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# Clostridioides difficile infection in patients with nonalcoholic fatty liver disease - Current Status.



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**Abstract**

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, leading to fibrosis, cirrhosis and hepatocellular carcinoma (HCC), factors known to be associated with increased cardiovascular disease mortality. The pathogenesis of NAFLD is not fully understood, although NAFLD is thought to be a hepatic form of metabolic syndrome. There is an increasing understanding of the role of microbiota disturbances in NAFLD pathogenesis, and as with many other conditions affecting the microbiota, NAFLD may be a novel risk factor for *C. difficile* colonization (CDC) and *C. difficile* infection (CDI). CDI is an emerging nosocomial disease, and community-acquired cases of infection are growing, probably due to an increase in CDC rates. The association of NAFLD with CDI has been shown in only 4 studies to date, three of which included less than 1000 patients, although the frequency of NAFLD in these studies was observed in almost 20% of the total patient cohort. These data revealed that NAFLD is a risk factor for CDI development and, moreover, is a risk factor for intestinal complications of CDI. More studies are needed to investigate this association and move forward CDC and CDI screening efforts for this group of patients.

**Key Words:** NAFLD; *Clostridioides difficile*; *Clostridioides difficile* colonization; *Clostridioides difficile* infection; Minireview

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**Core Tip:** The association of NAFLD with CDI has been shown in only 4 studies to date, three of which included less than 1000 patients, although the frequency of NAFLD in these studies was observed in almost 20% of the total patient cohort. These data revealed that NAFLD is a risk factor for CDI development and, moreover, is a risk factor for intestinal complications of CDI. More retrospective studies and systematic

reviews are needed to examine this group of patients as a risk factor for CDI, make recommendations to prevent CDI, and effectively screen and diagnose CDC within NAFLD patients.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized as a chronic liver disease with ≥5% hepatic fat accumulation and a natural progressive course from nonalcoholic fatty liver (NFL) to nonalcoholic hepatitis (NASH) and cirrhosis. The current epidemiology of NAFLD is not totally understood due to its underdiagnosis, as patients may remain asymptomatic even after the formation of cirrhosis and escape medical evaluation; however, NAFLD is thought to affect approximately 25% of the adult population, and the incidence of NAFLD is expected to increase in the future[1-3]. In NFL, the fibrosis progression rate averages 14 years per each stage of fibrosis *vs* 7 years per each stage of fibrosis in NASH. There are also rapid progressors with NASH in whom fibrosis progresses in less than 7 years. Among NAFLD patients, approximately 20% have NASH, and these patients should be diagnosed and receive proper treatment, as they can develop cirrhosis within 2-3 decades[4].

Patients with NAFLD are at risk for hepatocellular carcinoma (HCC), the fourth leading cause of cancer death worldwide, which may occur in the absence of cirrhosis in up to 50% of NAFLD patients, leading to late diagnosis and increased mortality[3,5,6]. In addition to cirrhosis and HCC, NAFLD is associated with an increased risk of cardiovascular disease (CVD), as these patients tend to have obesity, type 2 diabetes mellitus, and dyslipidemia, the hallmark of metabolic syndrome. Thus, these patients are at a higher risk for hypertension, coronary heart disease, cardiac arrhythmias, cardiomyopathy development and increased cardiovascular morbidity and mortality[7]. Nonobese patients with NAFLD have significantly lower rates of CVD than obese patients with NAFLD; however, even in the absence of obesity, patients with NAFLD are at a higher risk of CVD, with an incidence rate of 18.7 per 1000 persons-years[1]. In addition to the association of NAFLD with CVD and HCC, recent studies have shown

that patients with NAFLD are at risk for *Clostridioides difficile* infection (CDI) development[8-11].

## ABOUT *C. difficile*

*Clostridioides difficile* (*C. difficile*) is a gram-positive, spore-forming bacterium with transmission by the fecal-oral route. It is widespread in the environment and human population, may persist in the intestinal tract of asymptomatic carriers and animals and contaminate ambient objects, and can cause mild to severe diarrhea and colitis. In the last 30 years, CDI has become one of the most significant nosocomial infections and the leading cause of antibiotic-associated diarrhea (AAD), with increased severity, rate of recurrence (i.e., up to 10-30%) and mortality[12,13]. In 2011, 453,000 new cases of CDI and 29,300 associated deaths were identified in the USA; in 2017, the incidence was estimated at 223,900 with 12,800 deaths[14]. In Europe, the annual estimated number of cases is up to 189,256, according to a 2016-2017 study[12]. The increased incidence and severity associated with CDI can be attributed to the emergence and spread of a strain known as ribotype 027 (NAP1/BI/027) among hospitalized patients[15]. NAP1/BI/027 is highly resistant to fluoroquinolone, has increased toxin A and B production, produces a strain-specific binary toxin and persists in the USA and Europe; however, in Asia, the dominant strains include ribotype 017, 018 and 014[16]. Of note, drug resistance and severity of CDI also vary by ribotype and region. Developing diagnostic methods have led to an understanding of the heterogeneity of *C. difficile*, while molecular typing studies have demonstrated the presence of up to 98 different ribotypes in a single country[17,18].

