economic and clinical impact. Patients with inflammatory bowel diseases (IBD) were identified as a risk category for *Clostridioides difficile* infection (CDI). In addition to traditional risk factors for *C. difficile* acquisition, IBD-specific risk factors such as immunosuppression, severity and extension of the inflammatory disease were identified. *C. difficile* virulence factors, represented by both toxins A and B, induce the damage of the intestinal mucosa and vascular changes, and promote the inflammatory host response. Given the potential life-threatening complications, early diagnostic and therapeutic interventions are required. The screening for CDI is recommended in IBD exacerbations, and the diagnostic algorithm consists of clinical evaluation, enzyme immunoassays (EIAs) or nucleic acid amplification tests (NAATs). An increased length of hospitalization, increased colectomy rate and mortality are the consequences of concurrent CDI in IBD patients. Selection of CD strains of higher virulence, antibiotic resistance, and the increasing rate of recurrent infections make the management of CDI in IBD more challenging.

inflammatory bowel disease | Clostridioides difficile | ulcerative colitis | Crohn's disease

1. Introduction

The healthcare burden of *Clostridioides difficile* infection (CDI), especially in patients with underlying chronic disorders, dictates an increased vigilance for prompt diagnosis, appropriate treatment, and prevention measures to limit the spread. The economic and clinical impact of CDI is significant. Annual costs have been estimated to exceed $1.2 billion in the United States according to various reports [1], with increased length of hospitalization and hospital costs attributed to infection [2][3][4].

Given the significant health impact in the United States, *Clostridioides difficile* (*C. difficile*) has been recognized as one of the three "urgent" antibiotic resistance threats by the Centers for Disease Control and Prevention (CDC) [5]. The selection of strains of higher virulence (ribotype 027, ribotype 078) [6][7][8][9], the emergence of antibiotic resistance [10], and recurrent infections are major health threats. The risk of CDI recurrence after the primary episode is 25%, and the risk for subsequent recurrences further increases [11].

Inflammatory bowel diseases (IBD) represent chronic disorders with a complex pathogenesis involving environmental risk factors, genetic susceptibility, dysregulation of immune response to altered gut microbiome, and disruption of the epithelial barrier [12]. Inflammation of the gastrointestinal tract may have an unpredictable evolution, with periods of remissions and exacerbations. Nonsteroidal anti-inflammatory drugs and antibiotic use,

infections [13][14], smoking [15][16], psychological stress [17][18][19][20], high altitude journeys and flights [21] have all been described as triggers for ulcerative colitis (UC) or Crohn's disease (CD) flare-ups. By examining the stool during IBD relapses, *C. difficile* toxins were detected in 5.5% to 20% of patients [22][23].

Making the distinction between symptoms related to CDI and symptoms associated with an IBD flare can be a difficult task in practice. During IBD flares, patients experience abdominal pain, bloody diarrhea, and weight loss. Similarly, watery diarrhea, abdominal cramps, dehydration, loss of appetite and weight loss, and even rectal bleeding in severe cases, are typical symptoms of CDI. According to international guidelines, patients presenting with more than three episodes of unexplained unformed stools in 24 h should be tested for CDI [24]. Therefore, patients presenting with an IBD exacerbation should be screened for *C. difficile* toxins [25][26][27].

The risk factors for *C. difficile* acquisition, complex disease, and for recurrence, are older age (greater than 65 years old), current or recent exposure to broad-spectrum antibiotics (broad-spectrum penicillins, fluoroquinolones, cephalosporins, clindamycin), hospitalization [28][29][30], comorbidities, hypoalbuminemia, renal insufficiency [31], immunosuppression [32], chemotherapy [33][34][35][36], proton pump inhibitor (PPI) usage [37][38], nasogastric tubes [39], and gastrointestinal surgery [40]. Elderly patients, patients with immunosuppression, oncology patients, solid organ transplant recipients [41], and IBD patients also represent categories susceptible to infection.

