

Original Article/Liver

Risk factors for *Clostridium difficile* infection in cirrhotic patientsDong Yan^a, Yan-Di Huang^b, Yun-Bo Chen^a, Tao Lv^a, Si-Lan Gu^a, Yong-Tao Li^a, Jian-Rong Huang^a, Lan-Juan Li^{a,*}^a State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China^b Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China

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ABSTRACT

Background: Cirrhotic patients are susceptible to *Clostridium difficile* infection (CDI), however, the high risk factors are not clear. The present study aimed to identify the risk factors in cirrhotic patients with CDI.

Methods: A total of 526 cirrhotic patients admitted to our hospital between May 2015 and October 2015 were included in this study. Stool samples were collected upon admission for the detection of CDI and toxin. CDI was monitored during the hospital stay. In total, 34 cases showed CDI. Then we analyzed the effects of age, sex, *C. difficile* colonization (CDC), multiple hospitalization, extended hospital stay, elevation of total bilirubin (TBIL), creatinine (Cr), Child-Pugh grade C, hepatic encephalopathy, hepatorenal syndrome, upper gastrointestinal hemorrhage, and exposure of antibiotics and proton pump inhibitor (PPI) on the CDI in cirrhotic patients.

Results: Patients in the CDI group had more frequent CDC, multiple hospitalization, and extended hospital stay compared to those in the non-*C. difficile* infection (NCDI) group. Patients in the CDI group had higher TBIL and Cr, and higher frequency of Child-Pugh grade C, hepatic encephalopathy, upper gastrointestinal hemorrhage compared with those in the NCDI group. Multiple logistic regression analysis indicated that age >60 years (OR=1.689; 95% CI: 1.135–3.128), multiple hospitalization (OR=3.346; 95% CI: 1.392–8.043), length of hospital stay >20 days (OR=1.564; 95% CI: 1.113–2.563), hypoproteinemia (OR=4.962; 95% CI: 2.053–11.996), CDC (OR=18.410; 95% CI: 6.898–49.136), hepatic encephalopathy (OR=1.357; 95% CI: 1.154–2.368), and exposure of antibiotics (OR=1.865; 95% CI: 1.213–2.863) and PPI (OR=3.125; 95% CI: 1.818–7.548) were risk factors of CDI.

Conclusions: Age >60 years, multiple hospitalization, length of hospital stay >20 days, hypoproteinemia, CDC, hepatic encephalopathy, and exposure of antibiotics and PPI were risk factors for CDI in cirrhotic patients. These may contribute to the early diagnosis and monitoring of CDI in clinical practice.

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Introduction

Clostridium difficile infection (CDI) is one of the major causes for infectious diarrhea and pseudomembranous colitis (PMC) [1]. To date, several risk factors have been reported to be associated with the pathogenesis of CDI, including host immunity and bacterial virulence. The host immunity involves utilization of corticosteroid and/or immunosuppressive agents, anti-infection therapy and presence of *C. difficile* toxin A antibody. To our best knowledge, patients with high level of *C. difficile* toxin A antibody are at high risk of *C. difficile* colonization (CDC) [2]. CDC and CDI are closely related to

C. difficile strains. Strains with high toxicity are more likely to cause CDI. For instance, North American pulsed-field type 1 (NAP1) strain is superior to the other strains in terms of triggering CDI, however, it rarely induces CDC [3–5]. In addition to the host immunity and strain toxicity, several clinical features are considered to be risk factors of CDI, including advanced age, repetitive and extended hospitalization, utilization of antibiotics and anti-acid agents, nasal feeding, gastrointestinal tract surgery, and basal disorders [4]. About one third of the cases with cirrhosis may present bacterial infection [6–11], and the most common types of infection are abdominal infection, sepsis and intestinal infection. Cirrhotic patients in the list of liver transplantation are prone to abdominal infection, upper gastrointestinal hemorrhage and hepatic encephalopathy [7,12–14]. Previous studies indicated that ICU stay, antibiotic exposure and hepatic encephalopathy are the risk

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factors for CDI among cirrhotic patients; ICU stay and high blood ammonia are high risk factors for CDI-related mortality [15,16]. Nowadays, CDC also raised attention. Drekonja et al. [17] found 25% patients with CDC and a recurrence rate of 33% in CDI patients after antibiotics administration. In 2015, a meta-analysis showed that the incidence of CDI in patients carrying *C. difficile* was about 6-fold higher than that of the counterparts without *C. difficile* [18]. Kagan et al. [19] showed that the incidence of diarrhea in the CDC carriers was significantly higher than that of those without colonization (23.3% vs. 6.7%).

