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Colonic Diverticula Are Not Associated with Mucosal Inflammation or Chronic Gastrointestinal Symptoms

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Abstract

Background & Aim—Colonic diverticulosis has been reported to be associated with low-grade mucosal inflammation possibly leading to chronic gastrointestinal symptoms. However, there is poor evidence for this association. We aimed to determine whether colonic diverticula are associated with mucosal inflammation and whether diverticula are associated with chronic gastrointestinal symptoms. We explored whether inflammation was present among symptomatic participants with and without diverticula.

Methods—We analyzed data from a prospective study of 619 patients undergoing a screening colonoscopy from 2013 through 2015 at the University of North Carolina Hospital in Chapel Hill, North Carolina. Among our participants, 255 (41%) had colonic diverticula. Colonic mucosal biopsies were analyzed for levels of interleukin 6 (*IL6*), *IL10*, and tumor necrosis factor mRNAs by quantitative reverse-transcriptase PCR, and numbers of immune cells (CD4+, CD8+, CD57+, and mast cell tryptase) by immunohistochemistry. Gastrointestinal symptoms were assessed using Rome III criteria. Proportional odds models were used to estimate odds ratios (ORs) and 95% CIs.

Results—After adjustment for potential confounders, there was no association between diverticulosis and tumor necrosis factor (OR, 0.85; 95% CI, 0.63–1.16) and no association with CD4+ cells (OR, 1.18; 95% CI, 0.87–1.60), CD8+ cells (OR, 0.97; 95% CI, 0.71–1.32), or CD57+ cells (OR, 0.80; 95% CI, 0.59–1.09). Compared to controls without diverticulosis, biopsies from individuals with diverticulosis were less likely to express the inflammatory cytokine IL6 (OR, 0.59; 95% CI, 0.36–0.96). There was no association between diverticulosis and irritable bowel syndrome (OR, 0.53; 95% CI, 0.26–1.05) or chronic abdominal pain (OR, 0.68; 95% CI, 0.38–

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1.23). There was no evidence for inflammation in patients with symptoms, when those with vs without diverticulosis were compared.

Conclusion—We found no evidence that colonic diverticulosis is associated with mucosal inflammation or gastrointestinal symptoms. Among patients with symptoms and diverticula, we found no mucosal inflammation.

Keywords

symptomatic uncomplicated diverticular disease; painful diverticular disease; symptomatic diverticulosis; irritable bowel syndrome

Colonic diverticula are common in the United States. After the age of sixty, more than 50% of individuals in the Unites States have colonic diverticulosis.[1, 2] While colonic diverticulosis can be complicated by the overt inflammation of acute diverticulitis, there is some thought that colonic diverticulosis is associated with low-grade mucosal inflammation.[3] Moreover, this low-grade diverticular inflammation is believed to contribute to chronic gastrointestinal symptoms.[3] The term symptomatic uncomplicated diverticular disease (SUDD) has been applied to chronic gastrointestinal symptoms attributed to colonic diverticulosis in the absence of overt inflammation.[3]

Despite the growing literature associating colonic diverticulosis with low-grade mucosal inflammation and symptomatic uncomplicated diverticular disease, the evidence for these associations is poor. A single pilot study found that colonic diverticulosis was associated with an increase in colonic macrophages compared to controls.[4] This study was limited to bivariate comparisons that did not control for confounding variables. The evidence for chronic gastrointestinal symptoms attributed to colonic diverticulosis in Western populations is limited. A population-based study found an association between diverticulosis and diarrhea-predominant irritable bowel syndrome, but only in those over the age of 60.[5] Another study found an association, but diverticulosis status was abstracted from charts.[6] The evidence that low-grade diverticular inflammation is associated with chronic gastrointestinal symptoms is very low quality and includes a cases series[7] and case control studies with inappropriate controls.[4, 8] Determining whether colonic diverticulosis is associated with chronic mucosal inflammation and chronic gastrointestinal symptoms has clinical implications. The chronic inflammation argument is used as rationale for treating patients with SUDD with mesalamine.[9]

To assess whether colonic diverticula are associated with markers of colonic mucosal inflammation, we conducted a prospective study of patients undergoing a complete screening colonoscopy that included mucosal biopsies. We also examined whether colonic diverticula are associated with chronic gastrointestinal symptoms and explored whether markers of mucosal inflammation were increased among participants with chronic gastrointestinal symptoms. To focus on colonic diverticulosis, we excluded any individual with a history of diverticulitis or with overt diverticular inflammation.