2. Epidemiology of CDI in IBD Patients

Epidemiological data prove that the incidence of CDI among IBD patients has been increasing [42][43][44], with negative consequences on clinical outcomes (higher hospitalization rate, requirement for colectomy, and mortality). In a retrospective cohort study by Rodemann et al., the rate of CDI approximately doubled in CD patients (9.5 to 22.3/1000 admissions) and tripled in UC patients (18.4 to 57.6/1000) over a period of seven years (1998–2004) [45]. The increasing trend of CDI rate in IBD-hospitalized patients in the USA was also demonstrated in an analysis of data from the Nationwide Inpatient Sample: a 1.4% rate of infection in 1998, increased to 2.3% in 2004, and 2.9% in 2007, respectively [44].

The infection rate among IBD patients varies in different geographic regions: 3.4% in UC patients and 5.9% in CD patients were reported in the Netherlands [46]; 8.5% in CD and 24.7% in UC were reported in Chinese patients [47]. The rate of CDI among IBD patients with an ileal pouch anal anastomosis (IPAA) varies between 10.7 and 18.3% [48][49].

3. Pathogenic Mechanisms

C. difficile is an anaerobic, spore-forming, Gram-positive bacterium, identified as a causative agent of antibiotic-associated pseudomembranous colitis [50]. Infection comprises a spectrum of clinical manifestations that range from asymptomatic carriage or mild symptoms to life-threatening conditions (toxic megacolon, colonic perforation) and death [51].

Spores are transmitted by the fecal–oral route. Acquisition of C difficile in hospitalized patients occurs by ingestion of spores from an infected patient, transmission of pathogens via contaminated hands of healthcare personnel, or from contaminated surfaces. Antibiotic use affects gut microbiota composition and creates a favorable environment for C difficile colonization.

Virulence factors responsible for damage of intestinal mucosa are two monoglycosyltransferases, enterotoxin A (TcdA) and cytotoxin B (TcdB), released by CD toxigenic strains [52]. These two toxins are encoded by tcdA and tcdB genes, located on the 19.6 kb pathogenicity locus (PaLoc). The PaLoc contains three other genes involved in regulation of the toxin genes (tcdR, tcdC) and toxin secretion (tcdE) [53][54].

Toxin A and toxin B bind to a specific receptor on colonic epithelial cells and affect intestinal barrier integrity by disruption of epithelial tight junctions and promotion of inflammatory host responses by neutrophil recruitment and activation of pro-inflammatory cytokines [55]. Additionally, vascular changes caused by these toxins, with increased production of vascular endothelial growth factor A (VEGF-A) and increased colonic vascular permeability, are involved in disease pathogenesis [56]. Toxigenic strains may produce both toxin A and toxin B (causing the majority of CDIs), TcdB alone (A−B+strains), or TcdA alone (A+B−strains) [57][58][59][60]. Recent research proves the positive correlation between high serum levels of toxin A and the severity of CDI [61]. Toxemia could explain systemic clinical manifestations in severe cases of CDI [62][63]. However, since circulating anti-toxin antibodies prevent the detection of *C. difficile* toxins in blood, current methods used to detect serum toxins levels have a low sensitivity [64][65].

Cytokine profile could also serve as a predictive marker for CDI severity. Yu H et al. demonstrated that high serum levels of IL-2 and IL-15 are associated with severe disease and poor prognosis, while high serum levels of IL-5 and gamma interferon are encountered in less severe disease. Therefore, anti-inflammatory agents could play an important therapeutic role by controlling host response and consequently reducing intestinal injury [66].

A third toxin, named C difficile transferase or binary toxin CDT, also involved in disease severity, increases bacterial adherence to intestinal epithelial cells and uptake of TcdA and TcdB [67]. Of note, 23% of the toxigenic strains, including the hypervirulent ribotype 027 and 078, produce binary toxin [68].

Asymptomatic carriers of toxigenic strains represent a population reservoir that favors the spread of infection [69] [70]. *C. difficile* carriage was reported in 8.2% of IBD outpatients in clinical remission [71]. It is possible that the asymptomatic carriage of non-toxigenic strains confers protection against the toxigenic ones [72].