*C. difficile* toxins cause acute colonic inflammation *via* epithelial disruption and the release of proinflammatory cytokines and chemokines, resulting in CDI, which is clinically heterogeneous. The severity of CDI is thought to be dependent on both the host and strain characteristics[17,19]. The distal colon is the most frequently affected organ in CDI, resulting in mild diarrhea with spontaneous recovery after antibiotic withdrawal. However, some patients manifest profuse diarrhea, colonic ileus,

pseudomembranous colitis (PMC) and toxic megacolon, followed by fever, abdominal pain, sepsis, *etc.* Clinical and laboratory findings may vary between patients depending on CDI severity including dehydration, peritonitis, leukocytosis, a positive fecal occult blood test (FOBT), *etc.*; therefore, CDI should be suspected in any patient with acute diarrhea, recent antimicrobial exposure and a prolonged hospital stay[20,21]. Risk factors for CDI include non-CDI-active antimicrobial use, prolonged hospitalization, advanced age (≥65 yr.) and recent intake of acid-suppressive therapy[20,22-24].

Recurrent CDI (rCDI) is a new CDI episode occurring within eight weeks after a previous episode. Etiologically, rCDI may be due to relapse of the same strain as the first infection or reinfection by a different strain, and it develops in 15 to 30% of patients after initial CDI. The risk of further recurrence is much higher, as approximately 40% of patients with one episode of rCDI will develop the second episode, whereas the third episode will develop in 45-65% of patients. Thus, prevention of rCDI remains very important[25]. Risk factors for rCDI include advanced age (>76 yr.), antibiotic exposure, gastric acid suppression, CDI caused by a highly virulent strain (NAP1/BI/027), severe underlying diseases and a prolonged hospital stay[25,26].

CDI diagnosis depends on clinical findings and the detection of *C. difficile*, its toxin or toxin-producing gene in a stool specimen taken before the initiation of *C. difficile*-specific treatment to avoid false-negative results. The European Society of Clinical Microbiology and Infectious Disease (ESCMID) recommends a 2-step diagnostic algorithm for CDI confirmation. The first step is a highly sensitive screening method (i.e., the nucleic acid amplification test (NAAT) and the glutamate dehydrogenase (GDH) assay). Positive results are followed by the performance of a second step which includes detecting free toxins in stool (i.e., the enzyme immunoassay (EIA) for disease causing toxins or the cell cytotoxicity neutralization assay)[20,27].

Recently, there has been an interest in asymptomatic colonized individuals, acting as a reservoir for CDI and being at increased risk (i.e., 51.9 cases per 100,000 persons) of developing CDI[20]. *C. difficile* colonization (CDC) stands for the detection of *C. difficile* in the absence of CDI symptoms for 12 wk pre- or post-specimen collection; however,

many studies use the simple definition of a *C. difficile*-positive stool and the absence of CDI symptoms[28]. *C. difficile* colonizes the gut of 5% of the adult population and up to 70% of infants and does not affect the intestinal tract while the gut microbiome is intact; however, administration of antibiotics affects its composition and promotes the growth of vegetative forms, the germination of spores, and the production of toxins[15,18]. Approximately 4-10% of patients are colonized with *C. difficile* at the time of hospitalization, and the number of colonized patients increases during their stay[17]. Therefore, asymptomatic hospitalized patients require *C. difficile* screening to prevent microbe transmission and the development of strategies to mitigate the risks for developing active CDI[28,29].

