Mucosa-Associated Microbiota in Ileoanal Pouches May Contribute to Clinical Symptoms, Particularly Stool Frequency, Independent of Endoscopic Disease Activity

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INTRODUCTION: Pouchitis is a common complication after ileal pouch–anal anastomosis (IPAA). However, there is a poor correlation between symptoms and endoscopic appearance of the pouch, and many patients can have debilitating symptoms in the absence of overt inflammation. It is unknown whether these clinical symptoms are independently associated with the microbiota. The objective of this work was to examine whether the individual clinical components of the pouch activity scoring systems are associated with specific microbiota.

METHODS: Pouch biopsies from 233 patients (50% male, 100% IPAA/ulcerative colitis) post-IPAA were included. Clinical phenotyping was performed, and patients were classified using both clinical and endoscopic components of the Pouch Activity Scale. Scoring for symptoms examined 24-hour stool frequency, urgency, incontinence, and rectal bleeding as described by the Pouchitis Disease Activity Index Score.

RESULTS: In the absence of inflammation, an increase in stool frequency reported over 24 hours was associated with a decrease in Bacteroidetes relative abundance, and this was the strongest association found. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis in inflamed groups showed that an increase in 24-hour stool frequency was associated with an increase in biofilm formation.

DISCUSSION: These findings indicate that in patients with IPAA, the composition of mucosa-associated microbiota of the pouch may contribute to clinical symptoms, particularly stool frequency, independent of endoscopic disease activity.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A40, http://links.lww.com/CTG/A41, and http://links.lww.com/CTG/A42

INTRODUCTION
Ulcerative colitis (UC) is a chronic inflammatory disease of unknown etiology affecting the colon. Approximately 10%–35% of affected individuals require surgery over their lifetime (1). The most common surgical procedure in patients with UC is the creation of an ileal pouch, namely ileal pouch–anal anastomosis (IPAA) (2). However, serious postoperative complications, such as leak or strictures, may occur. Pouchitis can occur in 30%–50% of these pouches in various forms. However, pouchitis-like symptoms can occur in the absence of overt endoscopic inflammation. Pouchitis is the most common complication after IPAA and is estimated to affect up to 50% of patients (3). Previous studies have used the Pouchitis Disease Activity Index (PDAI) and Pouchitis Activity Score (PAS) to describe inflammation severity. The correlation between these 2 indexes has been evaluated, providing a means of objectively evaluating pouchitis severity (4). However, it has been observed that patients with objective endoscopic inflammation in the pouch may have minimal to no symptoms and vice versa (4). Indeed, our group has previously shown that there is a low level of correlation between clinical and endoscopic or histologic subscores by both the PDAI and PAS (4). Clinical symptoms do not necessarily reflect objective evidence of inflammation, and these indexes do not always identify patients with pouch inflammation.

Following IPAA surgery, a study has suggested that the physiology of the small intestine becomes similar to the colon (5). Similarly, the microbiota becomes more similar to the colonic community than the microbiota of the ileum (6). It remains unknown which of these factors might contribute to the development of pouchitis.

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The human gut microbiome has been shown to be highly associated with health and disease (7). Indeed, it allows human immune system stimulation, resistance to pathogens, or degradation of indigestible dietary components (8,9). The microbiome has also been associated with many inflammatory diseases including Crohn’s disease, UC, multiple sclerosis, and pouchitis (10,11). Our group has shown that several microbial taxa are associated with pelvic pouch outcomes (pouchitis and Crohn’s disease–like phenotype). Difference in genera relative abundance was shown to correlate poorly with time since surgery (12,13). However, it is unknown whether those taxa are specifically associated with clinical traits in individuals with a pelvic pouch.

Our objective was thus to assess the correlation between composition and function of the tissue-associated microbiota of the pelvic pouch and clinical components of the pouchitis activity indexes.

METHODS

Patient recruitment
Patients were recruited during regular pouch follow-up at Mount Sinai Hospital in Toronto, Canada, up to 2013 (14). Any patients with confirmed UC and who had undergone IPAA at least 1 year before recruitment were included in the study. Biopsies were taken from within the pouch itself (1 biopsy) and 5–10 cm into the afferent limb (1 biopsy) and were immediately placed into sterile, empty freezer vials and snap frozen in liquid nitrogen. During the pouchoscopy, physicians documented the appearance of the pouch and afferent limb using established criteria as previously reported (12,14). The original study describing this patient population was reported in Tyler et al. (13).

Outcome measures
All patients were classified into outcome groups based on a combination of long-term complications in conjunction with inflammatory activity at the time of the procedure. To assess inflammation of both the pouch and afferent limb, endoscopic appearance (erythema, friability, and ulceration) and histological (polymorphonuclear leukocyte infiltration, ulceration/erosions) scores at the time of the study endoscopy were documented (15,16). These traits were used as they appear to be objective categories within the PDAI and PAS. Using these defined categories, a score greater than 3 was applied as the cutoff indicative of inflammation (15–17). Thus, endoscopic and histologic activity was defined as endoscopic and histologic PAS components greater than 3, respectively (Table 1). Clinical activity was defined as clinical PAS component greater than 3. Clinical activity was recorded on the day of pouchoscopy. More details on the scoring system for PAS/PDAI were previously described (17).

Taxonomic profiling of the gut microbiota
A total of 233 patients provided biopsies that were processed using identical protocols at 2 separate time points as a subset of the pouch. All samples were included in a previous study (14). Total DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Santa Clarita, CA) according to the manufacturer’s protocol with slight modifications of an additional bead beating step performed using the FastPrep system (MP Biomedicals, Solon, OH). Samples underwent 16S sequencing of the V4 hypervariable region of bacterial 16S ribosomal RNA (16S ribosomal ribonucleic acid [rRNA]) on the MiSeq platform.

