Alcohol Enhances Symptoms and Propensity for Infection in Inflammatory Bowel Disease Patients and a Murine Model of DSS-Induced Colitis

Abigail R. Cannon[1,2,6], **Paulius V. Kuprys**[1,2,6], **Adrienne N. Cobb**[2,3], **Xianzhong Ding**[4], **Anai N. Kothari**[2,3], **Paul C. Kuo**[3,*], **Joshua M. Eberhardt**[3], **Adam M. Hammer**[1,2,6], **Niya L. Morris**[1,2,6], **Xiaoling Li**[1,2,3], **Mashkoor A. Choudhry**[1,2,3,4,5,6]

[1]Alcohol Research Program, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

[2]Burn & Shock Trauma Research Institute, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

[3]Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

[4]Department of Pathology, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

[5]Department of Microbiology and Immunology, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

[6]Integrative Cell Biology Program, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

Abstract

Over 1.4 million Americans have been diagnosed with Inflammatory Bowel Disease (IBD), and ulcerative colitis (UC) makes up approximately half of those diagnoses. As a disease, UC cycles between periods of remission and flare, which is characterized by intense abdominal pain, increased weight loss, intestinal inflammation, rectal bleeding, and dehydration. Interestingly, a widespread recommendation to IBD patients for avoidance of a flare period is 'Don't Drink Alcohol' as recent work correlated alcohol consumption with increased GI symptoms in patients

Corresponding Author: Mashkoor A. Choudhry, PhD, Department of Surgery, Burn & Shock Trauma Research Institute, Bldg. 115/ CTRE; Room 320, Loyola University Chicago Health Sciences Division, 2160 South First Ave, Maywood, IL 60153, Office: 708-327-2463, Fax: 708-327-2813, mchoudhry@luc.edu.
*Current Address -Department of Surgery, University of Tampa, FL
Contributions of each author:
ARC - study design, data collection, data analysis, data interpretation, writing
PVK - study design, data collection, data analysis, data interpretation, writing
ANC – human data analysis, interpretation, and writing
XD – data collection, interpretation, and writing
ANK – human data analysis, interpretation, and writing
PCK – human data analysis, interpretation, and writing
JME – human data analysis, interpretation, and writing
AHM – data collection, interpretation, and writing
NLM – data collection, interpretation, and writing
XL – data collection, interpretation, and writing
MAC – study design, data analysis, data interpretation, writing

with IBD. Alcohol alone not only induces a systemic pro-inflammatory response, but can also be directly harmful to gut barrier integrity. However, how alcohol could result in the exacerbation of UC in both patients and murine models of colitis has yet to be elucidated. Therefore, we conducted a retrospective analysis of patients admitted for IBD with a documented history of alcohol use in conjunction with a newly developed mouse model of binge alcohol consumption following DSS-induced colitis. We found that alcohol negatively impacts clinical outcomes of patients with IBD, specifically increased intestinal infections, antibiotic injections, abdomen CT scans, and large intestine biopsies. Furthermore, in our mouse model of binge alcohol consumption following an induced colitis flare we found alcohol exacerbates weight loss, clinical scores, colonic shortening and inflammation, and propensity to infection. These findings highlight alcohol's ability to potentiate symptoms and susceptibility to infection in UC and suggest alcohol as an underlying factor in perpetuating symptoms of IBD.

Summary Sentence:

This study builds on previous findings that alcohol has adverse effects in IBD and establishes these effects in a mouse model of colitis.

Introduction

Inflammatory Bowel Disease (IBD) is diagnosed in over 1.4 million Americans each year. Yet, the exact mechanisms behind disease onset remain elusive[1–3]. IBD is subdivided into two clinical categories of either Crohn's disease (CD) or Ulcerative Colitis (UC), characterized by inflammation of the entire gastrointestinal tract in CD or the large intestine only in UC[2,4]. UC accounts for over 700,000 IBD diagnoses and is most prevalent in patients under thirty; the age range where an estimated 70% of binge alcohol drinking episodes occur[1,3,5]. UC is a life-long disease characterized by cycles of asymptomatic remission and active disease flares. UC patients are subject to symptoms of bloody stool and subsequent anemia, bowel incontinence, and weight loss[2].

The precise etiology of UC is still unknown, but research has focused on genetic factors leading to over activation of the immune system with a break in tolerance to commensal intestinal bacteria, environmental factors resulting in dysbiosis of the bacterial population residing in the gut, or some combination thereof that could potentially be contributing to disease onset. As there is no cure for UC, patients are forced into maintenance regimens obtaining symptomatic relief through the use of immunosuppressive drugs, antibiotics, or surgical therapies[2,6–8]. Although these treatments aid in relieving symptoms of UC, the resulting immunosuppression could allow for the invasion of pathogenic bacteria that would have normally been defended against. Furthermore, changes in bacterial populations from antibiotic treatment could open specific niches more favorable to invading pathogens, which then gives rise to the increased susceptibility to bacterial infections seen in IBD/UC patients.

Maintenance of UC requires patients to avoid stress, certain foods, and alcohol, as all three can potentially induce flare periods of UC[2,9]. Specifically, physicians recommend UC patients maintain a sober lifestyle[9], but there exists a gap in knowledge as to how alcohol intoxication affects UC flare periods.

Alcohol consumption is well known to be both pro-inflammatory and directly harmful to gut barrier function as it breaks down the normal physical and immunological barrier provided by intestinal epithelial cells and gut associated lymphoid tissue (GALT), respectively[10–13]. Alcohol alone is known to induce intestinal erosion, which can impair intestinal absorption leading to increases in diarrhea and intestinal permeability, allowing for leakage of bacteria or bacterial endotoxins into the circulation[12,14,15]. Despite this, only a handful of studies have explored the impact of alcohol in the setting of UC. Some research has shown that alcohol has a deleterious role in UC by increasing gastrointestinal symptoms[16], inducing a flare[17] and promoting disease onset[18], while one study describes that alcohol has no effect in the onset of UC[19]. The inconsistency of evidence in either support or contradiction of alcohol's role in exacerbating UC flare and/or onset prompted us firstly to elucidate whether alcohol is a contributing factor in UC and UC research.

To further address this question, we conducted a retrospective analysis of patient databases to assess clinical outcomes of patients admitted with diagnoses for IBD, UC, and CD that either have or do not have documented history of alcohol use. The results of this analysis suggested that patients with documented history of alcohol use may have an increased risk of infections as well as require more diagnostic and therapeutic procedures.

These findings led us to generate two models that focus on the impact of alcohol in one form of IBD, UC with the hypothesis that not only does alcohol further exacerbate UC flares, but also increases susceptibility of UC patients to bacterial infections. In one model, mice were treated with DSS and a subsequent binge alcohol paradigm, allowing us to examine the ability of alcohol to exacerbate a flare period. In the other model, mice received the combined DSS-induced colitis and binge-alcohol paradigm followed by an inoculation of *Citrobacter rodentium* (*C. rod*), which allowed us to understand how alcohol increases susceptibility of UC patients to bacterial infections.

Methods

Patient database analysis

We obtained data from the Healthcare Cost and Utilization Project (HCUP) State in Patient Databases (SID) for New York and Florida from 2009–2013. Using the *International Classification of Diseases, 9th Revision, Clinical Modification*, (ICD-9-CM) diagnosis codes, we defined three study groups: IBD, UC, and CD. All patient admission data was retained if the patient had a primary diagnosis of IBD. All of the ICD-9-CM codes used in this study can be found in Table S1. In addition, we utilized the HCUP cost-to-charge ratio files, which provide a ratio of the actual cost relative to the total amount charged to insurance for each separate hospital. These files are used to estimate the actual cost for individual patient admissions.

Comorbidities were assigned to patients on the basis of the Agency for Healthcare Research and Quality (AHRQ) comorbidity measures. For each individual patient, if the presence of a comorbidity was indicated, then the comorbidity was applied to all of that patient's admissions. Patients were classified as smokers based on whether any admission contained ICD-9-CM codes for tobacco use disorder or personal history of tobacco use. To evaluate the

impact of alcohol on outcomes in IBD, patients were identified as having a documented history of alcohol use (+A) based on whether any admission contained ICD-9-CM codes relating to alcohol use or the AHRQ comorbidity measure for alcohol abuse, otherwise the patients were classified as not having a documented history of alcohol use (−A).

Following this, only admissions for which the primary cause was IBD, UC, or CD were retained and all other admissions were excluded. Patients aged 18 to 90 years of age were included for study. Admissions missing data elements for the median household income national quartile for patient ZIP code, unique VisitLink patient identifier, age, length of stay, total charges and patient race, were excluded. The −A and +A patients from each respective study group of IBD, UC or CD, were propensity score matched 1:1 at their first admission based on age, sex, race, primary expected payer, median household income national quartile for patient ZIP code, smoking status and various comorbidities (AIDS, congestive heart failure, chronic pulmonary disease, coagulopathy, depression, diabetes mellitus, drug abuse, hypertension (combined complicated and uncomplicated), neurological disorders, obesity, psychoses, renal failure). Subsequent admissions of propensity matched patients following their first recorded hospital admission were collected. The complete admission history of matched patients within each study group was used for data analysis, allowing for a five year perspective of what occurs when −A and +A patients are admitted for IBD, UC, or CD. The patient selection workflow is detailed in Figure S1.

