Nonalcoholic fatty liver disease is associated with worse intestinal complications in patients hospitalized for *Clostridioides difficile* infection

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**Abstract**

**BACKGROUND**

Nonalcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver disease with increasing prevalence worldwide. *Clostridioides difficile* infection (CDI) remains the most common cause of nosocomial diarrhea in developed countries.

**AIM**

To assess the impact of NAFLD on the outcomes of hospitalized patients with CDI.

**METHODS**

This study was a retrospective cohort study. The Nationwide Inpatient Sample database was used to identify a total of 7239 adults admitted as inpatients with a primary diagnosis of CDI and coexisting NAFLD diagnosis from 2010 to 2014 using ICD-9 codes. Patients with CDI and coexisting NAFLD were compared to those with CDI and coexisting alcoholic liver disease (ALD) and viral liver disease (VLD), individually. Primary outcomes included mortality, length of stay, and total hospitalization charges. Secondary outcomes were in-hospital complications. Multivariate regression was used for outcome analysis after adjusting for possible confounders.
Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous disease with a spectrum from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma[1,2]. With a prevalence of 10 to 46 percent in the United States and 6% to 35% worldwide[3,4], NAFLD has become the leading cause of chronic liver disease, and its prevalence continues to increase, paralleled by the increase of obesity and type 2 diabetes[5].

Clostridioides difficile (C. difficile) is a gram-positive, spore-forming bacterium, known as the most common pathogen causing nosocomial diarrhea in developed countries[6]. Symptoms of C. difficile infection (CDI) range from mild to severe diarrhea, which can progress to sepsis, fulminant colitis, and bowel perforation[7]. Severe colitis may also present as ileus and megacolon, which are characterized by symptoms of intestinal obstruction[8,9]. Gut microbiota dysbiosis due to the administration of antibiotics is the most prominent risk factor for the development of CDI. Advanced age, prolonged hospitalization and gastric acid suppression are some common additional risk factors for CDI[10,11].

CONCLUSION
Hospitalized CDI patients with NAFLD had more intestinal complications compared to CDI patients with VLD and ALD. Gut microbiota dysbiosis may contribute to the pathogenesis of intestinal complications.

Key Words: Nonalcoholic fatty liver disease; Clostridioides difficile infection; Gut microbiota; Intestinal complications; Alcoholic liver disease; Viral liver disease

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Core Tip: This study demonstrated that patients hospitalized with Clostridioides difficile infection (CDI) and coexisting nonalcoholic fatty liver disease (NAFLD) had more favorable overall outcomes but higher rates of intestinal complications when compared to those with alcoholic liver disease and viral liver disease individually, which suggests altering gut microbiota may play an essential role in the pathogenesis of both CDI and NAFLD. NAFLD-associated metabolic syndrome may contribute significantly to gut dysbiosis and increase risk for CDI and its complications. This study provides potential directions for future prospective clinical research to identify the clinical meaningfulness of interactions between the gut microbiota, gut immunity and systemic inflammation.

INTRODUCTION
Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous disease with a spectrum from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma[1,2]. With a prevalence of 10 to 46 percent in the United States and 6% to 35% worldwide[3,4], NAFLD has become the leading cause of chronic liver disease, and its prevalence continues to increase, paralleled by the increase of obesity and type 2 diabetes[5].

Clostridioides difficile (C. difficile) is a gram-positive, spore-forming bacterium, known as the most common pathogen causing nosocomial diarrhea in developed countries [6]. Symptoms of C. difficile infection (CDI) range from mild to severe diarrhea, which can progress to sepsis, fulminant colitis, and bowel perforation[7]. Severe colitis may also present as ileus and megacolon, which are characterized by symptoms of intestinal obstruction[8,9]. Gut microbiota dysbiosis due to the administration of antibiotics is the most prominent risk factor for the development of CDI. Advanced age, prolonged hospitalization and gastric acid suppression are some common additional risk factors for CDI[10,11].
Recently, a number of animal and human studies have revealed the role of the gut microbiota in the pathophysiology of NAFLD. It is proposed that dysbiosis-induced dysregulation of the gut barrier function and translocation of the bacteria link the gut microbiome to NAFLD\cite{12,13}. In addition, it has been well documented that patients with chronic liver disease are more susceptible to CDI due to frequent hospitalization and antibiotics use. Specifically, recent studies have observed that NAFLD is an independent risk factor for CDI by single-centered retrospective design\cite{14,15}.

Although a strong association between NAFLD and CDI has been observed, gut microbiota dysbiosis likely plays a vital role in the pathogenesis of both aforementioned diseases. However, the inpatient outcomes of CDI in the NAFLD population, have not been well studied in large populations. The aim of this nationwide study was to assess the impact of NAFLD on the outcomes of hospitalized patients with CDI.

**MATERIALS AND METHODS**

**Data source and study population**

The largest all-payer inpatient care database in the United States, the Nationwide Inpatient Sample (NIS) database was accessed. The NIS database represents approximately 20% of all inpatient hospitalizations. Weighted, it estimates more than 35 million hospitalizations nationally\cite{16}. It includes demographic information (age, sex, race, income), hospital characteristics (e.g., bed size, type), insurance status, discharge status, diagnoses and procedures (identified by The International Classification of Diseases-Ninth Edition Revision Clinical Modification (ICD-9 CM) codes), total hospitalization charges, length of stay (LOS), severity and other comorbidity measures. Yearly sampling weights are applied to generate national estimates.

This retrospective cohort study examined all adult (18-90 years old) patients hospitalized with CDI as the primary diagnosis from 2010 to 2014. Within this CDI population, patients with NAFLD were selected to compare to those with viral liver disease (VLD) (including hepatitis B infection and hepatitis C infection) and those with alcoholic liver disease (ALD). Notably, CDI was identified by ICD-9 CM code 008.45. NAFLD was identified by ICD-9 CM code 571.80 with the exclusion of all diagnostic codes for previous organ recipients and donors as well as other causes of chronic liver disease including hepatitis B and hepatitis C infection, ALD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases. The diagnosis of VLD was identified by the ICD-9 CM codes for hepatitis B and C caused liver diseases with the exclusion of previous organ recipients and donors, as well as other causes of chronic liver disease including NAFLD, ALD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases. The diagnosis of ALD was identified by the ICD-9 CM codes for ALD with the exclusion of previous organ recipients and donors as well as other causes of chronic liver disease including NAFLD, VLD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases (see Supplementary Table 1, supplemental digital content 1, which demonstrates ICD-9 diagnostic and procedure codes). VLD and ALD were assessed as separate groups which excluded patients with concomitant diagnoses of VLD and ALD. Information such as patients’ demographics, comorbidities, disposition, selected outcomes and surgical interventions were extracted from the NIS database. Elixhauser Comorbidity Index (ECI)\cite{17}, which measures 29 general medical conditions, then assigns different weights to compile a longitudinal score, allowing for further description of comorbidity burden.