METHODS

Study design

Data for this analysis came from a prospective study to assess risk factors for colonic diverticulosis (NIH R01DK094738). The study recruited outpatients undergoing a first-time screening colonoscopy between 2013–2015 at the University of North Carolina Hospital in Chapel Hill, North Carolina. The study included patients 30 years of age and older with at least a satisfactory preparation for colonoscopy and a complete examination to the cecum. The study excluded any patient with a prior colonoscopy, familial polyposis syndrome (defined as greater than 100 polyps or FAP gene test positive), evidence of colitis, previous colon resection, previous colon cancer or adenomas or indication other than screening. Informed consent was obtained from all participants. For this analysis, we excluded those with a history of diverticulitis (n=1) or overt diverticular inflammation (n=4). The University of North Carolina School of Medicine Institutional Review Board approved this study.

Participant interviews

Each participant completed a detailed telephone interview prior to the colonoscopy. The interview captured diet, physical activity, tobacco and alcohol use, non-aspirin nonsteroidal anti-inflammatory drug (NSAIDs), aspirin use, bowel habits and gastrointestinal symptoms. Dietary intake was collected using a 124-item food frequency questionnaire[10] and covered a 12-month period to avoid seasonal variation. Physical activity was assessed using a validated instrument and expressed as metabolic equivalents in minutes.[11] Race was self-reported. Bowel movement frequency was self-reported. Participants identified the stool form (using the Bristol Stool Scale) they had most of the time. Gastrointestinal symptoms were assessed using Rome III diagnostic criteria questions for irritable bowel syndrome. Chronic abdominal pain was defined as pain or discomfort at least 2–3 days a month for at least 6 months.

Colonoscopy and biopsy

A research assistant measured the participant’s height and weight the day of the colonoscopy. During the colonoscopy, the gastroenterologist assessed the colon for diverticula either on insertion or withdrawal. A research assistant was present during the entire examination to document the procedure on a standard data collection form. The gastroenterologist counted the number of diverticula in the colon by segment: cecum, ascending, hepatic flexure, transverse, splenic flexure, descending, and sigmoid colon and reported the findings to the research assistant. Biopsies were taken from normal appearing mucosa in the mid sigmoid in cases and controls for assessment of inflammatory markers. The biopsies (approximately 3–4 mm in diameter) were obtained using standard (8mm. wing) disposable, fenestrated colonoscopy forceps (Olympus, Center Valley, PA). Laboratory personnel were blinded to clinical information and diverticulosis status of subjects.

Intraepithelial lymphocytes

To evaluate the role of the mucosal immune system in the etiology of diverticulosis, we assessed immune markers and cytokine levels which have been implicated in inflammatory bowel disease and irritable bowel syndrome. We evaluated the following immune markers, CD4, CD8, CD57 and mast cell tryptase. Mouse monoclonal antibodies against human CD4 (NCL-L-CD4-368), CD8 (CD8-4B11-L-CE-S), and CD57 (NCL-NK1) were purchased from Leica Microsystems Inc. (Norwell, MA). Mouse monoclonal anti-human mast cell tryptase (M7052) was purchased from Dako (Carpinteria, CA).

Immunohistochemistry was carried out in the Bond fully automated slide staining system (Leica Microsystems Inc. Norwell, MA). Tissue sections were de-paraffinized in Bond dewax solution (AR9222) and hydrated in Bond wash solution (AR9590). Heat-induced antigen retrieval was performed for 30 min at 100ºC in Bond-Epitope Retrieval solution 1, pH-6.0 (AR9961; CD4, CD57 and MCT) or for 20 min in Bond-Epitope Retrieval solution 2, pH9.0, (AR9640; CD8). Slides were incubated in anti-CD4 or anti-CD8 for 30 min at a dilution of 1:200. Incubation in anti-CD57 was performed for 1 hour at a dilution of 1:100 and mast cell tryptase was used at a dilution of 1:3000 with an incubation time of 30 min. Detection of all antibodies was performed using the Bond Polymer Refine Detection System (DS9800). Stained slides were dehydrated and cover slipped. Positive and negative controls (no primary antibody) were included for each antibody.