Secondary diagnoses occurring during admission that were identified by ICD-9- CM codes, included *Clostridium difficile* infection, poorly defined intestinal infection, and all other intestinal infections, inclusive of *C. difficile* and poorly defined intestinal infection. Procedures during admission were identified by ICD-9-CM volume 3 and included CT scan of abdomen, small intestine biopsy, small intestine resection, colectomy, large intestine biopsy, rectal biopsy, and antibiotic injection.

Since the patient data within the HCUP SID is de-identified and publically available, the database analysis was exempt from institutional review board approval.

Induction of DSS Colitis

Male 8–9 week old (~23–25g body weight) C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, MA). Briefly, as can be seen in Figure 1A, mice were randomly separated into four experimental groups: Sham Vehicle, Sham Ethanol, DSS Vehicle, and DSS Ethanol. DSS treated mice received 2% (wt/vol) DSS (40,000 kDa; MP Biomedicals), *ad libitum*, in their drinking water for five days. Mice in the Sham group received water only for 5 days acting as a control. On day 5, DSS was discontinued and replaced with normal drinking water in both the DSS and Sham/control groups. On day 5, mice in both the DSS and Sham/control group were further subdivided into two subgroups: mice gavaged with alcohol (~3g/kg) or mice gavaged with water on days 5, 6, and 7 to mimic a binge alcohol abuse pattern. The amount of DSS water drank per animal was recorded and no differences in intake between groups were observed. Mice were weighed every day for the determination of percent weight change. This was calculated as: % weight change = (weight at day X- weight at day 0/weight at day 0) × 100. Animals were monitored

clinically for rectal bleeding, diarrhea, and general signs of morbidity, including hunched posture and failure to groom.

Citrobacter rodentium (C. rod) infection

Mice were subjected to the DSS-induced colitis and binge alcohol paradigm as described above. However, after the last gavage on day 7, mice were further divided into two subgroups: mice gavaged with 1×10^5 CFUs of *C. rod* suspended in 300 μL of PBS or 300 μL of PBS alone. This resulted in four experimental groups: DSS Vehicle, DSS Ethanol, DSS Vehicle + *C. rod,* and DSS Ethanol + *C. rod.*

The experiments described here were carried out in adherence with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and are approved by the Institutional Animal Care and Use Committee, Loyola University Chicago Health Sciences Division, Maywood IL.

Tissue Staining

For hematoxylin and eosin (H&E) staining, 2cm of colon tissue closest to the rectum was taken from each mouse and saved in 10% formalin. Tissue was fixed with 10% phosphate buffered formalin, paraffin embedded, sectioned at 5 μm, and stained with hematoxylin and eosin by AML laboratories (Saint Augustine, Florida). Images were taken on an Olympus BX43 Microscope using an Olympus DP26 camera.

Histopathology Scoring

H&E stained sections were analyzed and scored in a blinded manner by Dr. Xianzhong Ding. Dr. Ding is a trained pathologist at Loyola University Chicago and author on this study. Scoring was based on a modified 0–4 point scale examining exudate, epithelial damage, polymorphonuclear leukocyte invasion, and submucosal edema[20]. The values from each of the 4 categories were added to produce a combined histopathology score for each animal.

Colon Length and Average Clinical Scores

Immediately following euthanasia, colons were excised and length measured. Baseline clinical scores were determined using a modified protocol from Siegmund et al.[21,22]. Briefly, no weight loss was registered as 0, weight loss of 1–5% from baseline was assigned 1 point, 6–10% 2 points, 11–20% 3 points, and more than 20% 4 points. For stool consistency, 0 points were assigned for well-formed pellets, 2 points for pasty and semiformed stools that did not adhere to the anus, and 4 points for liquid stools that did adhere to the anus. For bleeding, 0 was assigned for no blood by using hemoccult (Beckman Coulter), 2 points for positive hemoccult, and 4 points for gross bleeding.

ELISA

Mice in all four groups were sacrificed three hours after the last gavage on day 7, as seen in Figure 1A. Large intestines were harvested and homogenized. The homogenates were analyzed for IL-18 (eBioscience), IL-1B (R&D Systems), IL-6 (R&D Systems), TNFα (eBioscience), and KC (BD Biosciences) using their respective ELISAs per the

manufacturer's instructions. The cytokine levels were expressed per milligram of total protein in the homogenates.

Statistics

For patient database analysis, we utilized a student's t-test for mean cost, Mann–Whitney U test for median length of stay with interquartile ranges (IQRs), and χ^2 test for categorical variables. Statistical analysis and propensity score matching were performed using STATA version 12 (StataCorp LP, College Station, TX). For the mouse model experiments, comparisons within groups were analyzed using a two-Way ANOVA with a Tukey post-hoc test. Analysis was done using GraphPad Prism software. A confidence level of $p < 0.05$ was considered statistically significant. Significance is represented throughout the manuscript as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Online Supplemental Material

Figure S1 is a schematic of the patient data selection process. Table S1 lists ICD-9 diagnosis codes used to define study populations and the variables examined in the study. Table S2 and S3 provide patient demographic and comorbidity statistics

Results

Within the patient database study groups, there were 41,810 IBD patients, 18,695 UC patients, and 24,059 CD patients that met the inclusion criteria. The baseline demographics of these groups are shown in Table 1, 2, and 3, respectively. Additional demographics are shown in Table S2. Before matching, the age, race, primary expected payer, and median household income, were nearly all significantly different between the −A and +A patients, notwithstanding the age of patients in the UC study group. Males, African Americans, and smokers were overrepresented within the +A patients. With regard to the primary expected payer, the +A patients were more likely to carry Medicaid and self-pay, while being less likely to carry private insurance. The lowest quartile for median household income contains more +A patients then −A patients, while the inverse can be seen for the highest income quartile. The +A patients had significantly increased comorbidities across all measures compared to the −A patients (Table S3).

Post propensity matching, the differences observed for age, sex, race, smoker status, primary expected payer, median household income, and comorbidities between the −A and +A patients within the three study groups were no longer statistically significant (Tables 1, S2 and S3). For the IBD study group, there were 2,021 matched patients (Table 1), that encompassed 4,965 IBD−A admissions and 4,013 IBD+A admissions (Table 4). For the UC study group, there were 992 matched patients (Table 2), that encompassed 1,602 UC−A admissions and 1,437 UC+A admissions (Table 4). For the CD study group, there were 1,083 matched patients (Table 3), that encompassed 3,089 CD−A admissions and 2,548 CD +A admissions (Table 4).

Examination of intestinal infection diagnoses during the admission for either the IBD, UC, or CD study groups revealed that +A patients had significantly increased intestinal infections (Table 4). The IBD+A patients had increased *Clostridium difficile* intestinal infection (2% vs

2.6%, p < 0.05), poorly defined intestinal infection (0.4% vs 0.8%, p < 0.01), and overall intestinal infections (2.6% vs 3.9%, p < 0.01). The UC+A patients had increased poorly defined intestinal infections (0.6% vs 1.5%, p < 0.05), while the CD+A patients had increased intestinal infections of all types (2.1% vs 3.1%, p < 0.05).

With regard to procedures during the admission (Table 4), all +A patients had significant increases of abdominal CT scans (IBD: 6.8% vs 9.6%, p < 0.001; UC: 6.9% vs 9.0%, p < 0.05; CD: 7.3% vs 9.9%, p < 0.01) and antibiotic injections (IBD: 3.1% vs 5.8%, p < 0.001; UC: 3.8% vs 5.5%, p < 0.05; CD: 3.4% vs 5.8%, p < 0.001). Large intestine and rectal biopsies were significantly increased in +A patients compared to −A patients within the IBD study group (18.1% vs 21.7%, p < 0.001; 3.1% vs 4.3%, p < 0.01). Within the UC and CD study group, large intestine and rectal biopsies were not significantly changed between the −A and +A patients, although UC+A patients did have increased large intestine biopsies (30.7% vs 32.6%, p = .272). Small intestine biopsies were unchanged between the −A and +A patients. Resection of the small intestine was significantly increased in −A patients with IBD (1.9% vs 1.1%, p < 0.01) and CD (2.4% vs 1.5%, p < 0.05). For the UC study group, −A patients displayed a trend towards increased colectomy (4.7% vs 3.4%, p = 0.077). The differences in percentages of colectomy and small intestine resection procedures for the UC and CD study groups support the appropriate separation of these patients into their respective groups.