In clinical practice, it is hard to control the host immunity and *C. difficile* toxicity. Nevertheless, some measures can be taken to attenuate or eliminate the risk factors. Investigation on these risk factors may contribute to the early diagnosis and decline in CDI incidence. In this study, we aimed to investigate the risk factors for CDI in cirrhotic patients.

Methods

Subjects

A total of 526 cirrhotic patients between May 2015 and October 2015 admitted to our hospital were included in this study. The inclusion criteria were as follows: (i) aged 18–70 years; (ii) hospital stay >7 days. Patients with liver cancer and other malignancies were excluded from this study. Each patient signed the informed consent. The study protocols were approved by the Ethical Committee of the First Affiliated Hospital, Zhejiang University School of Medicine.

Clinical information included age, sex, hospitalization within 1 year, peripheral white blood cell (WBC), hemoglobin (HGB), platelet (PLT), albumin (ALB), creatinine (Cr), total bilirubin (TBIL), and prothrombin activity (PTA) as well as Child-Pugh grade, complications, CDC and clinical treatment (i.e. administration of anti-acid agents and antibiotics). CDC is defined as culture positive for *C. difficile*. The patients were divided into the CDI group and non-CDI (NCDI) group. After admission, the frequency of diarrhea was recorded according to the previous description [20]. In cases of diarrhea, stool samples were collected for the *C. difficile* culture and toxin detection. CDI was defined as culture positive for *C. difficile* and toxin. We analyzed the WBC, HGB, PLT, ALB, Cr, TBIL, PTA, Child-Pugh grade, cirrhosis-related complications and treatment (administration of anti-acid agent and antibiotics) between the two groups.

Sample collection

Within 48 h after admission, stool samples were collected. In cases of diarrhea, stool samples were cultured and toxicity was measured.

Quality control strain

The quality control strains including ATCC700057 and PCR biotype 027 were kindly bequeathed by Professor Guo-Yong Yuan from Queen Mary Hospital, Hong Kong, China. These strains served as the positive control for the binary toxin gene.

Identification and culture of *C. difficile*

Stool samples were subject to toxin A and B measurements within 2 h after collection. Then the remnants were stored at -80°C for further analysis. Fresh samples (0.5 g) were mixed with 0.5 mL alcohol, stored at room temperature for 30 min, and centrifuged at 3500 rpm for 15 min. The pellets were incubated on cycloserine cefoxitin fructose agar (CCFA), and cultured at 37°C for 48 h under anaerobic environment.

Table 1

Clinical features of patients in the CDI group and NCDI group.

Characteristics	CDI group (n = 34)	NCDI group (n = 492)	P value
Age (yr, mean \pm SD)	55.8 \pm 12.7	53.2 \pm 12.0	0.141
Sex (male)	20 (58.8%)	271 (55.1%)	0.680
Multiple hospitalization	20 (58.8%)	132 (26.8%)	<0.001
Length of hospital stay (d)	20.26 \pm 7.08	12.59 \pm 4.64	0.002
Decrease of platelet	15 (44.1%)	152 (30.9%)	0.052
CDC	27 (79.4%)	77 (15.7%)	<0.001
WBC ($\times 10^9/\text{L}$)	6.16 \pm 2.90	5.53 \pm 4.19	0.367
HGB (g/L)	106.89 \pm 24.29	103.10 \pm 24.89	0.365
PLT ($\times 10^9/\text{L}$)	92.39 \pm 47.21	94.72 \pm 76.94	0.854
ALB (g/L)	31.84 \pm 6.04	32.60 \pm 4.12	0.293
Cr ($\mu\text{mol/L}$)	85.31 \pm 65.24	72.28 \pm 27.07	0.016
TBIL ($\mu\text{mol/L}$)	140.75 \pm 96.27	114.97 \pm 85.42	<0.001
PTA < 40%	13 (38.2%)	120 (24.4%)	0.126

SD: standard deviation; CDI: *Clostridium difficile* infection; CDC: *C. difficile* colonization; PTA: prothrombin activity; WBC: white blood cell; HGB: hemoglobin; PLT: platelet; ALB: albumin; TBIL: total bilirubin; Cr: creatinine.