Primary outcomes included mortality, length of stay, and total hospitalization charges. Secondary outcomes were CDI related complications and interventions.

**Statistical analysis**

SAS Survey Procedures (SAS 9.4, SAS Institute Inc, Cary, NC, United States) was utilized for all statistical analyses. The national estimates were calculated after accounting for sample design elements (clusters, strata, and trend weights) provided by the NIS. Continuous variables were reported as weighted mean ± SE; categorical variables were reported as weighted numbers (n) and percentages (%). The SEs of weighted means were estimated using the Taylor linearization method that incorporated the sample design. Weighted Student’s t-tests were used to analyze the normally distributed continuous variables, while Rao-Scott modified chi-square tests were used to test the difference of distribution for categorical variables. Wilcoxon Rank-Sum Tests were used to test the variables that are not normally distributed.
Multivariate linear regression was used to estimate the average change in LOS and total hospitalization charges after adjusting for patient demographics, hospital characteristics, insurance type, median household income, ECI score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma. Multivariate logistic regression was used to estimate the odds ratio (OR) of mortality, CDI complications and interventions after adjusting for the same confounding variables as noted above.

The statistical methods of this study were reviewed by Dr. Chunyi Wu, PhD of Epidemiology from University of Michigan Medical School.

RESULTS

Patient demographics and baseline characteristics

From 2010 to 2014, the numbers of patients hospitalized for CDI with coexisting NAFLD, VLD and ALD were 7239, 11857 and 5938, respectively. The CDI with NAFLD cohort in this study was predominantly Caucasian with an average age 56.3 years old. In the aforementioned cohort, 69.4% of the patients were female, 41.6% were admitted to southern hospitals, and 58.6% were admitted to large hospitals (Table 1). Compared to CDI with VLD or ALD individually, the CDI with NAFLD group had significantly more patients in the 18-39 and greater than 70-year-old age groups ($P < 0.0001$), were more likely to be female ($P < 0.0001$), from the southern hospital region ($P < 0.0001$), and less likely to be Medicaid insured ($P < 0.0001$). Additionally, the CDI with coexisting VLD group was associated with a higher percentage of African American patients and had less patients with a high household income (Q3 and Q4, median household income for ZIP code between 51th and 100th percentile) compared to the CDI with NAFLD group.

In regard to comorbidities (Table 2), when compared to the CDI with VLD or ALD groups individually, CDI patients with NAFLD had a greater prevalence of obesity ($P < 0.0001$, $P < 0.0001$), diabetes ($P < 0.0001$, $P < 0.0001$), hypertension ($P = 0.0006$, $P < 0.0001$) and dyslipidemia ($P < 0.0001$, $P < 0.0001$). CDI with NAFLD was also associated with a significantly lower rate of cirrhosis ($P < 0.0001$, $P < 0.0001$) when compared to the other two groups. None of the patients in the CDI with NAFLD group had cirrhosis-related ascites, esophageal varices bleeding, spontaneous bacterial peritonitis or hepatorenal syndrome. Moreover, a lower rate of hepatocellular carcinoma ($P < 0.0001$, $P = 0.0217$) was observed in the CDI with NAFLD group compared to the CDI with VLD or ALD groups individually.

Outcomes and regression analysis of CDI patients with NAFLD vs VLD

When compared to the CDI with NAFLD group, the CDI with VLD group was associated with higher rates of acute kidney injury (AKI) [adjusted OR (aOR) = 1.35, 95%CI: 1.10-1.67, $P = 0.0041$], respiratory failure (RF) (aOR = 1.83, 95%CI: 1.22-2.76, $P = 0.0036$), longer LOS (adjusted LOS ratio = 1.12, 95%CI: 1.06-1.18, $P < 0.0001$) and higher hospitalization charges (adjusted cost ratio = 1.13, 95%CI :1.06-1.2, $P < 0.0001$). However, a lower rate of intestinal perforation rate was observed in the CDI with VLD group (aOR = 0.12, 95%CI: 0.03-0.57, $P = 0.0075$). CDI with VLD was initially associated with higher rates of mortality, colectomy and ileostomy, however this difference no longer existed after adjusting for confounding factors (Table 3).

Outcomes and regression analysis of CDI patients with NAFLD vs ALD

When compared to CDI patients with NAFLD, CDI patients with ALD had higher rates of RF (aOR = 1.72, 95%CI: 1.09-2.72, $P = 0.0201$), mortality (aOR = 2.63, 95%CI: 1.25-5.51, $P = 0.0107$), longer LOS (adjusted LOS ratio = 1.18, 95%CI: 1.10-1.25, $P < 0.0001$) and higher hospitalization charges (adjusted cost ratio = 1.17, 95%CI: 1.09-1.26, $P < 0.0001$). However, a lower rate of intestinal obstruction (aOR = 0.45, 95%CI: 0.28-0.72, $P = 0.0010$) was found in the CDI with ALD group when compared to the CDI with NAFLD group. Higher rates of AKI and septic shock, and a lower rate of colectomy were initially observed in CDI with ALD group, but the difference no longer existed after adjusting for the aforementioned confounders (Table 4).
Table 1 Comparison of demographic data for patients hospitalized with *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease, viral liver disease and alcoholic liver disease