The median length of stay was unchanged between −A and +A patients in all study groups (4 days), however the interquartile range was significantly increased for the −A patients in the IBD (2–7 days vs 2–6 days, p < 0.01) and CD (2–7 days vs 2–6 days, p < 0.05) study groups. In terms of total cost, the −A patients had increased total cost during hospitalization for all study groups compared to the +A patients (IBD: $13,880.65 vs $12,262.51, p < 0.0001; UC: $14,564.63 vs $13,217.54, p < 0.05; CD: $13,480.92 vs $11,794.42, p < 0.001) (Table 4).

Having found that alcohol affects the clinical outcomes of IBD patients, we established a novel model of DSS-induced colitis in the context of a binge alcohol paradigm. DSS-induced colitis is a well-established model for studying an UC flare period[23], yet no model currently exists to study how alcohol intoxication could exacerbate a UC flare. Hence, our lab developed a murine model as seen in Figure 1. Previous studies on DSS-induced colitis have used concentrations of DSS from 1–3%[24–27]. In order to parse out minute differences between DSS-induced intestinal damage alone and DSS and alcohol induced intestinal damage, we utilized a 2% concentration of DSS.

As can be seen in Figure 1A, DSS treated mice received DSS *ad libitum* in their drinking water for 5 days to induce the UC disease state. Mice in sham/control group received water. On day 5, DSS was discontinued to allow entrance into UC remission. A binge alcohol paradigm was then employed where mice were gavaged with alcohol (~3g/kg) or gavaged with water on days 5, 6, and 7. This resulted in four overall experimental groups - Sham Vehicle, Sham Ethanol, DSS Vehicle, and DSS Ethanol.

Weight loss, histopathology score, colonic shortening, and average clinical score are the most common experimental observations used to assess UC disease severity. Therefore, we first monitored weight loss or gain in our newly adapted model of UC and binge alcohol. Consistent with previous reports, mice treated with DSS began losing body weight on day 5 after treatment as calculated by percent weight change from day 0. On day 6, mice receiving a combined insult of DSS Ethanol lost twice as much weight compared to those mice in the DSS Vehicle group, ~10% vs. ~5% respectively. By day 7, the weight loss in mice receiving DSS Ethanol reached 17% to that of their original body weight as compared to only 12% in DSS vehicle mice, Figure 1B.

To address differences in histopathology between the four experimental groups, sections of colon were taken closest to the rectum, stained via H&E, blinded, and scored by a pathologist. Figure 2A shows gross differences in large intestine morphology after DSS Ethanol treatment compared to all other groups. Inflammatory infiltrate, epithelial damage, and crypt damage are severely increased in the DSS Ethanol mice compared to that of the DSS Vehicle. Furthermore, combined histopathology scores in Figure 2B, show significant increases in DSS Ethanol compared to DSS Vehicle.

Increased weight loss and histopathology scores accompanied a significant decrease in colon length in our novel model of UC and binge alcohol. As has been observed by many others in the past[23], DSS Vehicle treated mice experienced a decrease in colon length compared to that of Sham Vehicle mice. Interestingly, the addition of alcohol to the DSS treated mice resulted in a more severe decrease in colon length compared to DSS Vehicle treated mice, Figure 3A. Clinical scores were obtained by combining weight loss, stool consistency, and blood in stool as described in the methods section above. Data in Figure 3B show that the addition of alcohol to DSS-induced colitis trends toward an increase in the average clinical score compared to DSS Vehicle, highlighting alcohol's detrimental effect on UC flares.

Colonic inflammation is a hallmark symptom of UC. To further delineate how alcohol could be exacerbating an UC flare, levels of large intestine pro-inflammatory cytokines were determined. We hypothesized that alcohol would further increase levels of large intestine pro-inflammatory cytokines following the addition of our alcohol binge paradigm. Our results revealed that in mice receiving DSS Ethanol, IL-18 (Figure 4A), IL-1β (Figure 4B), and KC (Figure 4E) trended to increase compared to DSS Vehicle treated mice. However, the cytokines, IL-6 (Figure 4C) and TNFα (Figure 4D) were not found to be increased in the colons of DSS Ethanol treated mice compared to mice receiving DSS vehicle. Although increases in inflammation following DSS Ethanol did not reach statistical significance, total large intestine homogenates were used for all ELISAs, and we anticipate specifically isolating inflamed areas in contrast to diluting inflamed areas with non-inflamed areas (as commonly occurs in the intestines of UC patients) will yield statistical significance. However, future work will focus on this and the specific cell types in the intestine responsible for the increases in inflammation that we do see in the total homogenates.

UC patients are at higher risk of developing bacterial infections[28,29]. To understand whether consumption of alcohol not only impacts UC patient's increased susceptibility to infection but also increases severity of symptoms related to bacterial infection, we utilized our model

of DSS-induced colitis and binge alcohol, as described above, along with a single inoculation of *C. rod*, a well-known Gram negative enteropathogen associated with colonic infection, at 1×10^5 CFUs three hours after the last gavage on day 7 (Figure 5A). As can be seen in Figure 5B, the percent survival of DSS Ethanol treated mice following *C. rod* infection fell to 50% by day 11 compared to 100% survival in the DSS Vehicle group with *C. rod* infection. Interestingly, DSS Ethanol mice with no *C. rod* infection also experienced a 20% reduction in survival compared to the 100% survival in the DSS Vehicle group with and without *C. rod* infection.

Weight loss and gain was also monitored as described previously by percent change from day 0 up till day 11 as only 50% of the mice in the DSS Ethanol + *C. rod* infection group survived till day 11. By day 11 following *C. rod* infection, mice in the DSS Ethanol group experienced a ~27% decrease from their original body weight compared to ~22% in the DSS Vehicle group (Figure 6A) giving evidence to our hypothesis of alcohol not only increasing susceptibility to infection with UC, but also increasing severity of symptoms associated with UC and infection. Increased weight loss accompanied increases in colonic shortening in the DSS Ethanol + *C. rod* group compared to mice in the DSS Vehicle + *C. rod* group, Figure 6B.

Again, to understand differences in histopathology following infection with *C. rod*, sections of colon were taken closest to the rectum, stained via H&E, blinded, and scored by a pathologist. Figure 7A shows gross differences in large intestine morphology after DSS Ethanol + *C. rod* treatment compared to all other groups. As in Figure 2B, inflammatory infiltrate and epithelial damage were assessed and were severely increased in the DSS Ethanol + *C. rod* mice compared to that of the DSS Vehicle + *C. rod* group. The combined histopathology scores in DSS Ethanol + *C. rod* treated mice were significantly increased compared to mice in the DSS Vehicle + *C. rod* group, Figure 7B.

As in Figure 4, we assessed colonic inflammation under the hypothesis that *C. rod* infection would further increase inflammation in mice receiving DSS Ethanol treatment, which could perpetuate increased colonization of *C. rod* We found that mice in the DSS Ethanol + *C. rod* group had increased levels of IL-18 (Figure 8A) and IL-1β (Figure 8B) compared to DSS Vehicle + *C. rod*. Furthermore, the pro-inflammatory cytokine IL-6 was increased in both the DSS Ethanol + *C. rod* and DSS Vehicle + *C. rod* compared to mice treated with DSS alone (Figure 8C). However, both TNFα and KC were not further increased following *C. rod* infection after DSS Ethanol treatment (Figure 8D and 8E).

Discussion

Alcohol is known to have numerous deleterious effects in a variety of settings, yet its effects in IBD are not well understood. In this study, we described a potential role for alcohol affecting the inpatient care of IBD patients as well as prolonging an inflammatory flare period and increasing pathogenic infection in a mouse model of UC.

Research has shown that IBD patients and alcoholic patients carry an intestinal bacterial dysbiosis[30,31]. A dysbiosis is believed to provide pathogens an opportunity to colonize and

proliferate[32]. Indeed, studies have shown IBD patients and alcoholics are at risk for increased infections[33,34]. Thus, the combination of alcohol use in a patient with IBD would likely lead to an increase in the amount of intestinal infections, which is exactly what we observe in our patient database analysis. Furthermore, the +A patients required increased antibiotic injections as well as increased diagnostic procedures. Despite these increases, the +A patients had decreased total costs compared to −A patients. This can likely be explained by the decrease in surgical procedures (colectomy and small intestine resection) observed in the +A patients. These patients might be less than ideal candidates for surgery, which is often more expensive. In addition, while the median length of stay was unchanged between the −A and +A patients, the interquartile range for the length of stay was increased for the −A patients, which may also be related to the increase in surgical procedures since surgical patients require longer hospitalization periods post-surgery. How this decrease in surgical interventions affects the clinical course of IBD patients with alcohol use remains to be further elucidated.

We would like to note that to be coded for an ICD-9 diagnosis for an alcohol related disorder, the patient must have had some history of significant alcohol intake, thus our +A study group likely comprises mostly heavy drinkers. Unfortunately, the databases do not contain information regarding the amount of alcohol a patient had prior to an admission. Furthermore, the databases do not contain the exact cause of the IBD admission, thus we cannot discern whether a patient was admitted for a flare or other complication of IBD. Therefore, the findings of this analysis warrant additional research of IBD patients that could provide more detailed information beyond what is available through ICD-9 codes.