Immunohistochemistry stained sections were digitally imaged (20x objective) using the Aperio ScanScope XT (Aperio Technologies, Vista, CA). Analysis of biomarkers was performed using Definiens Tissue Studio software (ver 3.6.1; Munich, Germany) with the Composer_Nuclei_and_Simulated_Cells algorithm. The algorithm was trained to digitally recognize cells from the stromal compartment of the colon tissue samples, greatly reducing the number of epithelial cells included in the analysis. The percent cells with strong (+3), medium (+2) and weak (+1) positive DAB signal was used to compare biomarker levels among tissue samples. The H score was calculated for each immune marker. The H score is a composite score that is based on the intensity of staining. The stained cells are counted and then multiplied by a constant based on the intensity of the stain, e.g. most intensely stained cells are multiplied by 3. H-Score = (% at 0) * 0 + (% at 1+) * 1 + (% at 2+) * 2 + (% at 3+) * 3. The H score is a continuous variable that ranges from 0 to 300.

We assessed mRNA expression levels of interleukin (IL)-6, IL-10 and tumor necrosis factor α by quantitative reverse-transcriptase (qRT) PCR using an established protocol.[12] Briefly, RNA was extracted from mucosal biopsies stored in RNALater using RNEasy Mini Kit (Qiagen, Valencia CA). RNA was quantified by Nanodrop spectrophotometry (ThermoScientific, Wilmington, DE) and the quality was assessed on the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). To ensure that the RNA was free of DNA contamination, we performed DNAse digestion using RQ1 RNAase Free DNAase (Promega, Madison WI) following the manufacturer's protocol. One microgram of RNA reversed transcribed to cDNA using Invitrogen cloned AMV (Invitrogen, Carlsbad, CA). qRT-PCR was performed in duplicates using SYBR Green (BioRad, Hercules, CA) and RT2 profiler (Qiagen, Valencia, CA) ready to use optimized primer sets specific for each gene. The

expression of each inflammatory cytokine was evaluated relative to hydroxymethylbilane synthase (HMBS), which served as housekeeping gene.

Statistical Analysis

Means and standard deviations were calculated for continuous variables, medians for skewed distributions of continuous variables, and proportions for categorical data. For analysis in the models, the immune markers and mRNA expression levels were converted into categories (quartiles). The 10% change-in estimate approach was used to identify confounding variables.[13] To assess the association between colonic diverticula and markers of mucosal inflammation or gastrointestinal symptoms, proportional odds models[14] were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). The models were adjusted for age, sex, and body mass index. We performed analyses with all diverticulosis cases and cases stratified by number of diverticula. All tests of significance were two-tailed and p-values <0.05 were considered significant. The analysis was performed using SAS 9.4 (SAS, Cary, North Carolina).

RESULTS

Our analysis included 619 participants. Among our participants, 255 (41%) had one or more colonic diverticula. Participants with colonic diverticula were more likely to be older, male, and overweight or obese than those without diverticula (Table 1). Among those with colonic diverticula, 28% had 1–3 diverticula, 36% had 4–10 diverticula, and 36% had 10 or more diverticula. Among those with colonic diverticula, 61% had only distal diverticula, 29% distal and proximal diverticula and 7% only proximal diverticula.

Colonic diverticulosis and mucosal inflammation

Adjusting for age, sex and body mass index, there was no association between all cases with diverticulosis and gene expression levels of tumor necrosis factor (TNF-α) (OR 0.85; 95% CI 0.63–1.16) and no association with CD4 (OR 1.18; 95% CI 0.87–1.60), CD8 (OR 0.97; 95% CI 0.71–1.32), or CD57 (OR 0.80; 95% CI 0.59–1.09). Furthermore, there was no association with mast cell tryptase (OR 1.02; 95% CI 0.75–1.39). There was a reduced likelihood of mucosal concentrations of IL-6 (OR 0.58; 95% CI 0.41–0.83) and IL-10 (OR 0.67; 95% CI 0.49–0.91) (Table 2).