| Variables                          | CDI with NAFLD | CDI with VLD | CDI with ALD | P value | CDI with NAFLD vs CDI with VLD | CDI with NAFLD vs CDI with ALD |
|------------------------------------|----------------|--------------|--------------|---------|-------------------------------|-------------------------------|
| **n (weighted)**                   | 7239           | 11857        | 5938         |         |                               |                               |
| Age (yr)                           | 56.32 ± 0.42   | 57 ± 0.26    | 56.13 ± 0.37 | 0.15    | < 0.0001                      | < 0.0001                      |
| 18-39                              | 1133 (15.6%)   | 791 (6.7%)   | 557 (9.4%)   |         |                               |                               |
| 40-49                              | 1290 (17.8%)   | 1811 (15.3%) | 1051 (17.7%) |         |                               |                               |
| 50-59                              | 1618 (22.4%)   | 4873 (41.1%) | 2021 (34%)   |         |                               |                               |
| 60-69                              | 1620 (22.4%)   | 2791 (23.5%) | 1439 (24.2%) |         |                               |                               |
| ≥ 70                               | 1578 (21.8%)   | 1591 (13.4%) | 870 (14.7%)  |         |                               |                               |
| **Sex**                            |               |              |              |         |                               |                               |
| Female                             | 5023 (69.4%)   | 5795 (48.9%) | 2300 (38.7%) |         |                               |                               |
| **Race**                           |               |              |              |         |                               |                               |
| Caucasian                          | 5427 (75%)     | 6920 (58.4%) | 4358 (73.4%) |         |                               |                               |
| African American                   | 482 (6.5%)     | 2773 (23.4%) | 525 (8.8%)   |         |                               |                               |
| Hispanic                           | 648 (9%)       | 1144 (9.6%)  | 515 (8.7%)   |         |                               |                               |
| **Hospital bed size**              |               |              |              |         | 0.033                         | 0.9                           |
| Large                              | 4241 (58.6%)   | 7414 (62.6%) | 3461 (58.3%) |         |                               |                               |
| **Hospital region**                |               |              |              |         | < 0.0001                      | < 0.0001                      |
| Northeast                          | 1091 (15.1%)   | 2618 (22.1%) | 1243 (20.9%) |         |                               |                               |
| Midwest                            | 1618 (22.3%)   | 2514 (21.1%) | 1584 (26.7%) |         |                               |                               |
| South                              | 3008 (41.6%)   | 4208 (35.5%) | 1671 (28.1%) |         |                               |                               |
| West                               | 1522 (21%)     | 2517 (21.2%) | 1440 (24.3%) |         |                               |                               |
| **Hospital type**                  |               |              |              |         | < 0.0001                      | 0.22                          |
| Urban teaching                     | 3401 (47%)     | 7207 (60.8%) | 3065 (51.6%) |         |                               |                               |
| **Insurance**                      |               |              |              |         | < 0.0001                      | < 0.0001                      |
| Medicare                           | 3086 (42.6%)   | 5493 (46.3%) | 2239 (37.7%) |         |                               |                               |
| Medicaid                           | 914 (12.6%)    | 3329 (28.1%) | 1261 (21.2%) |         |                               |                               |
| Private                            | 2526 (34.9%)   | 1835 (15.5%) | 1391 (23.4%) |         |                               |                               |
| Median household income for ZIP Code, % |           |              |              | < 0.0001 |                               |                               |
| Q1                                 | 1790 (24.7%)   | 4205 (35.5%) | 1592 (26.8%) |         |                               |                               |
| Q2                                 | 1824 (25.2%)   | 3128 (26.4%) | 1407 (23.7%) |         |                               |                               |
| Q3                                 | 1926 (26.6%)   | 2333 (19.8%) | 1503 (25.3%) |         |                               |                               |
| Q4                                 | 1511 (20.9%)   | 1657 (14%)   | 1252 (21.1%) |         |                               |                               |

Values reported as weighted mean ± SE and weighted number [n (%)]. CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; VLD: Viral liver disease; ALD: Alcoholic liver disease; Q1: Quartile 1, 0-25th percentile; Q2: Quartile 2, 26th-50th percentile; Q3: Quartile 3, 51th-75th percentile; Q4: Quartile 4, 76th-100th percentile.

**DISCUSSION**

This nationwide retrospective cohort study investigated the inpatient clinical characteristics and outcomes of CDI in hospitalized patients with coexisting liver diseases, with comparisons between NAFLD, VLD and ALD. We demonstrated that patients hospitalized with CDI and coexisting NAFLD had overall more favorable outcomes including a lower rate of RF, lower hospitalization charges and a shorter LOS when...
### Table 2 Comparison of comorbid conditions and complications for patients hospitalized with *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease, viral liver disease and alcoholic liver disease

| Variables                                      | CDI with NAFLD | CDI with VLD | CDI with ALD | P value       | CDI with NAFLD vs CDI with VLD | CDI with NAFLD vs CDI with ALD |
|------------------------------------------------|----------------|--------------|--------------|---------------|-------------------------------|-------------------------------|
| n (weighted)                                   | 7239           | 11857        | 5938         |               |                               |                               |
| Number of Elixhauser comorbidities             |                |              |              | < 0.0001      |                               |                               |
| 0                                              | 0 (0%)         | 114 (1%)     | -            |               |                               |                               |
| 1                                              | 244 (3.4%)     | 574 (4.8%)   | 116 (2%)     |               |                               |                               |
| 2                                              | 656 (9.1%)     | 1409 (11.9%) | 354 (6%)     |               |                               |                               |
| ≥ 3                                            | 6338 (87.6%)   | 9760 (82.3%) | 5463 (92%)   |               |                               |                               |
| Obesity                                        | 2012 (27.8%)   | 850 (7.2%)   | 372 (6.3%)   | < 0.0001      |                               |                               |
| Diabetes                                       | 2750 (38%)     | 3451 (29.1%) | 1170 (19.7%) | < 0.0001      |                               |                               |
| Hypertension                                   | 4300 (59.4%)   | 6347 (53.5%) | 2980 (50.2%) | 0.00058       |                               |                               |
| Dyslipidemia                                   | 2619 (36.2%)   | 1868 (15.8%) | 905 (15.2%)  | < 0.0001      | < 0.0001                      |                               |
| Hepatocellular carcinoma                       | -              | 253 (2.1%)   | 45 (0.8%)    | < 0.0001      |                               | 0.0217                        |
| Cirrhosis related comorbidities³               |                |              |              |               |                               |                               |
| Cirrhosis                                      | 401 (5.5%)     | 2508 (21.2%) | 3407 (57.4%) | < 0.0001      |                               |                               |
| Number of cirrhosis complications              |                |              |              | 0.0013        |                               |                               |
| 0                                              | 137 (34.2%)    | 1773 (70.7%) | 2105 (61.8%) |               |                               |                               |
| 1                                              | 244 (60.8%)    | 688 (27.4%)  | 1104 (32.4%) |               |                               |                               |
| v ≥ 2                                          | 20 (5.0%)      | 47 (1.9%)    | 198 (5.8%)   |               |                               |                               |
| Ascites                                        | 0 (0%)         | 0 (0%)       | 0 (0%)       | NA            | NA                            | NA                            |
| Esophageal varices bleeding                     | 0 (0%)         | -            | 20 (0.6%)    | NA            | NA                            | NA                            |
| Hepatic encephalopathy                         | 110 (27.4%)    | 60 (2.4%)    | 569 (16.7%)  | 0.003338      |                               |                               |
| Hepatorenal syndrome                           | 0 (0%)         | 15 (0.6%)    | 33 (1.0%)    | NA            | NA                            | NA                            |
| Portal hypertension                            | 175 (43.6%)    | 661 (26.4%)  | 843 (24.7%)  | < 0.0001      |                               | < 0.0001                      |
| Spontaneous bacterial peritonitis              | 0 (0%)         | 38 (1.5%)    | 40 (1.2%)    | NA            | NA                            | NA                            |

³Value reported as percentage of all cirrhotic patients. Values reported as weighted number [n (%)].