Overall, the results of this patient database analysis further support the suggestion that alcohol has some negative impacts on the clinical outcomes of patients with IBD[13], which led us to examine and characterize the mechanism by which alcohol exacerbates the flare period and infective capacity in a mouse model of DSS-induced colitis.

To our knowledge, this is the first time a murine model of UC and alcohol has been developed to allow a better understanding of how drinking alcohol could affect a patient with UC. Understanding potential environmental factors that could contribute to disease flares, either as a trigger or an exacerbation of symptoms, is critical to improving the quality of life of UC patients stuck in the maintenance of their disease hoping to avoid a flare or a worsening of symptoms during a flare period.

We were able to show that mice undergoing a binge alcohol paradigm following DSS-induced colitis had exacerbated symptoms of UC as shown by increases in weight loss, colon shortening, histopathology and clinical scores, and inflammation, all of which are standard assessment of UC severity in mouse models. Besides symptoms associated with UC itself, UC patients have a higher propensity to other co-morbidities such as infection. Hence, we adapted our model of UC and binge alcohol to include a low inoculate of *C. rodentium* in order to understand whether UC patients that drink alcohol would also have increased susceptibility to infection. Our results showed mice receiving *C. rodentium* following alcohol and DSS-induced colitis had decreased survival and increased weight loss, colon shortening, histopathology and clinical scores, and inflammation.

Reappearance of UC symptoms during a flare stem from increases in intestinal inflammatory cytokines such as IFN-γ, IL-1, IL-6, and TNF-α[35–40], which can directly lead to mucosal ulcerations, damage to the colonic epithelium, and crypt micro-abscesses[41]. Coupled with the knowledge that alcohol itself induces increases in inflammation it follows that alcohol consumption could either perpetuate a current UC flare, such as we've provided evidence for above, and/or trigger entrance into UC flare via initiation of the inflammatory cascade, which requires further investigation.

This inflammation induced damage to the intestine and thus intestinal defense mechanisms could have dramatic consequences to UC patients, especially in combination with alcohol. Recent studies found that alcohol could cause a dysbiosis of the intestinal microbiome, which, in turn, could alter the intestinal microenvironment making it more favorable to opportunistic pathogens[42]. Therefore, it was imperative to understand the susceptibility to intestinal pathogens specifically after alcohol and DSS-induced colitis. With our low inoculate *C. rodentium* model in combination with alcohol and DSS-induced colitis, we were able to show increases in susceptibility to infection in DSS Ethanol treated mice. We recognize the burden of utilizing the model pathogen, *C. rodentium*, which itself is used as a model of IBD, on top of our binge alcohol and DSS-induced colitis model. Yet, our adaptation of using a much lower inoculate, 1×10^5 CFUs vs. 1×10^{11} CFUs to induce true IBD symptoms, allowed us to shed light on the increased propensity for +A IBD patients to acquire intestinal infections. However, more analysis of patient data, both prospective and retrospective, is needed to understand the molecular implications of alcohol in UC patient infections.

The idea that alcohol could act in such a way to worsen UC flare periods provides evidence to the clinician's warning to UC patients to avoid drinking alcohol. However, future work will focus on elucidating the mechanism behind alcohol intensifying UC flare symptoms in order to potentially open doors to patients wanting to participate in social situations that involving drinking alcohol. One potential pathway that upon its activation could act in the amelioration of DSS-induced colitis is the aryl hydrocarbon receptor (AhR) pathway. Studies have shown that activation of the AhR pathway not only through chemical activation via TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) but also by specific probiotics is able to inhibit DSS-induced colitis[43,44]. Additionally, cytokines known to be induced upon AhR activation, specifically IL-22, are known to be upregulated during remission periods of UC[45,46]. In the intestine IL-22 can stimulate proliferation, mucous protection, and AMP secretion, which could drive entrance into the remission period from a UC flare[47–52]. Understanding the interplay between these pathways, UC flare, the gut microbiome, and intestinal inflammation in the context of binge alcohol is critical to the development of a therapeutic intervention that would allow improvements to UC patient's lifestyles especially those stuck in lifelong maintenance therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Conflicts of Interest and Funding Sources:

References

1. U.S. Department of Health and Human Services. Centers for disease control and prevention. http://www.cdc.gov/alcohol/fact-sheets/binge-drinking.htm. Updated October, 2015March, 2016.

2. Crohn's and Colitis Foundation. Managing flares and other IBD symptoms. http://www.ccfa.org/assets/pdfs/flares_brochure_final.pdfMarch, 2016.

3. Crohn's and colitis foundation of america. http://www.ccfa.org/resources/facts-about-inflammatory.html. Updated May, 2011March, 2016.

4. Hanauer SB. Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis. 2006;12 Suppl 1:S3–9. [PubMed: 16378007]

5. Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. JAMA. 2003;289(1):70–75. [PubMed: 12503979]

6. Dignass A, Lindsay JO, Sturm A, et al. Second european evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: Current management. J Crohns Colitis. 2012;6(10):991–1030. [PubMed: 23040451]

7. Loghman-Adham M Medication noncompliance in patients with chronic disease: Issues in dialysis and renal transplantation. Am J Manag Care. 2003;9(2):155–171. [PubMed: 12597603]

8. Higgins PD, Rubin DT, Kaulback K, Schoenfield PS, Kane SV. Systematic review: Impact of non-adherence to 5-aminosalicylic acid products on the frequency and cost of ulcerative colitis flares. Aliment Pharmacol Ther. 2009;29(3):247–257. [PubMed: 18945258]

9. Brown AC, Rampertab SD, Mullin GE. Existing dietary guidelines for crohn's disease and ulcerative colitis. Expert Rev Gastroenterol Hepatol. 2011;5(3):411–425. [PubMed: 21651358]

10. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. Nat Med. 2015;21(7):698–708. [PubMed: 26121198]

11. Ferrier L, Berard F, Debrauwer L, et al. Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. Am J Pathol. 2006;168(4):1148–1154. [PubMed: 16565490]

12. Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: A possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin Exp Res. 2009;33(10):1836–1846. [PubMed: 19645728]

13. Swanson GR, Sedghi S, Farhadi A, Keshavarzian A. Pattern of alcohol consumption and its effect on gastrointestinal symptoms in inflammatory bowel disease. Alcohol. 2010;44(3):223–228. [PubMed: 20682190]

14. Bode C, Bode JC. Effect of alcohol consumption on the gut. Best Pract Res Clin Gastroenterol. 2003;17(4):575–592. [PubMed: 12828956]

15. Yan AW, Fouts DE, Brandl J, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology. 2011;53(1):96–105. [PubMed: 21254165]

16. Swanson GR, Sedghi S, Farhadi A, Keshavarzian A. Pattern of alcohol consumption and its effect on gastrointestinal symptoms in inflammatory bowel disease. Alcohol. 2010;44(3):223–228. [PubMed: 20682190]

17. Jowett SL, Seal CJ, Pearce MS, et al. Influence of dietary factors on the clinical course of ulcerative colitis: A prospective cohort study. Gut. 2004;53(10):1479–1484. [PubMed: 15361498]

18. Hsu TY, Shih HM, Wang YC, et al. Effect of alcoholic intoxication on the risk of inflammatory bowel disease: A nationwide retrospective cohort study. PLoS One. 2016;11(11):e0165411. [PubMed: 27802288]

19. Bergmann MM, Hernandez V, Bernigau W, et al. No association of alcohol use and the risk of ulcerative colitis or crohn's disease: Data from a european prospective cohort study (EPIC). Eur J Clin Nutr. 2017;71(4):566. [PubMed: 28377581]

20. Hughes ER, Winter MG, Duerkop BA, et al. Microbial respiration and formate oxidation as metabolic signatures of inflammation-associated dysbiosis. Cell Host Microbe. 2017;21(2):208–219. [PubMed: 28182951]

21. Siegmund B, Lehr HA, Fantuzzi G, Dinarello CA. IL-1 beta -converting enzyme (caspase-1) in intestinal inflammation. Proc Natl Acad Sci U S A. 2001;98(23):13249–13254. [PubMed: 11606779]

22. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004;118(2):229–241. [PubMed: 15260992]

23. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004;118(2):229–241. [PubMed: 15260992]

24. Ito R, Kita M, Shin-Ya M, et al. Involvement of IL-17A in the pathogenesis of DSS-induced colitis in mice. Biochem Biophys Res Commun. 2008;377(1):12–16. [PubMed: 18796297]

25. Vlantis K, Polykratis A, Welz PS, van Loo G, Pasparakis M, Wullaert A. TLR-independent anti-inflammatory function of intestinal epithelial TRAF6 signalling prevents DSS-induced colitis in mice. Gut. 2016;65(6):935–943. [PubMed: 25761602]

26. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004;118(2):229–241. [PubMed: 15260992]

27. Alex P, Zachos NC, Nguyen T, et al. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. Inflamm Bowel Dis. 2009;15(3):341–352. [PubMed: 18942757]

28. Toruner M, Loftus EV Jr, Harmsen WS, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. Gastroenterology. 2008;134(4):929–936. [PubMed: 18294633]

29. Rodemann JF, Dubberke ER, Reske KA, Seo DH, Stone CD. Incidence of clostridium difficile infection in inflammatory bowel disease. Clin Gastroenterol Hepatol. 2007;5(3):339–344. [PubMed: 17368233]

30. Mutlu EA, Gillevet PM, Rangwala H, et al. Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol. 2012;302(9):G966–78. [PubMed: 22241860]

31. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A. 2007;104(34):13780–13785. [PubMed: 17699621]

32. Bien J, Palagani V, Bozko P. The intestinal microbiota dysbiosis and clostridium difficile infection: Is there a relationship with inflammatory bowel disease? Therap Adv Gastroenterol. 2013;6(1):53–68.