Individuals with one to three colonic diverticula had a reduced mRNA gene expression of interleukin 6 (IL-6) (OR 0.59; 95% CI 0.36–0.96) and tumor necrosis factor (TNF-α) (OR 0.55; 95% CI 0.34–0.90). There was a reduced likelihood of mucosal concentrations of the anti-inflammatory cytokine IL-10 (OR 0.50; 95% CI 0.31–0.82) (Table 2). There was no association between a greater burden of colonic diverticula and cytokine gene expression or immune cells (Table 2). Median cytokine expression levels and immunohistochemistry markers by case control status are provided in a supplemental table.

Because gross inflammation (diverticulitis) is more common in the sigmoid colon, we conducted an analysis stratified by location of colonic diverticula. Individuals with only distal diverticula compared with no colonic diverticula had a reduced likelihood of, interleukin 6 (IL-6) (OR 0.53; 95% CI 0.35–0.81) (Table 3). There were no other

associations with distal diverticula. Individuals with pan diverticula had a reduced likelihood of CD57 (OR 0.59; 95% CI 0.36–0.94). Individuals with only proximal diverticula had a reduced likelihood of CD8 cell density (OR 0.36; 95% CI 0.14–0.93) and increased likelihood of a CD4/CD8 ratio (OR 4.52; 95% CI 1.24–16.51).

Colonic diverticulosis and gastrointestinal symptoms

Our analysis included 448 participants who completed the symptom interview. Of these, 12% met Rome III diagnostic criteria for irritable bowel syndrome and 14% reported chronic abdominal pain (pain or discomfort at least 2–3 days a month for at least 6 months). Examining extremes of bowel movement frequency, 2.4% had two or fewer bowel movements per week while 0.9% had twenty-two or more bowel movements per week. Regarding bowel consistency, 11% reported type one and two Bristol Stool type (hard lumps) and 8% type six and seven (watery, mushy) most of the time. Participants with irritable bowel syndrome were younger, more likely to be female, and overweight or obese than those without irritable bowel syndrome (Supplemental Table).

Adjusting for age, sex and body mass index, individuals with one or more colonic diverticula had no significant association with irritable bowel syndrome (OR 0.53; 95% CI 0.26–1.05) (Table 4). Moreover, there was no association between the number of colonic diverticula and odds of irritable bowel syndrome (Table 4). Similarly, there was no association between having colonic diverticula and chronic abdominal pain (OR 0.68; 95% CI 0.38–1.23). Compared to Bristol Stool types three, four and five, there was no association between colonic diverticulosis and stool types one and two (OR 1.03; 95% CI 0.52–2.05) or types six and seven (OR 1.20; 95% CI 0.55–2.61).

Colonic diverticula, gastrointestinal symptoms and mucosal inflammation

We identified 42 participants who met criteria for Rome III irritable bowel syndrome and had mucosal biopsies. Among these participants, 11 had colonic diverticulosis and 31 did not have diverticulosis. In bivariate comparisons, there were no differences in mucosal inflammatory markers, IL-6, IL-10, tumor necrosis factor, CD4, CD8, or mast cell tryptase (Table 5). Likewise, we identified 63 participants with chronic abdominal pain. Among these participants, 22 had colonic diverticulosis and 41 did not have diverticulosis. In bivariate comparisons, there were no differences in mucosal inflammatory markers, IL-6, IL-10, tumor necrosis factor, CD4, CD8, or mast cell tryptase (Table 5).

DISCUSSION

In this large colonoscopy-based study of individuals without a history of diverticulitis or overt peri-diverticular inflammation, we found that colonic diverticulosis was not associated with mucosal inflammation. We also found no association between colonic diverticulosis and chronic gastrointestinal symptoms. We explored whether markers of mucosal inflammation were increased among participants with chronic gastrointestinal symptoms and diverticulosis compared to those without diverticulosis. There was no evidence for mucosal inflammation in individuals with diverticulosis and chronic gastrointestinal symptoms, so called symptomatic uncomplicated diverticular disease.