Compared to those with ALD and VLD individually. Interestingly, higher rates of intestinal complications were observed in the CDI with NAFLD group when compared to the CDI with ALD or VLD groups. Specifically, a significantly higher rate of intestinal obstruction was seen in the CDI with NAFLD group when compared to the CDI with ALD group, and a higher rate of intestinal perforation was seen when compared to CDI patients with concomitant VLD.

Our findings of worse intestinal complications in patients hospitalized with CDI and coexisting NAFLD compared to CDI patients with VLD and ALD, link the gut pathology to the liver. The crosstalk between the gut and liver is increasingly recognized as the gut-liver axis[18]. Receiving more than 70% of the blood supply from the intestinal venous outflow, the liver represents the first line of defense against gut derived antigens with a broad array of immune cells[19]. The liver also releases many bioactive mediators into the systemic circulation, allowing for communication with the intestine. In the intestine, the endogenous and exogenous products from host and microbial metabolism translocate to the liver through the portal venous system, ultimately influencing liver function[20].
### Table 3 Multivariate regression analysis of outcomes for patients hospitalized for *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease vs viral liver disease

| Outcomes                  | CDI with NAFLD | CDI with VLD | Unadjusted ratio (95%CI) | $P$ value | Adjusted ratio$^1$ (95%CI) | $P$ value |
|---------------------------|----------------|--------------|--------------------------|-----------|---------------------------|-----------|
| **n (weighted)** | 7239          | 11857        |                          |           |                           |           |
| Hospital mortality        | 59 (0.8%)     | 186 (1.6%)   | 1.94 (1.44, 2.6)         | $< 0.0001$| 1.87 (0.95, 3.7)          | 0.071     |
| Acute kidney injury       | 938 (13%)     | 2035 (17.2%) | 1.39 (1.28, 1.51)        | $< 0.0001$| 1.35 (1.1, 1.67)          | 0.0041    |
| Respiratory failure       | 192 (2.7%)    | 504 (4.2%)   | 1.63 (1.37, 1.92)        | $< 0.0001$| 1.83 (1.22, 2.76)         | 0.0036    |
| Septic shock              | 39 (0.5%)     | 115 (1%)     | 1.8 (1.25, 2.59)         | 0.0015    | 1.64 (0.67, 4.02)         | 0.27      |
| Intestinal perforation    | -             | -            | 0.3 (0.1, 0.89)          | 0.03      | 0.12 (0.03, 0.57)         | 0.0075    |
| Intestinal obstruction    | 331 (4.6%)    | 527 (4.4%)   | 0.97 (0.84, 1.12)        | 0.67      | 0.94 (0.66, 1.33)         | 0.725     |
| Peritonitis               | 61 (0.8%)     | 106 (0.9%)   | 1.06 (0.77, 1.45)        | 0.71      | 0.72 (0.35, 1.52)         | 0.39      |
| Colectomy                 | 45 (0.6%)     | 105 (0.9%)   | 1.43 (1.01, 2.03)        | 0.044     | 1.38 (0.6, 3.15)          | 0.44      |
| Ileostomy                 | -             | 41 (0.3%)    | 2.47 (1.24, 4.92)        | 0.01      | 2.62 (0.66, 10.41)        | 0.17      |
| LOS (days)                | 5.75 ± 0.16   | 6.77 ± 0.16  | 1.11 (1.06, 1.16)        | $< 0.0001$| 1.12 (1.06, 1.18)         | $< 0.0001$|
| Total hospitalization charges (dollars) | 38150.34 ± 1757.01 | 46326.72 ± 1899.82 | 1.14 (1.07, 1.2) | $< 0.0001$ | 1.13 (1.06, 1.2) | $< 0.0001$ |

$^1$Adjusted for age, sex, race, primary insurance payer, hospital type, hospital bed size, hospital region, income quartile, Elixhauser Comorbidity Index score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma.

< Numbers were not displayed due to extremely small numbers were associated with increased risk for identification of persons. Values reported as weighted mean ± SE and weighted numbers [n (%)]; CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; VLD: Viral liver disease; CI: Confidence interval; LOS: Length of stay.

How does NAFLD influence the intestinal complications of CDI through the gut-liver axis? Convincing evidence has shown that NAFLD is associated with significantly increased gut permeability and inflammation in both animal[21] and human models. Miele et al.[22] found that NAFLD patients had significantly increased gut permeability measured by urine radiolabeled markers and immunohistochemical analysis of zona occludens -1 expression in intestinal biopsy specimens, compared with healthy volunteers. They also discovered that both gut permeability and the prevalence of small intestinal bacterial overgrowth are correlated with the severity of steatosis. Verdam et al.[23] found that plasma immunoglobulin G levels against endotoxin were increased in NASH patients, which positively correlated with the severity of inflammation. Furthermore, transmission electron microscopy observed irregular microvilli and widened tight junctions in the gut mucosa of the NAFLD patients[24]. In addition, decreased numbers of CD4+ and CD8+ T lymphocytes and increased levels of TNF-α, IL-6 and IFN-γ were detected in the NAFLD patient group compared to healthy control. All of these results suggested impaired gut permeability and increased levels of inflammation at both the tissue and cellular levels in NAFLD disease models.

The gut microbiota-mediated inflammation, the related disturbance of the intestinal integrity and the impairment in mucosal immune function have been reported to play important roles, not only in the pathophysiology of CDI[25] but also in the pathogenesis of NAFLD[13,24,26]. The gut microbiota normally exerts significant influence on intestinal epithelial cell health, nutrient metabolism and mucosal defense[19,27]. Early evidence in animal studies demonstrated that altered gut microbiota composition[28] independently contributed to the development of NAFLD in mice. In addition, altered interaction between the gut and the host (produced by defective inflammasome sensing in inflammasome-deficient mouse models) may govern the rate of progression of multiple metabolic syndrome-associated abnormalities[29]. With the recent developments in genome sequencing technologies, bioinformatics, and culturomics; it has been recognized that NAFLD and NASH are associated with decreased richness of the gut flora and increased risk of pathogenic flora in pediatric and adult patients[30-34], which are both well known risk factors for CDI. Although it is still unclear which specific microorganisms are harmful given conflicting results in
Table 4 Multivariate regression analysis of outcomes for patients hospitalized for Clostridioides difficile infection with coexisting nonalcoholic fatty liver disease vs alcoholic liver disease