About 48 h after bacterial culture, the suspicious colony was subcultured to the blood plates. Two days later, we selected the colony featured by thin and flat profile with slight bulge, in a grey ground glass pattern presenting a flavor of horseshit, without hemolytic crisis. Semi-quantitative analysis was carried out to the number of suspicious colony on the CCFA plates. The semi-quantitative analysis criteria were as follows: few, <50; moderate, 50–100; many, >100. The identification of *C. difficile* was conducted using mass spectrograph (Bruker Daltonics, Billerica, Massachusetts, USA).

Detecting *C. difficile* toxin genes

Single colony DNA was extracted as previously described [21]. *C. difficile* toxin *tcd A* and *tcd B* genes were determined using PCR assay [22]. Genes encoding the enzymatic (*cdtA*) and binding (*cdtB*) components of the binary toxin were determined by PCR assay [23].

Statistical analysis

SPSS 18.0 software was used for the statistical analysis (SPSS Inc., Chicago, IL, USA). Measurement data were presented as mean \pm standard deviation (SD). Student's *t* test was used for the inter-group comparison. Enumeration data were presented as percentage and frequency, and were analyzed using Chi-squared test. Logistic regression analysis was used to identify the risk factors of CDI. Odds ratios (OR) and their 95% confidence intervals (95% CI) were reported where appropriate. A $P < 0.05$ was considered statistically significant.

Results

Comparison of clinical characteristics in the CDI and NCDI groups

Among the 526 patients, 34 (6.5%) patients showed CDI. Table 1 showed the general information and laboratory test results for the subjects of the two groups. There were no statistical differences in the age, sex, and PTA, WBC, HGB, PLT and ALB between the two groups ($P > 0.05$). CDC prevalence in the CDI group was significantly higher than that of the NCDI group (79.4% vs. 15.7%, $P < 0.001$). The length of hospital stay in the CDI group was significantly longer than that of the NCDI group (20.26 \pm 7.08 days vs. 12.59 \pm 4.64 days, $P = 0.002$). The multiple hospitalization was significantly increased in the CDI group compared with the NCDI group (58.8% vs. 26.8%, $P < 0.001$). Compared with the NCDI group, TBIL (140.75 \pm 96.27 $\mu\text{mol/L}$ vs. 114.97 \pm 85.42 $\mu\text{mol/L}$, $P < 0.001$) and

Table 2
Comparison of hepatic cirrhosis complications and interference factors in the CDI group and NCDI group.

Characteristics	CDI group (n = 34)	NCDI group (n = 492)	P value
Child-Pugh grade C	17 (50.0%)	143 (29.1%)	0.019
Spontaneous peritonitis	13 (38.2%)	151 (30.7%)	0.346
Hepatic encephalopathy	12 (35.3%)	51 (10.4%)	<0.001
Hepatorenal syndrome	12 (35.3%)	47 (9.6%)	0.070
Upper gastrointestinal hemorrhage	6 (17.6%)	30 (6.1%)	0.022
Antibiotics	25 (73.5%)	203 (41.3%)	<0.001
Third generation cephalosporin	7 (20.6%)	98 (19.9%)	1.000
Penicillin	10 (29.4%)	89 (18.1%)	0.113
Carbapenems	9 (26.5%)	45 (9.1%)	0.004
Quinolones	5 (14.7%)	55 (11.2%)	0.573
Vancomycin	2 (5.9%)	10 (2.0%)	0.178
Antifungal drug	3 (8.8%)	44 (8.9%)	1.000
Proton pump inhibitors	28 (82.4%)	255 (51.8%)	0.001

CDI: *Clostridium difficile* infection; CDC: *C. difficile* colonization.

Cr (85.31±65.24 μmol/L vs. 72.28±27.07 μmol/L, $P=0.016$) were significantly increased in the CDI group.

Comparison of cirrhosis-related complications and prognosis

Compared with the NCDI group, patients in the CDI group had higher frequency of Child-Pugh grade C (50.0% vs. 29.1%, $P=0.019$), hepatic encephalopathy (35.3% vs. 10.4%, $P<0.001$), and upper gastrointestinal hemorrhage (17.6% vs. 6.1%, $P=0.022$) (Table 2).

The incidence of antibiotic exposure (73.5% vs. 41.3%, $P<0.001$), carbapenem antibiotics (26.5% vs. 9.1%, $P=0.004$) and proton pump inhibitor (PPI, 82.4% vs. 51.8%, $P=0.001$) in the CDI group was significantly higher compared with those in the NCDI group (Table 2).