The evidence that colonic diverticulosis without overt inflammation in an asymptomatic population without a history of diverticular disease is associated with low-grade mucosal inflammation is limited. A study from Italy assessed whether colonic mucosa from asymptomatic patients with diverticula was associated with markers of inflammation compared to those without diverticula.[4] Similar to our work, there was no difference in T-cell and mast cell counts between groups. Colonic macrophages were significantly increased in patients with colonic diverticula compared to those without diverticula. Notably, this was a pilot study of 30 participants and was limited to bivariate comparisons that did not control for confounding variables.

In Western countries, there is a rising incidence of chronic inflammatory diseases of the gastrointestinal tract in the absence of overt infection.[15] To evaluate a potential role for the mucosal immune system in the etiology of diverticulosis, we assessed immune markers and cytokine levels which have been implicated in inflammatory bowel disease and irritable bowel syndrome. The inflammatory response in Crohn's disease consists mainly of T cells and macrophages while the inflammation of ulcerative colitis is restricted to the mucosa with predominant infiltration by neutrophils.[15] There is some evidence for an imbalance of mucosal proinflammatory tumor necrosis factor α and anti-inflammatory IL-10 in irritable bowel syndrome, a condition previously considered a motility disorder.[16] Increased mucosal mast cell infiltration has also been reported in several studies of patients with irritable bowel syndrome.[17] Despite assessing an array of immune markers and cytokine levels, our work found no evidence that colonic diverticulosis is associated with mucosal inflammation, which suggests that chronic mucosal inflammation is not likely to be responsible for the development of diverticulosis.

SUDD has been defined as a subtype of diverticular disease in which there are persistent abdominal symptoms in the absence of macroscopically overt colitis or diverticulitis.[18, 19] Once colonic diverticula form, there is the potential for numerous complications. The most common diverticular complication is acute diverticulitis. Beyond diverticulitis, there is growing evidence for a spectrum of chronic diverticular-related bowel disorders. Many of these disorders remain ill defined however each likely has a separate pathophysiology. SUDD should not be confused with chronic or smoldering diverticulitis, segmental colitis associated with diverticular disease or the chronic gastrointestinal symptoms experienced after an episode of diverticulitis.

Whether SUDD is a valid condition independent of irritable bowel syndrome is controversial. There are no consistent definitions of SUDD in the literature. Some define SUDD as abdominal pain and changes in bowel habits attributed to diverticula in the absence of alternate etiologies.[5, 20, 21] While others define SUDD as abdominal pain in patients with diverticulosis in the absence of any complications (stenosis, abscess, fistulas) and where the presence of abdominal pain is noted in the left lower quadrant lasting for more than 24 hours.[18, 22, 23] Because of the possible overlap between IBS and SUDD, clinical criteria have been developed to differentiate the two entities.[22] Patients with SUDD are older than IBS patients, lack the female predominance of IBS, and have prolonged episodes of pain.[22] The pain is thought to arise from muscular contractions. Based on 24 hour manometry studies, patients with diverticulosis had a significant increase in regular

patterns of phasic pressure activity compared to controls, and 30% reported cramping and lower abdominal pain during colonic contractions.[24] The study was based on small numbers (12 patients) and painful episodes, although associated with contractions, lasted for 5–10 minutes.

The evidence for colonic diverticulosis and associated gastrointestinal symptoms is limited. A population-based colonoscopy-based study from Sweden found that participants with diverticulosis were more likely to report loose stools (OR 1.88; 95% CI, 1.20–2.96) and high stool frequency (OR 2.02; 95% CI, 1.11–3.65). There was no significant association with abdominal pain, irritable bowel syndrome or irritable bowel syndrome subtypes. In analyses limited to those over the age of 60, participants with diverticulosis had an increased risk of abdominal pain (OR 2.10; 95% CI, 1.01–4.37) and diarrhea-predominant irritable bowel syndrome (OR 9.55; 95% CI, 1.08–84.1) compared to those without diverticulosis.[5] In this study, colonic diverticula were not assessed in a standard manner and the gastroenterologist who performed the colonoscopy many times also performed the pre-procedure medical examination.[25] If the gastroenterologist believed that a patient's gastrointestinal symptoms were associated with colonic diverticulosis, they may have been biased to over-detect and over-report colonic diverticula in those with symptoms. The prevalence of colonic diverticulosis was substantially lower in this Swedish study (10–25% of participants 50–59 years old) compared with the prevalence of diverticulosis in US populations (33–40% of adults 50–59 years old).[1, 2] This may reflect a difference in the prevalence of diverticulosis in these two countries or under-reporting in the Swedish study.