33. Sundaram V, May FP, Manne V, Saab S. Effects of clostridium difficile infection in patients with alcoholic hepatitis. Clin Gastroenterol Hepatol. 2014;12(10):1745–52.e2. [PubMed: 24681081]

34. Ananthakrishnan AN, McGinley EL, Binion DG. Excess hospitalisation burden associated with clostridium difficile in patients with inflammatory bowel disease. Gut. 2008;57(2):205–210. [PubMed: 17905821]

35. Schett G, Elewaut D, McInnes IB, Dayer JM, Neurath MF. How cytokine networks fuel inflammation: Toward a cytokine-based disease taxonomy. Nat Med. 2013;19(7):822–824. [PubMed: 23836224]

36. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology. 2005;129(2):550–564. [PubMed: 16083712]

37. Masuda H, Iwai S, Tanaka T, Hayakawa S. Expression of IL-8, TNF-alpha and IFN-gamma m-RNA in ulcerative colitis, particularly in patients with inactive phase. J Clin Lab Immunol. 1995;46(3):111–123. [PubMed: 8926619]

38. Casini-Raggi V, Kam L, Chong YJ, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. J Immunol. 1995;154(5):2434–2440. [PubMed: 7868909]

39. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. Nat Med. 2015;21(7):698–708. [PubMed: 26121198]

40. Obermeier F, Kojouharoff G, Hans W, Scholmerich J, Gross V, Falk W. Interferon-gamma (IFN-gamma)- and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. Clin Exp Immunol. 1999;116(2): 238–245. [PubMed: 10337013]

41. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427–434. [PubMed: 17653185]

42. Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The gastrointestinal microbiome: Alcohol effects on the composition of intestinal microbiota. Alcohol Res. 2015;37(2):223–236. [PubMed: 26695747]

43. Takamura T, Harama D, Matsuoka S, et al. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. Immunol Cell Biol. 2010;88(6): 685–689. [PubMed: 20231854]

44. Takamura T, Harama D, Fukumoto S, et al. Lactobacillus bulgaricus OLL1181 activates the aryl hydrocarbon receptor pathway and inhibits colitis. Immunol Cell Biol. 2011;89(7):817–822. [PubMed: 21321579]

45. Monteleone I, MacDonald TT, Pallone F, Monteleone G. The aryl hydrocarbon receptor in inflammatory bowel disease: Linking the environment to disease pathogenesis. Curr Opin Gastroenterol. 2012;28(4):310–313. [PubMed: 22450895]

46. Yamamoto-Furusho JK, Miranda-Perez E, Fonseca-Camarillo G, Sanchez-Munoz F, Dominguez-Lopez A, Barreto-Zuniga R. Colonic epithelial upregulation of interleukin 22 (IL-22) in patients with ulcerative colitis. Inflamm Bowel Dis. 2010;16(11):1823. [PubMed: 20222141]

47. Zindl CL, Lai JF, Lee YK, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. Proc Natl Acad Sci U S A. 2013;110(31):12768–12773. [PubMed: 23781104]

48. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity. 2008;29(6): 947–957. [PubMed: 19100701]

49. Wolk K, Witte E, Wallace E, et al. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: A potential role in psoriasis. Eur J Immunol. 2006;36(5):1309–1323. [PubMed: 16619290]

50. Sugimoto K, Ogawa A, Mizoguchi E, et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest. 2008;118(2):534–544. [PubMed: 18172556]

51. Pickert G, Neufert C, Leppkes M, et al. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med. 2009;206(7):1465–1472. [PubMed: 19564350]

52. Nagalakshmi ML, Rascle A, Zurawski S, Menon S, de Waal Malefyt R. Interleukin-22 activates STAT3 and induces IL-10 by colon epithelial cells. Int Immunopharmacol. 2004;4(5):679–691. [PubMed: 15120652]
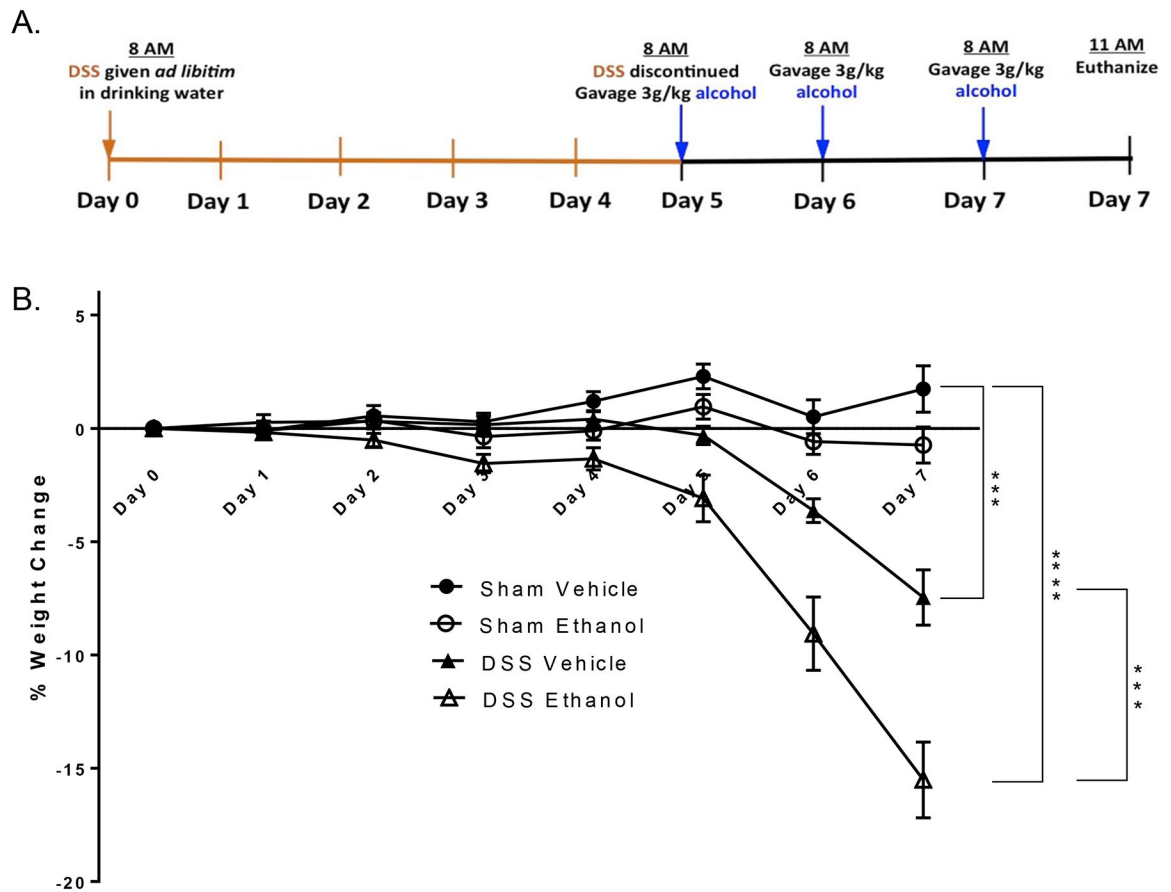
A.

B.

Figure 1.).