Univariate analysis for the risk factors of CDI in cirrhotic patients

Univariate analysis showed that age (>60 years) (OR=3.385; 95% CI: 1.440–7.954), multiple hospitalization (OR=3.896, 95% CI: 1.913–7.937) and the length of hospital stay >20 days (OR=4.040; 95% CI: 1.819–8.970) were the risk factors for CDI in cirrhotic patients, together with hypoproteinemia (OR=1.919; 95% CI: 1.109–3.412) and CDC (OR=20.788; 95% CI: 8.743–49.429) upon admission. Moreover, hepatorenal syndrome (OR=5.861; 95% CI: 2.757–12.460), hepatic encephalopathy (OR=4.717; 95% CI: 2.204–10.093) and Child-Pugh grade C (OR=2.441; 95% CI: 1.212–4.914) were the risk factors for CDI. Antibiotics (OR=1.887; 95% CI: 1.138–4.127), carbapenems (OR=3.576; 95% CI: 1.573–8.129), and utilization of PPI (OR=4.337; 95% CI: 1.765–10.660) were the risk factors for CDI during the hospitalization (Table 3).

Logistic regression analysis for CDI

Table 4 showed the multivariate logistic regression analysis. Aged >60 years (OR=1.689; 95% CI: 1.135–3.128), multiple hospitalization (OR=3.346; 95% CI: 1.392–8.043), length of hospital stay >20 days (OR=1.564; 95% CI: 1.113–2.563), hypoproteinemia (OR=4.962; 95% CI: 2.053–11.996), CDC (OR=18.410; 95% CI: 6.898–49.136), hepatic encephalopathy (OR=1.357; 95% CI: 1.154–2.368), and exposure of antibiotics (OR=1.865; 95% CI: 1.213–2.863) and PPI (OR=3.125; 95% CI: 1.818–7.548) were the risk factors for CDI in hepatic cirrhosis patients.

Discussion

The prevalence of CDI is on an increasing trend worldwide. In a previous National Hospital Discharge Survey (NHDS) in USA, the

Table 3
Univariate analysis for the risk factors of CDI.

Parameters	OR	95% CI	P value
Age >60 years	3.385	1.440–7.954	0.005
Multiple hospitalization	3.896	1.913–7.937	<0.001
Length of hospital stay > 20 days	4.040	1.819–8.970	0.001
Hypoproteinemia	1.919	1.109–3.412	0.033
Decrease of platelet	1.766	0.874–3.568	0.113
CDC	20.788	8.743–49.429	<0.001
Hepatorenal syndrome	5.861	2.757–12.460	<0.001
Spontaneous peritonitis	1.398	0.682–2.866	0.360
Hepatic encephalopathy	4.717	2.204–10.093	<0.001
Upper gastrointestinal hemorrhage	3.300	0.869–8.584	0.112
Child-Pugh grade C	2.441	1.212–4.914	0.012
Antibiotics	1.887	1.138–4.127	0.014
Carbapenems	3.576	1.573–8.129	0.002
Quinolones	1.370	0.509–3.685	0.533
Proton pump inhibitors	4.337	1.765–10.660	0.001

CDI: *Clostridium difficile* infection; CDC: *C. difficile* colonization; 95% CI: 95% confidence interval; OR: odds ratio.

Table 4
Multivariate logistic regression analysis for the risk factors of CDI.

Parameters	OR	95% CI	P value
Age >60 years	1.689	1.135–3.128	0.045
Multiple hospitalization	3.346	1.392–8.043	0.007
Length of hospital stay > 20 days	1.564	1.113–2.563	0.046
Hypoproteinemia	4.962	2.053–11.996	<0.001
CDC	18.410	6.898–49.136	<0.001
Hepatic encephalopathy	1.357	1.154–2.368	0.021
Antibiotics	1.865	1.213–2.863	0.025
Proton pump inhibitor	3.125	1.818–7.548	0.043

CDI: *Clostridium difficile* infection; CDC: *C. difficile* colonization; OR: odds ratio; CI: confidence interval.

mortality and morbidity of hospital and community CDI are increasing, with a prevalence of 0.8% among the adult patients [24]. To our best knowledge, about one third of the cirrhotic patients may present bacterial infection during the hospital stay [6–11], with CDI as the predominant type of infection [25]. In clinical practice, patients with hepatic disorders are more likely to develop CDI. In a retrospective analysis, among the cirrhotic patients presented upper gastrointestinal hemorrhage and received antibiotics, the prevalence of CDI was about 5.7%, while the prevalence was 5% in the other patients receiving broad-spectrum antibiotics [7]. In this prospective analysis, we reported a CDI prevalence of 6.5% among the cirrhotic patients (34/526), which was higher than the previous study [26]. This may be related to the variance of subjects, severity of the disease and the geographic differences [27]. Retrospective analysis in previous study may also play a role [28].