Diverticulosis was also associated with irritable bowel syndrome in a study that utilized a survey and chart review.[6] Again, colonic diverticulosis was not assessed in a standard manner and there is the same potential for bias as in the Swedish study. In a study design similar to our own, distal diverticulosis was associated with irritable bowel syndrome in a Japan.[26] In contrast with prior work, we found no association between colonic diverticulosis and chronic abdominal pain or irritable bowel syndrome.

The evidence that low-grade diverticular inflammation is associated with chronic gastrointestinal symptoms is very low quality. The most commonly cited evidence is a case series of 17 patients with diverticulosis and gastrointestinal symptoms, 94% had histopathology findings of chronic non-specific inflammation.[7] This case series is published in abstract form only, did not include controls and included patients with a spectrum of gastrointestinal symptoms (diarrhea, constipation, rectal bleeding, weight loss, bloating). A small Italian study found no evidence for mucosal inflammation when comparing patients with SUDD and asymptomatic controls.[8] A pilot study found that patients with SUDD compared to asymptomatic controls without diverticula had higher levels of mucosal mast cells and macrophage counts.[4] Notably, the controls in both studies did not have colonic diverticula. Because there is evidence for mucosal inflammation in irritable bowel syndrome, these were not appropriate controls.[16]

We explored whether markers of mucosal inflammation were increased among participants with chronic gastrointestinal symptoms who had diverticulosis compared to those with symptoms who did not have diverticulosis. Because there is evidence that irritable bowel

syndrome is associated with mucosal inflammation, we did not compare those with and without irritable bowel syndrome. Instead, we were interested in whether there might be a subtype of individuals with irritable bowel syndrome (or chronic abdominal pain) with low-grade inflammation secondary to colonic diverticula. We found no evidence for mucosal inflammation in individuals with diverticulosis and the symptoms of irritable bowel syndrome or chronic abdominal pain. This finding has clinical implications. Based on the theory that SUDD is the result of chronic peridiverticular mucosal inflammation, some recommend treatment with mesalamine.[9] Our findings strongly question the rationale for treating SUDD with mesalamine.

Our work has notable strengths. This was a prospective study designed to study factors associated with colonic diverticula. Every participant had a complete colonoscopy and assessment for diverticula. Colon biopsies were obtained in a uniform manner. Gastrointestinal symptoms were assessed using Rome Criteria. Confounding variables were measured and accounted for in our analyses. The panel of inflammatory markers allowed us to assess both local inflammation and immune activation in the colonic mucosa. Our work has limitations. Because we included only patients presenting for a screening colonoscopy, we have a small number of participants with IBS in our sample. Moreover, we do not have a measure of IBS severity or IBS subtypes. While we studied several immune markers and cytokines, there are other potential markers that may be associated with chronic inflammation. Multi-analyte profiling could be used to assess an array of cytokines and markers for macrophages (CD68), global T cells (CD3), and B cells (CD19) could be studied. Whether there is utility in further study given our negative results is debatable.

In summary, we have found no evidence that colonic diverticula are associated with mucosal inflammation or chronic gastrointestinal symptoms. Our data challenge the concept that colonic diverticulosis is a state of chronic inflammation and suggest that chronic mucosal inflammation is not likely to be responsible for the development of diverticulosis. Our work also raises questions about the whether symptomatic uncomplicated diverticular disease is a legitimate condition. We found no evidence of inflammation suggesting that there is no basis for anti-inflammatory agents like mesalamine in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics Among Those With and Without Colonic Diverticula