A. *Murine model of DSS-induced colitis and ethanol.* A 2% DSS concentration was administered ad libitum in drinking water for 5 days to mimic symptoms of UC. On day 5, DSS was discontinued to allow entrance into UC remission. A binge alcohol paradigm was employed where mice were gavaged with alcohol or water on days 5, 6, and 7. Mice were euthanized 3 hours post last gavage on day 7. *B. Ethanol Increased DSS-induced weight loss.* Percent weight change of animals was determined by the following equation: % weight change = (weight at day X- weight at day 0/weight at day 0)*100. Values are mean ± SEM, n=8–14 animals/group. ***p<0.001 DSS Ethanol compared to DSS Vehicle, ***p<0.001 DSS Vehicle compared to Sham Vehicle; ****p<0.001 DSS Ethanol compared to Sham Vehicle by ANOVA on day 7.
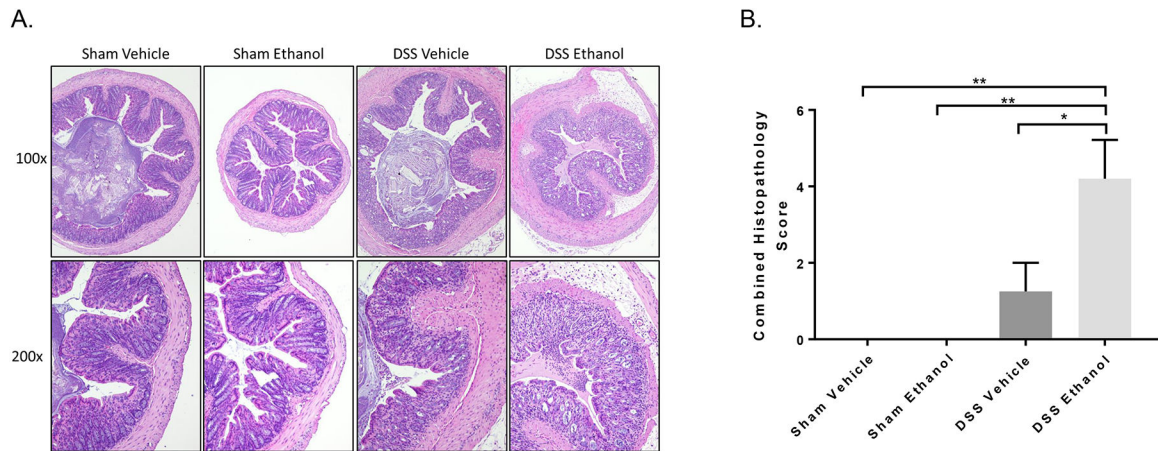
A.

B.

Figure 2.). Gross histological pathologies increased following combined ethanol and DSS treatment.

A. Representative H&E stained sections of the colon on day 7 (Top row x100, Bottom row x200). B. Combined Histopathology Score following blinded histological scoring as described in detail in Methods section above. Values are mean ± SEM, n=4–5 animals/ group. **$p<0.01$ DSS Ethanol compared to Sham Vehicle and Sham Ethanol,; *$p<0.05$ DSS Ethanol compared to DSS Vehicle by ANOVA.
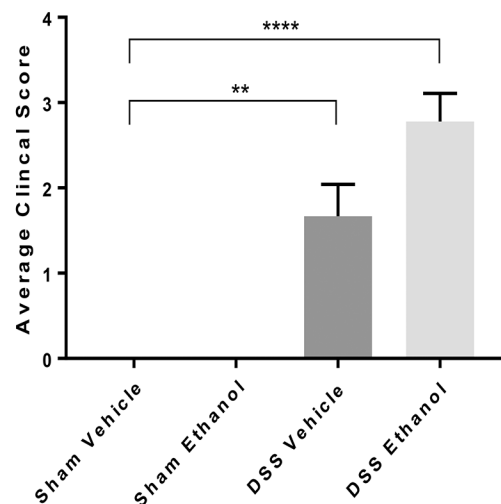
A.
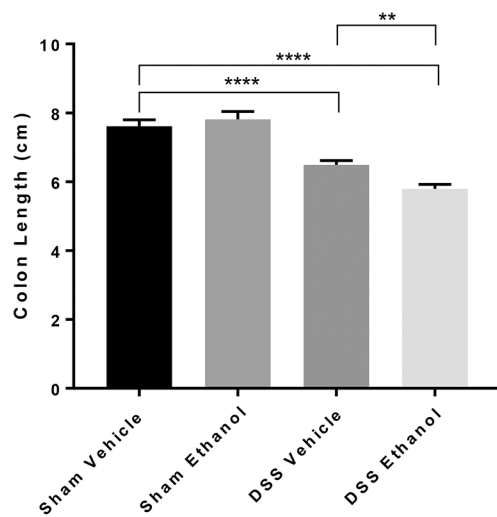
B.

Figure 3.). Increased colon length shortening and average clinical scores following ethanol and DSS-induced colitis.

A. Colon length measured in centimeters (cm) on day 7. Values are mean ± SEM, n=8–14 animals/group. **p<0.01 DSS Ethanol compared to DSS Vehicle, ****p<0.0001 DSS Vehicle compared to Sham Vehicle; ****p<0.0001 DSS Ethanol compared to Sham Vehicle by Two-way ANOVA. B. Clinical scores as described above. Briefly scores were determined by the average of % body weight loss, stool consistency, and presence of blood in the stool. Values are mean ± SEM, n=4–6 animals/group. **p<0.01 DSS Vehicle compared to Sham Vehicle; ***p<0.001 DSS Ethanol compared to Sham Vehicle by ANOVA.
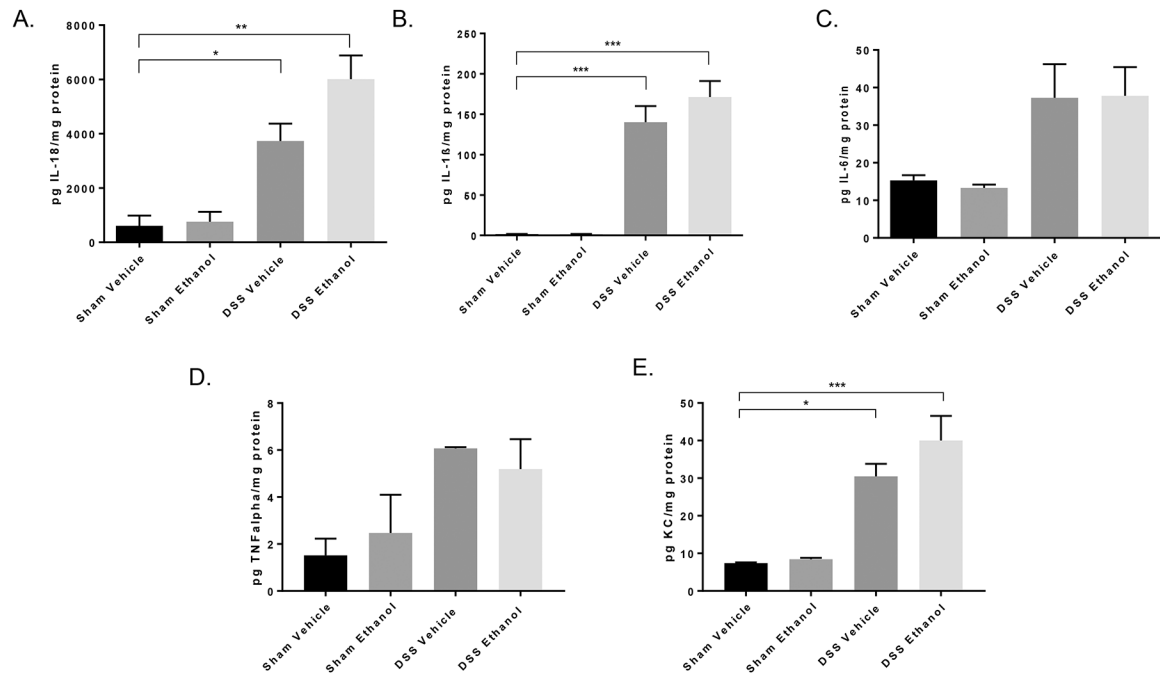
Figure 4.). Increased colonic inflammation after DSS-induced colitis and ethanol treatment.
Colons were harvested, homogenized, and processed on day 7 for the analysis of inflammatory mediators using respective ELISAs. A. IL-18, B. IL-1β, C. IL-6, TNFα, E. KC by ELISA. Values are mean ± SEM 3–6 animals per group *p<0.05; **p<0.01; ***p<0.001 all groups compared to Sham Vehicle by ANOVA.
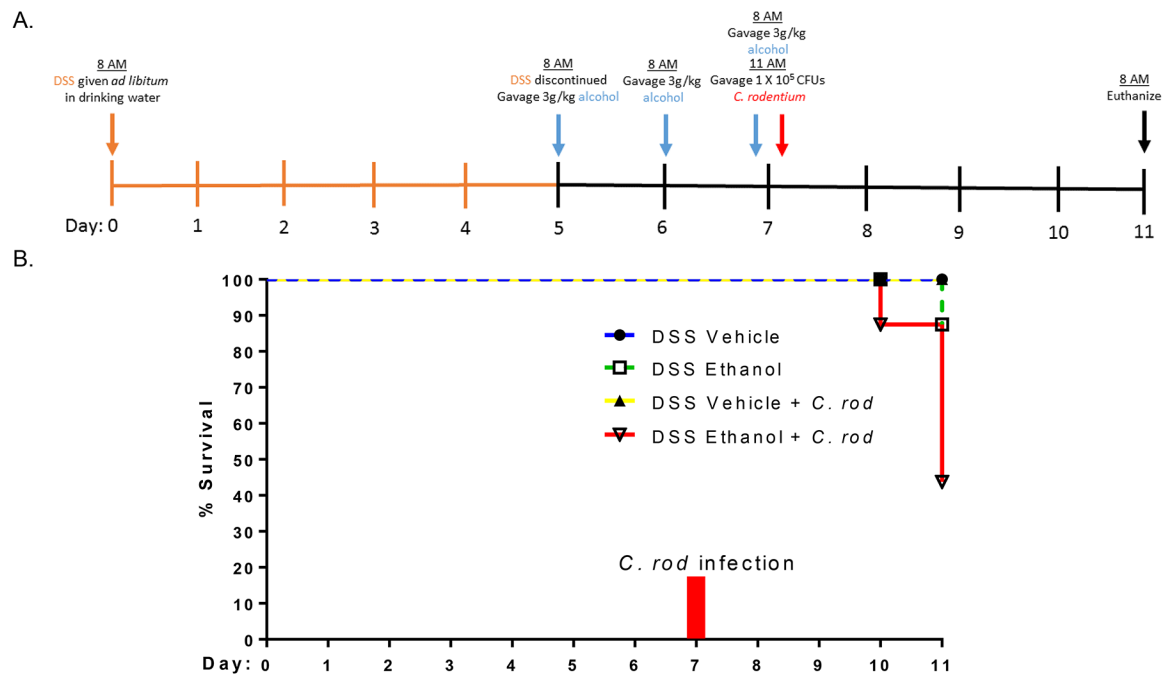
Figure 5.).