| Outcomes                          | CDI with NAFLD | CDI with ALD | Unadjusted ratio (95%CI) | P value  | Adjusted ratio (95%CI) | P value  |
|-----------------------------------|----------------|--------------|--------------------------|----------|------------------------|----------|
| n (weighted)                      | 7239           | 5938         |                          |          |                        |          |
| Hospital mortality                | 59 (0.8%)      | 159 (2.7%)   | 3.34 (2.48, 4.52)        | < 0.0001 | 2.63 (1.25, 5.51)      | 0.0107   |
| Acute kidney injury               | 938 (13%)      | 935 (15.8%)  | 1.26 (1.14, 1.39)        | < 0.0001 | 1.2 (0.93, 1.54)       | 0.15     |
| Respiratory failure               | 192 (2.7%)     | 249 (4.2%)   | 1.61 (1.33, 1.94)        | < 0.0001 | 1.72 (1.09, 2.72)      | 0.0201   |
| Septic shock                      | 39 (0.5%)      | 79 (1.3%)    | 2.48 (1.69, 3.64)        | < 0.0001 | 2.14 (0.84, 5.46)      | 0.109    |
| Intestinal perforation            | -              | 0 (0%)       | NA                       | NA       | NA                     | NA       |
| Intestinal obstruction            | 331 (4.6%)     | 133 (2.2%)   | 0.48 (0.39, 0.59)        | < 0.0001 | 0.45 (0.28, 0.72)      | 0.0010   |
| Peritonitis                       | 61 (0.8%)      | 69 (1.2%)    | 1.38 (0.97, 1.95)        | 0.071    | 0.54 (0.25, 1.18)      | 0.12     |
| Colectomy                         | 45 (0.6%)      | 15 (0.3%)    | 0.42 (0.23, 0.74)        | 0.003    | 0.44 (0.14, 1.39)      | 0.16     |
| Ileostomy                         | -              | -            | 0.65 (0.23, 1.85)        | 0.42     | 0.99 (0.15, 6.61)      | 0.98     |
| LOS (days)                        | 5.75 ± 0.16    | 6.84 ± 0.23  | 1.14 (1.08, 1.21)        | < 0.0001 | 1.18 (1.1, 1.25)       | < 0.0001 |
| Total hospitalization charges     | 38150.34 ± 1757.01 | 44641.74 ± 1660.66 | 1.14 (1.07, 1.22)        | < 0.0001 | 1.17 (1.09, 1.26)      | < 0.0001 |

1 Adjusted for age, sex, race, primary insurance payer, hospital type, hospital bed size, hospital region, income quartile, Elixauser Comorbidity Index score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma.

Values reported as weighted mean ± SE and weighted numbers [n (%)]. -: Numbers were not displayed due to extremely small numbers were associated with increased risk for identification of persons. CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; ALD: Alcoholic liver disease; LOS: Length of stay.

In human and animal studies[35], it is believed that gut microbiota-derived signatures extracted by whole-genome shotgun sequencing of DNA can be used for diagnosis of advanced fibrosis in NAFLD[36], and modification of gut microbiota analyzed by 16S ribosomal RNA pyrosequencing can be used for therapeutic purposes in NASH patients[37]. Additionally, increased pathogenic flora in NAFLD and NASH further disturb the immune balance and cause worsened dysbiosis through various mechanisms involving short-chain fatty acids[38], lipopolysaccharide[21], choline metabolism[39], bile acid metabolism[40] and bacteria-derived ethanol[41]. Collectively, NAFLD and NASH related alterations of gut microbiota its downstream dysbiosis pathways may contribute to CDI risk and worse intestinal complications.

On the other end, we sought to identify the characteristics of gut microbiota changes in ALD and VLD. Compared to NAFLD, ALD is remarkably similar histologically[42] and initiated directly from the gut by alcohol intake or binges. It has been well documented that alcohol intake can lead to changes in gut microbiota composition[43] and gut permeability[44] early on, even before the development of liver disease. These alterations involve multiple physical and biochemical layers of defense in the intestinal barrier[19]. In VLD, the gut microbiome works as an effective tool early on for immunity against the hepatitis virus, and helps with viral clearance[45]. In chronic VLD, large translocations of intestinal microbiota were observed and thought to contribute to not only dysregulation of immune cells and dysfunction of the intestinal barrier, but also viral replication[27]. Comparison analysis revealed that, compared to other cirrhosis etiologies, alcoholic cirrhosis is associated with worse gut dysbiosis after adjusting for Model For End-Stage Liver Disease score and body mass index[46]. In two other studies[47,48], which primarily compared the gut microbiota composition in HBV/HCV related and alcoholic cirrhosis, no difference was observed at the phylum and class level.

Intriguingly, in our study, the majority (94.5%) of patients in CDI with NAFLD group were non-cirrhotic; the percentage of cirrhotic patients in CDI with NAFLD group was significantly less than those in CDI with ALD or VLD group. CDI with NAFLD group was associated with a higher rate of intestinal complications after adjusting for cirrhosis and its complications. These results suggested that NAFLD is...
associated with altered gut microbiota that is predisposed to CDI and its complications, likely independent from the liver disease severity. In fact, NAFLD has been reported as an independent risk factor for CDI[14]. Although ALD and VLD cirrhosis was previously found to be associated with worse gut dysbiosis than NAFLD cirrhosis, this finding should be treated cautiously for non-cirrhotic patients, because the alteration of the gut microbiome is associated with the severity of liver disease, as significant differences in gut microbiota have been found between non-cirrhotic, compensated and decompensated cirrhotic patients[49,50]. Importantly, the standard of care therapies in cirrhotic patients such as lactulose, rifaximin, antibiotics and acid-suppressants that can affect the gut microbiota, may be playing a critical role[51]. In summary, our study suggested that NAFLD may be associated with worse dysbiosis in early liver disease stages and therefore a higher risk for CDI and its complications compared to ALD and VLD.