4. Risk of CDI in IBD Patients

Risk factors for *Clostridioides difficile* acquisition in IBD patients were analyzed in various and heterogenous studies and some contradictory data were obtained. IBD-related risk factors for CDI (such as disease location, severity and disease extent, and immunosuppressive therapy) were assessed, together with traditional risk factors (age, antibiotic exposure, hospitalization, and comorbidities). IBD patients with superimposed CDI showed distinct

features, compared to the general population: younger age, with no recent exposure to antibiotics, presenting more frequently with a community-acquired infection [73].

4.1. The Role of Traditional Risk Factors

Increasing age and comorbidities were identified as risk factors for CDI in IBD patients, similarly to the general population [45]. The predisposition of patients with IBD colitis towards *C. difficile* acquisition could be explained by gut microbiota disturbance [74], leading to impaired resistance to *C. difficile*, even in the absence of recent antimicrobial exposure [73]. The reported rate of prior antibiotic exposure as a risk factor for CDI in IBD varies: 42% of IBD patients (compared to 69% of non-IBD patients) were exposed to antibiotics within three months before CDI in one report [42], whereas no prior antibiotic therapy was identified in 39% of CDI-IBD cases in another report [75]. Other authors identified the recent use of antibiotics in IBD as a risk factor for both CDI and for recurrent CDI as well [76]. Exposure to nonsteroidal anti-inflammatory drugs within two months prior to hospital admission for IBD flare was a risk factor for CDI in a study performed by Regnault et al. [77]. Although CDI is mostly a healthcare-associated infection, some authors demonstrated that CDI in IBD is more frequently community-acquired [45]. Maharshak et al. identified recent hospitalization (within the prior two months) and the use of antacids as significant risk factors for CDI in IBD patients [78].

4.2. The Role of IBD-Related Risk Factors

The rate of CDI is higher in UC compared to CD [45], and colonic involvement represents a significant risk factor for CD acquisition (OR 3.12; 95% CI: 1.28–5.12) [75]. Patients with pancolitis or left-sided colitis, more than patients with limited colonic involvement, together with patients with severe forms of IBD, are at greater risk for CDI [79][80].

Safety concerns have been raised regarding IBD-specific therapy. In a retrospective study of serious bacterial infections among 10,662 IBD patients undergoing immunomodulating therapy, corticosteroid initiation, with or without concomitant immunomodulators, was associated with a threefold increased risk for CDI compared with other immunosuppressants (RR = 3.4; 1.9–6.1). No significant association was found with infliximab use. Antibiotic therapy in the previous six months was found in 57.2% of patients with CDI [81].

Corticosteroids and biologics (infliximab and adalimumab) were identified as risk factors for CDI in IBD patients, as well as metronidazole, hospitalizations, higher ambulatory care visits, shorter duration of IBD, and a higher number of comorbidities [82]. In a systematic review and meta-analysis, Balram et al. showed that biologic therapy, antibiotic use, and colonic involvement are all risk factors for CDI in IBD [83]. Maintenance therapy with immunomodulators (azathioprine, 6-mercaptopurine, methotrexate) was an independent risk factor for CDI in one report [75], whereas presence of a fistula, antibiotics (metronidazole and cephalosporins), and infliximab (particularly in combination with antibiotics) were identified as risk factors for CDI in another report [84].

4.3. Genetic and Immunologic Susceptibility

Additional risk factors for CDI in IBD were studied for identification of susceptible patients. Connelly et al. showed that interleukin-4 gene-associated single-nucleotide polymorphism rs2243250 was significantly associated with CDI in IBD patients [85].

The susceptibility for recurrent CDI can be explained by altered host humoral immunity, while high-serum anti-toxin A or anti-toxin B antibodies are protective against recurrent infections [86][87]. In older IBD patients, an impaired capacity to produce toxin-specific antibodies and memory B-cell responses could explain the increased risk for CDI [88].

5. Diagnosis of CDI in IBD

5.1. Clinical Assessment

Common symptoms of CDI are: new onset diarrhea, ranging from 3 loose stools to more than 10 watery stools within a 24 h period, abdominal pain, fever, dehydration, and blood in the stool. Severe complications occur in fulminant disease and can evolve to adynamic ileus, hypotension, shock, toxic megacolon, colonic perforation and peritonitis. In IBD patients who underwent total colectomy, an increased ileostomy output with associated dehydration occurs in acute enteritis caused by *C. difficile*, together with fever and leukocytosis [89]. An increasing number of stools in patients with IPAA should raise the suspicion for *C. difficile* pouchitis [48][49].