## ***C. DIFFICILE* AND LIVER DISEASES**

It is widely known that cirrhosis is associated with an increased risk of CDI and a severe disease course as cirrhotic patients have a high rate of hospitalization, an immunocompromised state, and are often prescribed to take antibiotics due to an increased risk of infection[30-32]. The average hospitalization stay in patients with CDI and cirrhosis is 14 days, inpatient mortality is ≥14%, and 30-day readmission rates occur in 35% of patients compared to the results for noncirrhotic patients, which are 13 days, 8% and 20%, respectively[33]. CDI is an independent mortality risk factor in cirrhotic patients as evident from the fact that mortality in a cohort of patients with cirrhosis and concurrent CDI were demonstrated to be higher (13.8%) than mortality in cirrhosis (8.2%) and CDI (9.6%) patients alone[34]. Moreover, hypoalbuminemia and admission to the intensive care unit (ICU) are independent predictors for short-term mortality[35]. Sahra *et al.*[36] revealed that patients with cirrhosis were more likely to develop CDI than noncirrhotic patients. Interestingly, the etiology of cirrhosis also affects CDI prevalence. For instance, patients with cirrhosis due to alcoholic liver disease (ALD) and NAFLD were more prone to CDI than patients with viral hepatitis B and C cirrhosis (174.0 *vs* 184.9, *vs* 81.7 *vs* 117.9 persons per 100,000, respectively)[36].

In contrast to cirrhosis, the association between NAFLD and CDI is not fully understood. To the best of our knowledge, there are currently only four studies examining this question, even though NAFLD is the most common cause of chronic liver disease and CDI is one of the most common nosocomial infections.

In November 2019, Nseir *et al.*[9] published their retrospective cross-sectional study, revealing that NAFLD is a risk factor for C. difficile-associated diarrhea (CDAD). Patients with NAFLD accounted for 66% of all patients with confirmed CDAD. Moreover, the authors revealed that metabolic syndrome, which is commonly seen in patients with NAFLD, is associated with severe CDAD[9]. A similar retrospective study by Papić *et al.*[8] confirmed that NAFLD is a risk factor for inpatient CDI, with an incidence rate of 16.9% *vs* 7.4%, as seen in the control group.

In 2021, Jiang *et al.*[10] presented a large retrospective study that included 7239 patients with CDI and coexisting NAFLD (with a total of 94.5% that were noncirrhotic) and compared them to patients with coexisting ALD and viral liver disease (VLD). The analysis showed that patients in the NAFLD group had a lower incidence of respiratory failure (2.7%), septic shock (0.5%), acute kidney injury (AKI) (13%), hospital mortality (0.8%) and length of stay (LOS) (5.75±0.16 days) than those in the ALD and VLD groups; however, the rates for intestinal complications were increased in the NAFLD group. Specifically, intestinal obstruction was seen in 4.6% of patients with NAFLD compared to 2.2% of patients with ALD. Additionally, a higher rate of intestinal perforation was observed in the NAFLD group compared to the VLD group[10].

Recently, Šamadan *et al.*[11] revealed that NAFLD is not only a risk factor for inpatient CDI in elderly patients exposed to systemic antibiotics but also a risk factor for rCDI (47.4% in the NAFLD group compared to 27.9% in the non-NAFLD group). Interestingly, the authors found a decreased rCDI ratio in patients taking statins in both the NAFLD and non-NAFLD groups, possibly due to their modulatory effect on the microbiome[11].

## GUT MICROBIOTA DISTURBANCES IN NAFLD AND CDI PATHOGENESIS

Although the association of NAFLD with CDI has not been fully studied, biological plausible links may lie in their shared pathogenesis (i.e., the gut microbiota disturbances).

It is widely accepted that microbiota disturbances play a main role in *C. difficile* colonization and infection; therefore, it is not surprising that most patients develop CDI after a course of antibiotics. The pathogenesis of *C. difficile* colonization and infection includes intermicrobial interactions. For instance, *C. difficile* produces quorum signals, inducing Proteobacteria metabolite production leading to Bacteroidetes inhibition. *C. difficile* can also produce inhibitors of indigenous microbiota, such as proline-base cyclic dipeptides[37]. Secondary bile acids have been shown to inhibit toxin activity and the growth of vegetative forms of *C. difficile*, while antibiotics affect microbes producing these acids. In contrast, primary bile acids promote *C. difficile* spore germination. Therefore, a low level of secondary bile salts (and consequently a low concentration of secondary bile acid-producing bacteria) and a high level of primary bile salts, results in CDI and its recurrence[38].

Multiple studies have shown that smaller microbial diversity and decreases in certain species are often seen in patients with CDI and CDC. For example, stool samples of patients with CDI revealed an increase in Proteobacteria, Firmicutes and Enterobacteriales, and a decrease in Bacteroidetes and butyrate-producing Ruminococcaceae and Lachnospiraceae families in comparison to healthy individuals[28].