CDI affects the prognosis of cirrhotic patients. In a previous study, CDI, variceal bleeding and hepatic encephalopathy were independent risk factors for cirrhosis related death [12]. In 2010, Bajaj et al. included 1165 cirrhotics with and 82 065 without *C. difficile* associated diarrhea (CDAD), in which cirrhotics with CDAD had a significantly higher mortality, and length of hospital stay compared with those without CDAD [12]. Utilization of antibiotics and PPI were the risk factors for CDAD among cirrhotic patients. Cirrhotic patients, especially those admitted with complications of cirrhosis or infections requiring antibiotics should be aggressively monitored and treated for possible *C. difficile*.

These studies suggested that close monitoring is necessary to the patients requiring antibiotics [12,17]. In this study, we included CDC into the risk factor analysis, and *C. difficile* detection was performed to all the cases together with CDI in order to avoid the misdiagnosis. This contributed to the early diagnosis and treatment of *C. difficile* infection.

CDI was reported to affect the prognosis of cirrhotic patients, and cirrhosis may affect the pathogenesis of CDI [9,12,28]. Increasing evidence indicated that liver diseases may increase the risk of CDI, while utilization of antibiotics and PPI was the risk factor for CDI [12,29–31]. Abdominal infection, hepatic encephalopathy, ascites and upper gastrointestinal hemorrhage were the major causes for CDI. Generally, PPI and antibiotics are for the cirrhotic patients. However, PPI and antibiotics cause dysbiosis of the gut microbiota. In normal individuals, the gut microbiota may modulate the immune defense to avoid the invasion of spoilage organisms through releasing compounds with antiseptic activities. When using the broad-spectrum antibiotics, the structure of gut microbiota may alternate and the resistance to colonization may decrease, which triggered the CDI. Recently, one study reported that clindamycin is closely related to the pathogenesis of CDI, together with other antibiotics such as Fluoroquinolones, third generation cephalosporin, and carbapenems [12].

The effects of PPI on CDI in cirrhotic patients are well documented [32]. Using a Cox model, Bajaj et al. [33] evaluated the risk ratio of infections in patients with decompensated cirrhosis the effects of PPI and H2 receptor antagonists (H2RAs) on spontaneous bacterial peritonitis, bacteremia, *C. difficile* and pneumonia. They found that about more than half of the decompensated cirrhotic patients received gastric acid inhibitors, in which 25.3% of them showed severe infection. H2RAs and PPI are high risk factors of CDI in cirrhotic patients [33]. The present study further demonstrated that PPI was an independent risk factor for CDI in cirrhotic patients.

Smith et al. [16] compared cirrhotic patients with CDI and those without CDI, and found that frequent utilization of antibiotics and ICU stay were the risk factors for CDI. They also found that hypoproteinemia and ICU stay were strong risk factors for CDI related death. Our data showed that the prevalence of CDI was high in the patients with Child-Pugh grade C, and multivariate analysis showed that hypoproteinemia and hepatic encephalopathy were risk factors for CDI. Khanna et al. [24] found that, after adjusting for age, sex, and comorbid conditions, CDI was still an independent predictor of longer hospital stay and all-cause mortality. In addition, they found that the prevalence of CDI among the aged population was higher than that of the young population, and that the outcome in the aged population was poorer. The predictors for all-cause mortality for CDI patients included age (>65 years), colectomy and coagulation disorder. On this basis, an age of >65 years should be added to the severity criteria of CDI [24]. Our data showed that the prevalence of CDI in the cirrhotic patients aged >60 years was obviously higher than that of the NCDI group, while regression analysis showed that advanced age was an independent risk factor for CDI.

In conclusion, our study showed age >60 years, multiple hospitalization, length of hospital stay >20 days, hypoproteinemia, CDC, hepatic encephalopathy, and exposure of antibiotics and PPI were risk factors for CDI in cirrhotic patients. These may help the clinician to diagnose the patients with CDI in early stage.

Contributors

LLJ proposed the study. YD and LLJ performed the research and wrote the first draft. HYD collected and analyzed the data. All authors contributed to the design and interpretation of the study and to further drafts. YD, HYD and CYB contributed equally to this work. LLJ is the guarantor.

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Ethical approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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