A. *Murine model of DSS-induced colitis and ethanol.* A 2% DSS concentration was administered *ad libitum* in drinking water for 5 days to mimic symptoms of UC. On day 5, DSS was discontinued to allow entrance into UC remission. A binge alcohol paradigm was employed where mice were gavaged with alcohol or water on days 5, 6, and 7. Mice were further subdivided and were gavaged with either 1×10^5 CFUs *C. rodentium* or water 3 hours post last gavage on day 7. Mice were euthanized on day 11.

B. *Ethanol decreased % survival following C. rodentium infection.* n=7–8 animals/group.

A.

B.
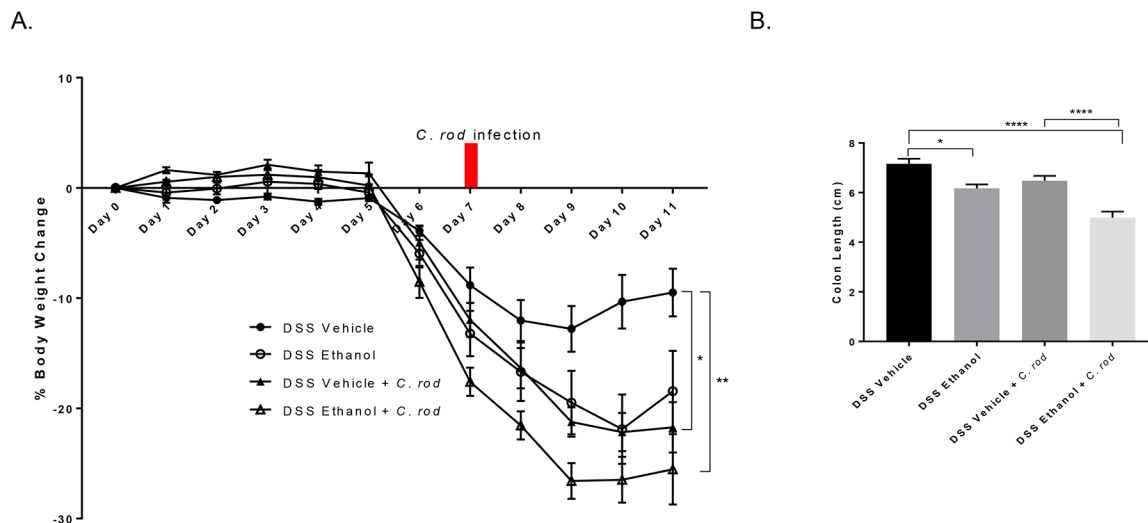
Figure 6.).

A. A*lcohol consumption increases weight loss following C. rodentium infection in DSS-induced colitis.* Values are calculated as average % weight change, *p<0.05 DSS Vehicle + *C. rod* compared to DSS Vehicle; **p<0.01 DSS Ethanol + *C. rod* compared to DSS Vehicle by Two-way ANOVA, n=7–8 animals/group. B. *Increased colonic shortening with C. rodentium infection after alcohol consumption and DSS-induced colitis.* Values are means ± SEM, n=7–8 animals/group. *p<0.05 DSS Ethanol compared to DSS Vehicle; ****p<0.0001 DSS Ethanol + *C. rod* compared to DSS Vehicle and DSS Vehicle + *C. rod* ANOVA.
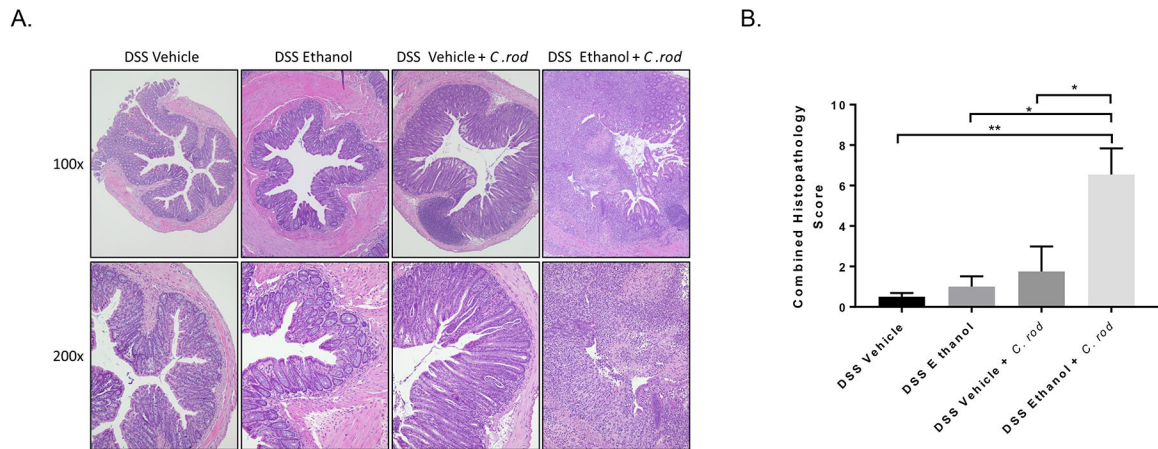
Figure 7.).

A. *Increased colonic damage and inflammatory infiltrate with C. rodentium infection after alcohol consumption and DSS-induced colitis.* Representative H&E images, n=6–12 animals/group. B. Combined Histopathology Score following blinded histological scoring as described in detail in Methods section above. Values are mean ± SEM, n=6–12 animals/group. **p<0.01 DSS Ethanol + *C. rod* compared to DSS Vehicle; *p<0.05 DSS Ethanol + *C. rod* compared to DSS Vehicle + *C. rod* and DSS Ethanol by ANOVA.
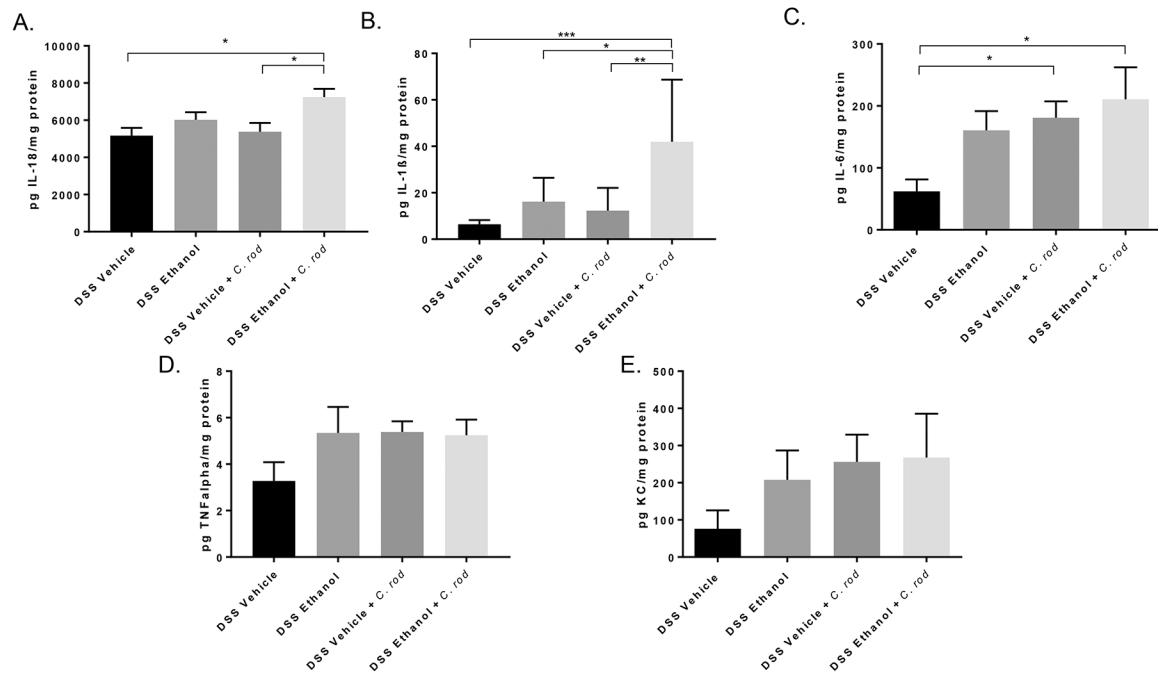
Figure 8.). C. rodentium further increases colonic inflammation after DSS-induced colitis and ethanol treatment.