5.2. Laboratory Tests

Laboratory diagnosis is recommended in symptomatic patients, and consists in the detection of toxins in a liquid stool sample. The culture for toxin-producing *Clostridioides difficile* (toxigenic culture TC) and cell culture cytotoxicity neutralization assay (CCNA) are methods with the highest sensitivity, but these require time and are expensive [90][91]. Compared to these methods, enzyme immunoassay (EIA) for toxins has the advantage of a rapid and easy-to-perform test, but with poor sensitivity [92]. Nucleic acid amplification tests (NAATs) represent sensitive methods to detect the presence of CD toxins genes. However, NAAT alone cannot make the distinction between colonization and infection and may lead to overdiagnosis and unnecessary treatment of asymptomatic carriers [93][94]. The glutamate dehydrogenase (GDH) test is a useful screening test that detects the enzyme GDH, expressed by toxigenic and non-toxigenic CD strains [95]. Additional laboratory tests are white blood cell count, serum albumin, creatinine, and electrolytes. Leukocytosis (>15,000 cells/mL) and increased serum creatinine level (>1.5 mg/dL) are markers of severe CDI. Patients with low albumin levels are at a higher risk of developing severe infection and recurrent CDI [96][97].

A new serological assay has been proposed to predict the risk for recurrent infection: an ELISA test measuring serum levels of toxin-A- and -B-specific antibodies, potentially identifying those patients with low levels of antibodies who are more susceptible to recurrence. However, extended research is needed to evaluate the applicability of this assay in current practice [98].

5.3. Diagnostic Algorithm

The diagnostic algorithm for CDI consists in EIA for GDH detection or NAAT, followed by EIA testing for toxins of the stool sample. Another approach is to test the stool sample simultaneously for GDH and free toxins (EIA), followed by NAAT or TC. According to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the Infectious Diseases Society of America (IDSA), and the Society for Healthcare Epidemiology of America (SHEA) guidelines, a combination of diagnostic tests is recommended [24][99]. Clinical judgement is advocated in making the distinction between colonization versus infection in PCR positives/toxins negative cases [99]. This approach prevents unnecessary antibiotic use that may cause disruption of normal gut microbiota and selection of vancomycin-resistant enterococcus species [100][101]. A positive GDH test with a negative EIA test for toxins demands immediate treatment in selected cases with severe symptoms and a high index of suspicion for CDI in IBD patients [102].

5.4. Endoscopic and Histopathological Evaluation

The classic morphologic features of CDI, consisting in the detection of inflammatory pseudomembranes on endoscopic and histological evaluation, are generally not found in IBD patients. Pseudomembranes represent a layer of fibrin, necrotic epithelial cells, mucus, leukocytes, that create the typical endoscopic appearance of elevated whitish or yellowish plaques on the colonic mucosal surface [75][103]. It is speculated that an altered mucosal inflammatory response in active IBD, or as a result of immunomodulator use, may result in the inability to form pseudomembranes [104].

A lower gastrointestinal endoscopy with biopsies is useful to rule out other potential causes of diarrhea, such as cytomegalovirus colitis, ischemic colitis or amoebiasis [105], and to evaluate the extension and the severity of IBD.

5.5. Imagistic Evaluation

Radiologic evaluation (abdominal X rays and computed tomography CT) represents an effective diagnostic method in severe, complicated cases, by detecting loss of haustration, dilated transverse or right colon in toxic megacolon, air-fluid levels, or free intraperitoneal gas in perforation. Imaging provides clues in severe colonic inflammation: bowel wall thickening, thumbprinting, accordion sign, pericolonic stranding and ascites [106][107][108]. Similar findings, consisting of small bowel dilatation, wall thickening, and ascites, have been described in CD enteritis [109][110]. Abdominal ultrasound allows the assessment of disease extent and severity, and specific features for both CDI and IBD can be detected: thickening of the bowel wall, colonic dehaustration, free fluid between bowel loops, colonic dilatation, or free air in complicated cases.

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