	Diverticula	No diverticula
n (%) or mean ± standard deviation	n = 255	n=364
Age		
≤49 y	20 (8)	38 (10)
50–59	171 (67)	270 (74)
60–69	55 (22)	48 (13)
70–79	8 (3)	8 (2)
≥80	1 (0.4)	0 (0)
Sex		
Male	120 (47)	150 (41)
Female	135 (53)	214 (59)
Race		
White	195 (76)	272 (76)
Black	50 (20)	75 (21)
Other	1 (0.4)	11 (3)
Smoking status		
Never	104 (54)	157 (60)
Former	59 (31)	76 (29)
Current	29 (15)	30 (11)
Body mass index, kg/m^2		
Underweight (<18.5)	6 (2)	7 (2)
Normal (18.5–25)	66 (26)	124 (34)
Overweight (25–30)	83 (33)	111 (30)
Obese (>30)	100 (39)	118 (32)
Waist circumference, centimeters	97.4 ± 17.0	93.1 ± 15.8
Physical activity per week, metabolic equivalent task-minutes	3,578 ± 4,732	3,330 ± 4,137
Daily dietary intake		
Total energy intake, kilocalories	2,110 ± 798	2,041 ± 770
Total fiber, grams	20.3 ± 8.7	20.6 ± 10.1
Nonsteroidal anti-inflammatory drug use per month		
Never	99 (52)	155 (59)
1–4 times	52 (27)	47 (18)
>4 times	39 (21)	59 (23)
Aspirin use per month		
Never	104 (54)	163 (62)
1–4 times	73 (38)	81 (31)
>4 times	14 (7)	19 (7)
Daily alcohol use, drinks	0.84 ± 1.51	0.76 ± 1.57

	Diverticula	No diverticula
Number of diverticula		
1–3	72 (28)	-
4–10	91 (36)	-
≥10	92 (36)	-
Location of diverticula		
Proximal only	19 (7)	-
Distal only	156 (61)	-
Both	75 (29)	-

Table 2

Colonic diverticulosis and association with cytokine expression and immunohistochemistry

	Adjusted odds ratio (95% confidence interval)¶				
	Controls	All cases	Cases by number of diverticula		
	(n=334)	(n=241)	1–3 diverticula (n=65)	4–10 diverticula (n=88)	≥10 diverticula (n=89)
Cytokine expression					
IL-6	Reference	0.58 (0.41, 0.83)	0.59 (0.36, 0.96)	0.86 (0.55, 1.34)	0.69 (0.44, 1.09)
IL-10	Reference	0.67 (0.49, 0.91)	0.50 (0.31, 0.82)	0.72 (0.46, 1.12)	0.77 (0.49, 1.20)
TNF-α	Reference	0.85	0.55 (0.34,	0.75 (0.48,	1.45 (0.93,
Immunohistochemistry - H score					
CD4	Reference	1.18 (0.87, 1.60)	0.88 (0.55, 1.42)	1.29 (0.84, 1.97)	1.35 (0.87, 2.09)
CD8	Reference	0.97 (0.71, 1.32)	0.75 (0.46, 1.21)	1.05 (0.69, 1.61)	1.08 (0.70, 1.67)
CD57	Reference	0.80 (0.59, 1.09)	0.64 (0.40, 1.05)	0.85 (0.56, 1.30)	0.91 (0.59, 1.41)
Mast cell tryptase	Reference	1.02 (0.75, 1.39)	0.90 (0.56, 1.45)	0.83 (0.54, 1.26)	1.44 (0.93, 2.23)
Immunohistochemistry - %					
positive cells					
CD4	Reference	1.25 (0.92, 1.70)	0.89 (0.55, 1.43)	1.42 (0.93, 2.18)	1.45 (0.93, 2.24)
CD8	Reference	1.02 (0.75, 1.39)	0.74 (0.45, 1.20)	1.05 (0.69, 1.62)	1.25 (0.81, 1.93)
CD57	Reference	0.79 (0.58, 1.08)	0.55 (0.34, 0.89)	0.91 (0.60, 1.39)	0.93 (0.60, 1.44)
Mast cell tryptase	Reference	1.03 (0.76, 1.40)	0.98 (0.60, 1.57)	0.79 (0.51, 1.20)	1.45 (0.94, 2.24)
CD4/CD8 ratio	Reference	1.18 (0.87, 1.60)	1.45 (0.89, 2.37)	1.17 (0.76, 1.79)	1.00 (0.64, 1.55)

¶Adjusted for age, sex and body mass index

Table 3

Colonic diverticulosis by location and association with cytokine expression and immunohistochemistry