The CDC microbiome disturbances were similar to those of CDI patients; however, in regard to the degree of changes seen, they were closer to healthy individuals. In addition, a higher level of some bacterial families were noted in CDC microbiomes, including Clostridiales family XI incertae sedis, Clostridium, and Eubacterium, but were significantly decreased in the infected individuals[39]. This data confirms that CDI occurrence is dependent on the presence of certain bacterial species and that colonization with these species may prevent CDC and CDI[40]. Studies on murine models have already confirmed that intestinal colonization with Lachnospiraceae

significantly reduced CDC and that administration of *Clostridium scindens* prevented CDI development in antibiotic treated mice with *C. difficile* spores[41,42]. From these studies it can be inferred that any condition connected with gut microbiota disturbance is a risk factor for CDC and CDI.

Recently, there has been increasing evidence of the role of microbiota disturbances in NAFLD pathogenesis and progression[43-45]. For example, it was shown that the transfer of the microbiome from mice with fasting hyperglycemia and insulinemia to germ-free mice led to the development of NAFLD. These conventionalized NAFLD mice had Lachnospiraceae bacterium 609 and *Barnesiella intestinihominis* overrepresented in their feces, whereas *Bacteroides vulgatus* was underrepresented in comparison to the control group[46].

Changes in the gut microbiota were also found in humans with NAFLD. Moreover, the composition of the gut microbiota varied not only between the control group and patients with NAFLD but also between patients with NAFLD, NASH and NAFLD cirrhosis[47]. Loomba *et al.*[48] revealed the dominance of Firmicutes and Bacteroidetes in NAFLD patients; however, the progression of the disease from mild/moderate to advanced fibrosis led to an increase in Proteobacteria and a decrease in Firmicutes. *Eubacterium rectale* and *Bacteroides vulgatus* were shown to be the most abundant species in mild/moderate NAFLD, and *B. vulgatus* (2.2%) and *Escherichia coli* were the most abundant in advanced fibrosis, suggesting a shift toward gram-negative microbes in which LPS is thought to cause the progression of fibrosis. Proteobacteria, Enterobacteria, Escherichia and Bacteroides were found in abundance in patients with NASH, while Gammaproteobacteria and Prevotella were more prevalent in the stool of obese children with NAFLD in comparison to non-NAFLD obese children[46,49]. Zhu *et al.*[50] found an increased representation of an alcohol-producing Escherichia, followed by increased blood alcohol concentration, in NASH patients compared to obese and healthy individuals, which may play a role in NASH pathogenesis. Zhang *et al.*[51] showed the association of a high-fat/high-cholesterol (HFHC) diet with progression of NAFLD and the concomitant changes in the microbiota of mice. Thus, enrichment of

Mucispirillum schaedleri_Otu038, Desulfovibrio_Otu047, Anaerotruncus_Otu107, Desulfovibrionaceae_Otu073, Clostridium celatum_Otu070, C. ruminantium_Otu059, C. cocelatum_Otu036 and C. methylpentosum_Otu053, and the depletion of Bifidobacterium_Otu026, Akkermansia municiphila_Otu034, Lactobacillus_Otu009, Bacteroides acidifaciens_Otu032, Bacteroides_Otu012, B. uniformis_Otu080 and B. eggerthii_Otu079 in the microbiota were observed with the progression of NAFLD to NASH and HAFLD-HCC. The authors also revealed a possible role of Helicobacter ganmanii_Otu031 enrichment and Bacteroides_Otu012 depletion in HCC development in mice. Lastly, fecal microbiome transplantation (FMT) from NAFLD patients to germ-free mice confirmed a role of gut microbiota in NAFLD pathogenesis as these mice showed hepatic steatosis, inflammation and multifocal necrosis on a high-fat diet (HFD), while germ-free mice from the control group only had minor liver inflammation and fat accumulation on the same HFD[52]. Therefore, the gut microbiota disturbances seen in both NAFLD and CDC/CDI and preexisting microbiota changes in patients with NAFLD may explain its association with CDI and rCDI[8-10].

## CONCLUSION

NAFLD is the most common chronic liver disease with an estimated prevalence of 20% in the general population. NAFLD is a well-known risk factor for cirrhosis and HCC development and is also associated with cardiovascular mortality. Although the pathogenesis of NAFLD is not fully understood, the past decade of research has led to an understanding of the role of the gut microbiota in NAFLD development and progression toward cirrhosis. As with any condition associated with microbiota disturbance, NAFLD has been shown to be associated with CDI severity. Despite NAFLD being such a common, chronic liver disease and *C. difficile* being an emerging nosocomial infection with increasing community-acquired cases, only 4 studies have examined this issue, to date (Table 1). More retrospective studies and systematic reviews are needed to examine this group of patients as a risk factor for CDI, make

recommendations to prevent CDI, and effectively screen and diagnose CDC within NAFLD patients.

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