Colons were harvested, homogenized, and processed on day 11 for the analysis of inflammatory mediators using respective ELISAs. A. IL-18 *p<0.05 DSS Ethanol + *C. rod* compared to DSS Vehicle + *C. rod* and DSS Vehicle by ANOVA, B. IL-1β *p<0.05; **p<0.01; ***p<0.001 DSS Ethanol + *C. rod* compared to all other groups by ANOVA, C. IL-6 *p<0.05 DSS Ethanol + *C. rod* compared to DSS Vehicle and DSS Vehicle + *C. rod* compared to DSS Vehicle by ANOVA, TNFα, E. KC by ELISA. Values are mean ± SEM 7–8 animals per group.

Table 1.

IBD Patient Demographics and Comorbidities Pre and Post Match

	Pre match			Post match		
	IBD-A (n=39,730)	IBD+A (n=2,080)	p value	IBD-A (n=2,021)	IBD+A (n=2,021)	p value
Mean Age	47.05	48.21	**0.0009**	48.19	48.3	0.8251
Sex			**< 0.001**			0.948
Male	17,658 (44.4%)	1,343 (64.6%)		1,289 (63.8%)	1,287 (63.7%)	
Female	22,072 (55.6%)	737 (35.4%)		732 (36.2%)	734 (36.3%)	
Race			**< 0.001**			0.728
White	28,440 (71.6%)	1,435 (69.0%)		1,409 (69.7%)	1,398 (69.2%)	
African American	4,112 (10.3%)	315 (15.1%)		302 (14.9%)	303 (15.0%)	
Hispanic	4,715 (11.9%)	221 (10.6%)		218 (10.8%)	214 (10.6%)	
Asian or Pacific Islander	518 (1.3%)	19 (0.9%)		12 (0.6%)	19 (0.9%)	
Native American	74 (0.2%)	<10 (%) [*]		<10 (%) [*]	<10 (%) [*]	
Other	1,871 (4.7%)	87 (4.2%)		79 (3.9%)	84 (4.2%)	
Smoking Status			**< 0.001**			0.139
Non smoker	26,956 (67.8%)	466 (22.4%)		427 (21.1%)	466 (23.1%)	
Smoker	12,774 (32.2%)	1,614 (77.6%)		1,594 (78.9%)	1,555 (76.9%)	

[*] AHRQ restricts reporting of values when n<10

Table 2.

UC Patient Demographics and Comorbidities Pre and Post Match

	Pre match			Post match		
	UC-A (n=17,653)	UC+A (n=1,042)	p value	UC-A (n=992)	UC+A (n=992)	p value
Mean Age	50.48	50.45	0.9519	50.68	50.72	0.9515
Sex			< 0.001			0.569
Male	7,712 (43.7%)	706 (67.8%)		649 (65.4%)	661 (66.6%)	
Female	9,941 (56.3%)	336 (32.2%)		343 (34.6%)	331 (33.4%)	
Race			< 0.001			0.698
White	12,039 (68.2%)	665 (63.8%)		647 (65.2%)	636 (64.1%)	
African American	1,926 (10.9%)	172 (16.5%)		152 (15.3%)	162 (16.3%)	
Hispanic	2,443 (13.8%)	136 (13.1%)		143 (14.4%)	130 (13.1%)	
Asian or Pacific Islander	293 (1.7%)	11 (1.1%)		10 (1.0%)	11 (1.1%)	
Native American	40 (0.2%)	<10 (%) [*]		<10 (%) [*]	<10 (%) [*]	
Other	912 (5.2%)	56 (5.4%)		39 (3.9%)	51 (5.1%)	
Smoking Status			< 0.001			0.502
Non smoker	12,546 (71.1%)	258 (24.8%)		245 (24.7%)	258 (26.0%)	
Smoker	5107 (28.9%)	784 (75.2%)		747 (75.3%)	734 (74.0%)	

[*] AHRQ restricts reporting of values when n<10

Table 3.

CD Patient Demographics and Comorbidities Pre and Post Match

	Pre match			Post match		
	CD-A (n=22,956)	CD+A (n=1,103)	p value	CD-A (n=1,083)	CD+A (n=1,083)	p value
Mean Age	44.26	45.88	**0.0005**	45.35	45.82	0.4906
Sex			**< 0.001**			0.826
Male	10,332 (45.0%)	682 (61.8%)		657 (60.7%)	662 (61.1%)	
Female	12,624 (55.0%)	421 (38.2%)		426 (39.3%)	421 (38.9%)	
Race			**< 0.001**			0.666
White	16,994 (74.0%)	804 (72.9%)		792 (73.1%)	790 (72.9%)	
African American	2,298 (10.0%)	163 (14.8%)		166 (15.3%)	159 (14.7%)	
Hispanic	2,392 (10.4%)	91 (8.3%)		94 (8.7%)	89 (8.2%)	
Asian or Pacific Islander	236 (1.0%)	<10 (%)[*]		<10 (%)[*]	<10 (%)[*]	
Native American	35 (0.2%)	<10 (%)[*]		<10 (%)[*]	<10 (%)[*]	
Other	1,001 (4.4%)	36 (3.3%)		23 (2.1%)	36 (3.3%)	
Smoking Status			**< 0.001**			0.664
Non smoker	14,935 (65.1%)	215 (19.5%)		207 (19.1%)	215 (19.9%)	
Smoker	8,021 (34.9%)	888 (80.5%)		876 (80.9%)	868 (80.1%)	

[*]
AHRQ restricts reporting of values when n<10

Table 4.

Outcomes of Patients During IBD, UC, or CD Admission

	IBD admissions			UC admissions			CD admissions		
	IBD-A (n=4,965)	IBD+A (n=4,013)	p value	UC-A (n=1,602)	UC+A (n=1,437)	p value	CD-A (n=3,089)	CD+A (n=2,548)	p value
Diagnoses during admission									
C. difficile intestinal infection	98 (2.0%)	105 (2.6%)	**0.042**	61 (3.8%)	47 (3.3%)	0.427	52 (1.7%)	57 (2.2%)	0.133
Poorly defined intestinal infection	18 (0.4%)	31 (0.8%)	**0.009**	10 (0.6%)	21 (1.5%)	**0.022**	<10 (%) [*]	10 (0.4%)	0.514
All intestinal infection	131 (2.6%)	158 (3.9%)	**0.001**	75 (4.7%)	79 (5.5%)	0.304	65 (2.1%)	78 (3.1%)	**0.023**
Procedures/Surgery									
Abdomen CT Scan	338 (6.8%)	386 (9.6%)	**< 0.001**	110 (6.9%)	130 (9.0%)	**0.026**	226 (7.3%)	252 (9.9%)	**0.001**
Biopsy Small Intestine	432 (8.7%)	384 (9.6%)	0.155	158 (9.9%)	134 (9.3%)	0.616	306 (9.9%)	246 (9.7%)	0.752
Resection Small Intestine	92 (1.9%)	44 (1.1%)	**0.004**	12 (0.7%)	5 (0.3%)	0.139	73 (2.4%)	39 (1.5%)	**0.026**
Biopsy large intestine	897 (18.1%)	872 (21.7%)	**< 0.001**	492 (30.7%)	468 (32.6%)	0.272	475 (15.4%)	399 (15.7%)	0.771
Colectomy	79 (1.6%)	62 (1.5%)	0.861	75 (4.7%)	49 (3.4%)	0.077	11 (0.4%)	13 (0.5%)	0.376
Biopsy Rectum	156 (3.1%)	173 (4.3%)	**0.003**	133 (8.3%)	125 (8.7%)	0.695	59 (1.9%)	46 (1.8%)	0.772
Antibiotic injection	154 (3.1%)	234 (5.8%)	**< 0.001**	61 (3.8%)	79 (5.5%)	**0.027**	105 (3.4%)	148 (5.8%)	**< 0.001**
Median Length of Stay (Days)	4 (IQR 2–7)	4 (IQR 2–6)	**0.0091**	4 (IQR 3–7)	4 (IQR 3–7)	0.1797	4 (IQR 2–7)	4 (IQR 2–6)	**0.0139**
Mean Total Cost ($)	13,880.65	12,262.51	**< 0.0001**	14,564.63	13,217.54	**0.0293**	13,480.92	11,794.42	**0.0001**

[*]
AHRQ restricts reporting of values when n<10