Aside from aforementioned gut microbiota changes that directly link NAFLD to CDI and intestinal complications, NAFLD related metabolic syndrome and systemic inflammation also play crucial roles in intestinal pathology. Recently, metabolic dysfunction-associated fatty liver disease has been proposed as a more appropriate name to replace NAFLD by an international panel of experts, with emphasis on the underlying metabolic dysfunction[52,53]. Clinical evidence has demonstrated that NAFLD, along with other components of metabolic syndrome, such as diabetes and obesity, are associated with an increased prevalence of small intestinal bacterial overgrowth (SIBO)[54,55] by insulin resistance, oxidative stress and chronic low grade inflammation[56]. Subsequently, the dysmotility induced by SIBO can further promote NAFLD in NAFLD patients, causing a vicious cycle[57]. In fact, dysmotility itself is associated with NAFLD and may be a potential therapeutic target for NAFLD from a Japanese study[58,59]. Moreover, diabetes, a component of metabolic syndrome which may cause vasculopathies and neuropathies in the intestines, also contributes to dysmotility[60]. Additionally, diverticular disease, irritable bowel disease[61] and inflammatory bowel disease[62], together with SIBO and dysmotility have all been shown to have increased prevalence in NAFLD patients. Not surprisingly, the structural and functional abnormalities in the gut associated with NAFLD and metabolic dysfunction further increase the risk of CDI and its complications.

The strengths of this study include the utilization of the NIS database to provide a unique opportunity to investigate a nationwide population hospitalized for CDI. To the best of our knowledge, this study is a leading clinical research analysis that provided a comprehensive nationwide comparison of outcomes between NAFLD and other common chronic liver diseases, ALD and VLD, in hospitalized CDI patients. There are also limitations in this study. Particularly, NIS data acquisition relies on the accuracy ICD-9-CM codes for medical diagnoses and no lab results, biopsy or image studies were available for NAFLD diagnosis and severity stratification. It is also difficult to determine which cases of CDI were hospital acquired or community acquired because ICD-9 codes are assigned at discharge. To strengthen the validity of ICD-9 codes for NAFLD, VLD and ALD, we used not only diagnostic codes but also excluded the codes for all other chronic liver diseases (Supplementary Table 1)[63]. The ICD-9 codes for CDI were validated previously with good diagnostic accuracy[64,65].

**CONCLUSION**

In conclusion, this study found more favorable overall outcomes but higher rates of intestinal complications in patients hospitalized with CDI and coexisting NAFLD, compared to CDI with coexisting ALD and VLD, individually. These results suggested that NAFLD may be associated with a higher risk of CDI associated intestinal complications through alteration of gut microbiota. Our study also suggested that NAFLD associated metabolic syndrome may contribute significantly to the gut dysbiosis even in the early liver disease stages and cause increased risk for CDI and its complications. During the last few years, the novel and rapidly evolving research technologies for the gut microbiome have been opening up an exciting era in the microbiota therapeutics for different disease models[66]. Tremendous progress has been observed in the treatment of NAFLD and CDI through gut microbiome manipulation. Our study may help increase awareness and diagnose intestinal complications in patients with two common diseases: CDI and NAFLD. Unraveling the significance of interactions between gut microbiota, gut immunity and systemic metabolic impact of NAFLD with prospective studies will provide more insights into the future microbiota therapeutics.
for CDI and NAFLD.

**ARTICLE HIGHLIGHTS**

**Research background**
The ongoing exploration of liver-gut axis has discovered strong association between gut dysbiosis and nonalcoholic fatty liver disease (NAFLD) in both basic science and clinical research. Small-scaled studies have observed that NAFLD is an independent risk factor for *Clostridioides difficile* infection (CDI).

**Research motivation**
CDI, as the most common cause of nosocomial diarrhea in developed countries, carries high hospitalization burden. NAFLD, as the leading cause of chronic liver disease, is commonly seen in hospitalized patients with CDI. So far the inpatient outcomes of CDI in the NAFLD population have not been well studied.

**Research objectives**
The authors aimed to examine the impact of NAFLD on the inpatient outcomes of hospitalized patients with CDI, by comparing the effect of NAFLD with alcoholic liver disease (ALD) and viral liver disease (VLD) individually.

**Research methods**
This nationwide retrospective cohort study was conducted according to STROBE statement using the National Inpatient Sample database. Inpatient CDI with coexisting NAFLD cases were selected using ICD-9 codes. Multivariate regression analysis was used with adjustment for a large group of possible confounders. Elixhauser Comorbidity Index (ECI) was used for a full description of comorbidity burden.

**Research results**
CDI with NAFLD was independently associated with lower rates of acute respiratory failure, shorter length of stay and lower hospitalization charges when compared to CDI with VLD and CDI with ALD. However, CDI with NAFLD was associated with a higher rate of intestinal perforation when compared to VLD, and a higher rate of intestinal obstruction when compared to ALD.

**Research conclusions**
CDI and coexisting NAFLD is associated with favorable overall outcomes, but higher rates of intestinal complications compared to CDI with coexisting ALD and VLD, individually.

**Research perspectives**
This finding suggests that alteration of gut microbiota may play an important role in the pathogenesis of both CDI and NAFLD. NAFLD associated metabolic syndrome may contribute significantly to the gut dysbiosis and cause increased risk for CDI and its complications. This study provides potential directions for future prospective clinical research to identify the clinical meaningfulness of interactions between gut microbiota, gut immunity and systemic inflammation. The study may open the door for potential microbiota therapeutic targets and manipulation as future treatment options for chronic liver diseases.

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**REFERENCES**
1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes.
2 Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021; 18: 223-238 [PMID: 33349658 DOI: 10.1038/s41575-020-00381-6]

3 Williams CD, Stengel J, Askie ML, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011; 140: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.035]

4 Vernon G, Baranov A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011; 34: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

5 Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol 2019; 70: 531-544 [PMID: 30414863 DOI: 10.1016/j.jhep.2018.10.033]

6 Evans CT, Saefdar N. Current Trends in the Epidemiology and Outcomes of Clostridium difficile Infection. Clin Infect Dis 2015; 60 Suppl 2: S66-S71 [PMID: 25922403 DOI: 10.1093/cid/civ140]

7 Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol 2009; 7: 526-536 [PMID: 19528959 DOI: 10.1038/nrmicro2164]

8 McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 2018; 66: e1-e48 [PMID: 29462280 DOI: 10.1093/cid/cix1083]

9 Nwachuku E, Shan Y, Senthil-Kumar P, Braun T, Shadis R, Kirton O, Vu TQ. Toxic Clostridiodes (formerly Clostridium) difficile colitis: No longer a diarrhea associated infection. Am J Surg 2021; 220: 240-242 [PMID: 32600621 DOI: 10.1016/j.amjsurg.2020.06.026]

10 Kistangari G, Lopez R, Shen B. Frequency and Risk Factors of Clostridium difficile Infection in Hospitalized Patients With Pouchitis: A Population-based Study. Inflamm Bowel Dis 2017; 23: 661-671 [PMID: 28296825 DOI: 10.1097/MIB.000000000001057]

11 Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Zalewski P, Olszewska M, Matejka P, Mordarska-Biczek D. Epidemiology of Clostridium difficile infection: a systematic review and meta-analysis. Int J Infect Dis 2020; 92: 105787 [PMID: 32065334 DOI: 10.1016/j.ijid.2020.03.007]