	Adjusted odds ratio (95% confidence interval)[¶]			
	Controls	Cases by Location		
	(n=334)	Proximal diverticula only (n=17)	Distal diverticula only(n=148)	Pan diverticula (n=73)
Cytokine expression				
IL-6	Reference	0.44 (0.15, 1.28)	0.53 (0.35, 0.81)	0.74 (0.43, 1.27)
IL-10	Reference	0.50 (0.18, 1.41)	0.74 (0.49, 1.12)	0.80 (0.47, 1.35)
TNF-α	Reference	0.71 (0.29, 1.71)	0.91 (0.63, 1.31)	0.82 (0.51, 1.31)
Immunohistochemistry - H score				
CD4	Reference	0.80 (0.33, 1.92)	1.37 (0.96, 1.95)	1.09 (0.68, 1.73)
CD8	Reference	0.36 (0.14, 0.93)	0.92 (0.64, 1.31)	1.39 (0.87, 2.21)
CD57	Reference	0.49 (0.20, 1.20)	0.94 (0.66, 1.34)	0.59 (0.36, 0.94)
Mast cell tryptase	Reference	0.86 (0.36, 2.08)	1.06 (0.74, 1.51)	0.95 (0.60, 1.51)
Immunohistochemistry - % positive cells				
CD4	Reference	0.95 (0.40, 2.29)	1.28 (0.90, 1.82)	1.46 (0.91, 2.32)
CD8	Reference	0.33 (0.13, 0.85)	0.98 (0.69, 1.40)	1.38 (0.87, 2.20)
CD57	Reference	0.42 (0.17, 1.04)	0.92 (0.64, 1.31)	0.61 (0.38, 0.98)
Mast cell tryptase	Reference	0.86 (0.36, 2.07)	1.03 (0.72, 1.47)	1.05 (0.66, 1.68)
CD4/CD8 ratio	Reference	4.52 (1.24, 16.51)	1.31 (0.88, 1.97)	1.09 (0.64, 1.86)

[¶]Adjusted for age, sex and body mass index

Table 4

Colonic diverticulosis and association with symptoms

	Adjusted odds ratio (95% confidence interval)¶				
	Controls	All cases	Cases by number of diverticula		
	(n=259)	(n=189)	1–3 diverticula (n=46)	4–10 diverticula (n=73)	≥10 diverticula (n=70)
Rome III Irritable Bowel Syndrome	Reference	0.53 (0.26, 1.05)	0.47 (0.14, 1.62)	0.54 (0.21, 1.41)	0.57 (0.22, 1.47)
Chronic Abdominal Pain	Reference	0.68 (0.38, 1.23)	0.86 (0.34, 2.16)	0.65 (0.28, 1.50)	0.58 (0.24, 1.43)
Bristol Stool Scale					
Type 1 & 2	Reference	1.03 (0.52, 2.05)	1.41 (0.49, 4.08)	1.51 (0.64, 3.55)	0.45 (0.14, 1.44)
Type 6 & 7	Reference	1.20 (0.55, 2.61)	2.49 (0.89, 6.95)	0.80 (0.23, 2.77)	0.82 (0.25, 2.67)

¶Adjusted for age, sex and body mass index

Table 5

Mucosal Inflammation Among Symptomatic Participants With and Without Diverticulosis

	IBS with diverticulosis	IBS without diverticulosis	p-value¶	Abdominal pain with diverticulosis	Abdominal pain without diverticulosis	p-value¶
	(n=11)	(n=31)		(n=22)	(n=41)	
Cytokine expression (median)						
IL-6	1.84	0.95	0.57	0.61	1.12	0.98
IL-10	0.16	0.21	0.45	0.16	0.21	0.34
TNF-α	1.39	2.26	0.43	1.39	2.26	0.40
Immunohistochemistry- H score (median)						
CD4	102.0	101.0	0.34	103.0	100.0	0.30
CD8	26.0	30.0	0.28	26.0	31.0	0.16
CD57	10.0	16.0	0.05	11.0	16.0	0.16
Mast cell tryptase	46.0	40.0	0.75	48.0	41.0	0.68

¶Wilcoxon Two-Sample Test