12 Safari Z, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). Cell Mol Life Sci 2019; 76: 1541-1558 [PMID: 30683985 DOI: 10.1007/s00018-019-03011-w]

13 Aron-Wisnewsky J, Vignollet C, Witjes J, Le P, Holleboom AG, Verheij J, Nieuwdorp M, Clément K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. Nat Rev Gastroenterol Hepatol 2020; 17: 279-297 [PMID: 32152478 DOI: 10.1038/s41575-020-0269-9]

14 Nseir WB, Hussein SHH, Farah R, Mahamid MN, Khatib HH, Mograbi JM, Peretz A, Amara AE. Nonalcoholic fatty liver disease as a risk factor for Clostridium difficile-associated diarrheea. QJM 2020; 113: 320-332 [PMID: 31688897 DOI: 10.1093/qjmed/hcz283]

15 Papić N, Ivelović F, Karlović M, Marić LS, Vince A. Nonalcoholic fatty liver disease as a risk factor for Clostridiodes difficile infection. Eur J Clin Microbiol Infect Dis 2020; 39: 569-574 [PMID: 31782025 DOI: 10.1007/s10096-019-03759-w]

16 Agency for Healthcare Research and Quality. Healthcare Cost and Utilization Project (HCUP). 2019. Available from: https://www.ahrq.gov/data/hcup/index.html

17 Moore BJ, White S, Washington R, Coenen N, Elshaxera A. Identifying Increased Risk of Readmission and In-hospital Mortality Using Hospital Administrative Data: The AHRQ Elixhauser Comorbidity Index. Med Care 2017; 55: 698-705 [PMID: 28498196 DOI: 10.1097/MRLR.0000000000000735]

18 Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018; 15: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0111-z]

19 Son G, Kremer M, Hines IN. Contribution of gut bacteria to liver pathobiology. Gastroenterol Res Pract 2010; 2010 [PMID: 20706692 DOI: 10.1155/2010/453563]

20 Stärkel P, Schnabl B. Bidirectional Communication between Liver and Gut during Alcoholic Liver Disease. Semin Liver Dis 2016; 36: 331-339 [PMID: 27999753 DOI: 10.1055/s-0036-1593882]

21 Mao JW, Tang HY, Zhao T, Tan XY, Bi J, Wang BY, Wang YD. Intestinal mucosal barrier dysfunction participates in the progress of nonalcoholic fatty liver disease. Int J Clin Exp Pathol 2015; 8: 3648-3658 [PMID: 26097546]

22 Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Gasparrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

23 Verdam FJ, Rensen SS, Driessen A, Greve JW, Buurman WA. Novel evidence for chronic exposure to endotoxin in human nonalcoholic steatohepatitis. J Clin Gastroenterol 2011; 45: 149-152 [PMID: 20661154 DOI: 10.1097/MCG.0b013e3181e12c24]

24 Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J, Liu Y. Dysbiosis gut microbiota.
associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015; 5: 8096 [PMID: 25644696 DOI: 10.1038/srep08096]

25 Theriot CM, Koenigsknecht MJ, Carlson PE Jr, Hatton GE, Nelson AM, Li B, Huffnagle GB, Z Li J, Young VB. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nat Commun 2014; 5: 3114 [PMID: 24445449 DOI: 10.1038/ncomms4114]

26 Campo L, Eisele S, Apfel T, Pyrsopoulos N. Fatty Liver Disease and Gut Microbiota: A Comprehensive Update. J Clin Transl Hepatol 2019; 7: 56-60 [PMID: 30944821 DOI: 10.14218/JCTH.2018.00068]

27 Sehgal R, Bedi O, Trehanpati N. Role of Microbiota in Pathogenesis and Management of Viral Hepatitis. Front Cell Infect Microbiol 2020; 10: 341 [PMID: 32850467 DOI: 10.3389/fcimb.2020.00341]

28 Le Roy T, Llopis M, Lapage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier AM, Gérard P. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut 2013; 62: 1787-1794 [PMID: 23197411 DOI: 10.1136/gutjnl-2012-303816]

29 Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporese JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammamose-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 2012; 482: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]

30 Tsai MC, Liu YY, Lin CC, Wang CC, Wu YJ, Yong CC, Chen KD, Chua KH, Yao CC, Huang PY, Chen CH, Hu TH, Chen CL. Gut Microbiota Dysbiosis in Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease: A Cross-Sectional Study in Taiwan. Nutrients 2020; 12 [PMID: 32204538 DOI: 10.3390/nu12030820]

31 Schwimmer JB, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, Bross C, Durelle J, Goyal NP, Hamilton G, Holtz ML, Lavine JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic permeability. inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut microbiota using a nutritional intervention. Gut 2019; 68: 608-618 [PMID: 30380572 DOI: 10.1136/gutjnl-2018-314878]

32 Del Chierico F, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnan N, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. Hepatology 2017; 65: 451-464 [PMID: 27028797 DOI: 10.1002/hep.28572]

33 Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. Hepatobiliary Pancreat Dis Int 2017; 16: 375-381 [PMID: 28823367 DOI: 10.1016/S1499-3872(17)60019-5]

34 Wang B, Jiang X, Cao M, Ge J, Bao Q, Tang L, Chen Y, Li L. Altered Fecal Microbiota Correlates with Liver Biochemistry in Nonobese Patients with Non-alcoholic Fatty Liver Disease. Sci Rep 2016; 6: 32002 [PMID: 27550547 DOI: 10.1038/srep32002]

35 Jennison E, Byrne CD. The role of the gut microbiota and diet in the pathogenesis of non-alcoholic fatty liver disease. Clin Mol Hepatol 2021; 27: 22-43 [PMID: 33291863 DOI: 10.3350/cmh.2020.0129]

36 Loomba R, Seguirvan V, Li W, Long T, Kiltgard N, Bhatt A, Dalai PS, Causby C, Bettencourt R, Highland SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-Alcoholic Fatty Liver Disease. Cell 2017; 170: 1054-1062.e5 [PMID: 28467925 DOI: 10.1016/j.cell.2017.04.001]

37 Wong VW, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sun GJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis—an longitudinal study. PLoS One 2013; 8: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]

38 Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Fearnside J, Tatoud R, Blanc L, Neirynck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control susceptibility to Clostridium difficile infection. Gut 2009; 58: 1091-1103 [PMID: 19240062 DOI: 10.1136/gut.2008.165886]

39 Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguet D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 2006; 103: 12511-12516 [PMID: 16895997 DOI: 10.1073/pnas.0601056103]

40 Parséus A, Sommer N, Sommer F, Caesar R, Molinaro A, Ståhlman M, Greiner TU, Perkins R, Bäckhed F. Microbiota-induced obesity requires farnesoid X receptor. Gut 2017; 66: 429-437 [PMID: 26740296 DOI: 10.1136/gutjnl-2015-310283]

41 Aragonés G, Gonzalez-Garcia S, Aguilar C, Richart C, Auguet T. Gut Microbiota-Derived Mediators as Potential Markers in Nonalcoholic Fatty Liver Disease. Biomed Res Int 2019; 2019: 8507583 [PMID: 30719448 DOI: 10.1155/2019/8507583]

42 Brunt EM, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. World J Gastroenterol 2010; 16: 5286-5296 [PMID: 21072891 DOI: 10.3748/wjg.v16.i42.5286]
Bajaj JS. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2019; 16: 235-246 [PMID: 30643227 DOI: 10.1038/s41575-018-0099-1]

Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; 32: 742-747 [PMID: 10845660 DOI: 10.1016/S0168-8278(00)00242-1]

Xu D, Huang Y, Wang J. Gut microbiota modulate the immune effect against hepatitis B virus infection. *Eur J Clin Microbiol Infect Dis* 2015; 34: 2139-2147 [PMID: 26272175 DOI: 10.1007/s10096-015-2464-0]

Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; 60: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]

Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; 54: 562-572 [PMID: 21574172 DOI: 10.1002/hep.24423]

Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittorno H, Ridlon JM, White MB, Noble NA, Monteith P, Fuchs M, Thacker LR, Sikaroodi M, Bajaj JS. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013; 58: 949-955 [PMID: 23333527 DOI: 10.1016/j.jhep.2013.01.003]

Qin N, Yang F, Li A, Priifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014; 513: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

Qin N, Le Chatelier E, Guo J, Priifti E, Li L, Ehrlich SD. Qin et al. reply. *Nature* 2015; 525: E2-E3 [PMID: 26381989 DOI: 10.1038/nature14852]

Bajaj JS, Betrapally NS, Gillevet PM. Decompensated cirrhosis and microbiome interpretation. *Nature* 2015; 525: E1-E2 [PMID: 26381988 DOI: 10.1038/nature14851]

Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiga G, Tairbili C, Yki-Järvinen H, Fan JG, Gronthk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouda Y, Chan WK, Mendez-Sanchez N, Ahe SH, Castera L, Bugianesi E, Ratsu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; 73: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; 158: 1999-2014.e1 [PMID: 32124434 DOI: 10.1053/j.gastro.2019.11.312]

Ferolla SM, Amrilato GN, Coutu CA, Ferrari TC. The role of intestinal bacteria overgrowth in obesity-related nonalcoholic fatty liver disease. *Nutrients* 2014; 6: 5583-5599 [PMID: 25479248 DOI: 10.3390/nu6125583]

Ghoshal UC, Goel A, Quigley EMM. Gut microbiota abnormalities, small intestinal bacterial overgrowth, and non-alcoholic fatty liver disease: An emerging paradigm. *Indian J Gastroenterol* 2020; 39: 9-21 [PMID: 32291578 DOI: 10.1007/s12664-020-01027-w]

Augustyn M, Grys I, Kukla M. Small intestinal bacterial overgrowth and nonalcoholic fatty liver disease. *Clin Exp Hepatol* 2019; 5: 1-10 [PMID: 30915401 DOI: 10.5114/ceh.2019.83151]

Wu WC, Zhao W, Li S. Small intestinal bacterial overgrowth decreases small intestinal motility in the NASC rats. *World J Gastroenterol* 2008; 14: 313-317 [PMID: 18186574 DOI: 10.3748/wjg.v14.i3.131]

Kessoku T, Imajo K, Kobayashi T, Ozaki A, Iwaki M, Honda Y, Kato T, Ogawa Y, Tomono W, Kato S, Higurashi T, Yoneda M, Kirikoshi H, Kubota K, Taguri M, Yamanaka T, Usuda H, Wada K, Kobayashi N, Saito S, Nakajima A. Lubiprostone in patients with non-alcoholic fatty liver disease: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet Gastroenterol Hepatol* 2020; 5: 996-1007 [PMID: 32805205 DOI: 10.1016/S2468-1253(20)30216-8]

Schattenberg JM. Intestinal motility: a therapeutic target for NAFLD? *Lancet Gastroenterol Hepatol* 2020; 5: 957-958 [PMID: 32805206 DOI: 10.1016/S2468-1253(20)30204-1]

Gotfried J, Priest S, Schey R. Diabetics and the Small Intestine. *Curr Treat Options Gastroenterol* 2017; 15: 490-507 [PMID: 28913777 DOI: 10.1007/s10620-017-9555-3]

Weaver MJ, McHenry SA, Sayuk GS, Gyawali CP, Davidson NO. Bile Acid Diarrhea and NAFLD: Shared Pathways for Distinct Phenotypes. *Hepatol Commun* 2020; 4: 493-503 [PMID: 32258945 DOI: 10.1002/hepc.14485]

Reddy SK, Zhan M, Alexander HR, El-Kamary SS. Nonalcoholic fatty liver disease is associated with benign gastrointestinal disorders. *World J Gastroenterol* 2013; 19: 8301-8311 [PMID: 24363521 DOI: 10.3748/wjg.v19.i45.8301]

Bush H, Golabi P, Otgonsuren M, Rafiq N, Venkatesan C, Younossi ZM. Nonalcoholic Fatty Liver Disease is Contributing to the Increase in Cases of Liver Disease in US Emergency Departments. *J Clin Gastroenterol* 2019; 53: 58-64 [PMID: 29608451 DOI: 10.1097/MCG.0000000000001026]

Dubberke ER, Reske KA, McDonald LC, Fraser VJ. ICD-9 codes and surveillance for Clostridium difficile-associated disease. *Emerg Infect Dis* 2006; 12: 1576-1579 [PMID: 17176576 DOI: 10.3201/eid1210.060016]
65 Scheurer DB, Hicks LS, Cook EF, Schnipper JL. Accuracy of ICD-9 coding for Clostridium difficile infections: a retrospective cohort. *Epidemiol Infect* 2007; 135: 1010-1013 [PMID: 17156501 DOI: 10.1017/S0950268806007655]

66 Khoruts A, Staley C, Sadowsky MJ. Faecal microbiota transplantation for Clostridioides difficile: mechanisms and pharmacology. *Nat Rev Gastroenterol Hepatol* 2021; 18: 67-80 [PMID: 32843743 DOI: 10.1038/s41575-020-0350-4]