Table 1. Clinical and endoscopic characteristics of the investigated cohorts

| Variable                                      | Endoscopically active<sup>a</sup> and clinically active<sup>b</sup> (n = 72) | Clinically active<sup>b</sup> but no endoscopic activity<sup>a</sup> (n = 82) | Endoscopically active<sup>a</sup> but no clinical activity<sup>b</sup> (n = 27) | Clinically active<sup>b</sup> and endoscopically active<sup>a</sup> inactive (n = 52) |
|-----------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Median PAS clinical score (range)             | 6.0 (4.0–7.0)                                                            | 5.0 (4.0–6.5)                                                            | 0.0 (0.0–2.0)                                                            | 0.0 (4.5–7.0)                                                            |
| Median stool frequency (range)                | 8.0 (6.75–11.0)                                                          | 9.0 (7.0–12.0)                                                           | 6.0 (5.0–7.5)                                                            | 6.0 (4.5–7.0)                                                            |
| Proportion with rectal bleeding (%)           | 54.0                                                                     | 47.6                                                                     | 0.0                                                                     | 0.0                                                                     |
| Proportion with abnormal frequency > 8 (%)   | 68.0                                                                     | 68.3                                                                     | 0.0                                                                     | 0.0                                                                     |
| Proportion with urgency/incontinence (%)      | 73.6                                                                     | 68.2                                                                     | 0.0                                                                     | 0.0                                                                     |
| Proportion PAS clinical score > 3 (%)         | 72.0                                                                     | 82.0                                                                     | 0.0                                                                     | 0.0                                                                     |
| Median PAS endoscopic score (range)           | 5.0 (3.0–6.0)                                                            | 0.0 (0.0–0.0)                                                            | 4 (3.0–5.5)                                                              | 0.0 (0.0–1.0)                                                            |
| Proportion PAS score endoscopic > 3 (%)       | 100.0                                                                    | 0.0                                                                     | 96.0                                                                    | 0.0                                                                     |
| Median PAS histologic score (range)           | 6.0 (4.0–6.25)                                                           | 3.0 (1.0–4.0)                                                            | 4.0 (3.0–5.0)                                                            | 2.0 (1.0–5.0)                                                            |
| Age (median ± SD)                             | 47.5 ± 13.1                                                              | 48.5 ± 12.2                                                              | 48.6 ± 12.2                                                             | 53.2 ± 14.1                                                             |
| Sex (M%)                                      | 55.0                                                                     | 40.0                                                                     | 57.0                                                                    | 55.0                                                                    |
| Proportion of antibiotic usage at biopsy extraction (%) | 31.0                                                                     | 29.0                                                                     | 26.0                                                                    | 21.0                                                                    |
| Proportion of probiotic consumption (%)       | 4.0                                                                      | 0.0                                                                     | 4.0                                                                     | 6.0                                                                     |
| Proportion of biologics usage (%)             | 4.0                                                                      | 0.0                                                                     | 4.0                                                                     | 0.0                                                                     |

Groups were classified according to the Materials and Methods section. M%, male percentage; PAS, Pouchitis Activity Score.

<sup>a</sup>Endoscopic and histologic activities were defined as endoscopic and histologic PAS components >3, respectively. Symptom groups with excessive stool frequency (>8 bowel movements/24 hours), daily rectal bleeding, and daily urgency/incontinence were also evaluated separately. The clinically active represented the active group, whereas the clinically inactive represented the “asymptomatic pouchitis” or “remission/quietness” groups (3).

<sup>b</sup>Clinical activity was defined as clinical PAS component >3.
were considered significant. Thus, the COG functions with a zero proportion higher than 90% were removed from the analysis. The remaining 4,309 COG functions were considered significant.

**Imputation of bacterial function**

The function of the microbial communities was imputed using the Li and Ji method (23). Thus, the COG functions were considered significant. Analyses using R software v 3.2.2 were restricted to merged OTUs with the same taxonomic assignment. P values of less than 5% after false discovery rate correction (q) were considered significant.

**Statistical analysis**

Samples were split into 4 groups according to the clinical and endoscopic activity observed: (i) clinically active and endoscopically active, (ii) clinically active but endoscopically inactive, (iii) nonsymptomatic and noninflamed, and (iv) nonsymptomatic but endoscopically active, as described above (Table 1). Filtering of 16s sequences was performed using phyloseq (24). Relative abundance of bacterial taxa obtained from sequencing of bacterial DNA were analyzed as a function of total stool frequency over 24 hours, and the 3 individual components of the PAS: rectal bleeding, stool frequency, and fecal urgency, and overall symptomatic behavior (dichotomized as being active or not active). R 3.2.2 was used for the analysis (25). A linear regression model was used to analyze the association between bacterial taxa counts and the clinical values, with correction for age, sex, and concomitant antibiotic use (within the previous 4 weeks before sampling). The linear model was used to obtain P values within all 4 clinical and endoscopic activity groupings over different taxonomic ranks. This was repeated within all 4 groups over COG terms.

Association of the general microbial composition and traits was assessed on a rarefied OTU table at a sequencing depth of 3,000. Beta diversity was measured using the weighted UniFrac distance. Association of general microbial composition in individuals with a low stool frequency (less than 5 bowel movements) and individuals with a high stool frequency (more than 15 bowel movements) was assessed using a PERMANOVA with 1,000 permutations.

**Sequence accession numbers and availability**

16S sequences are available under the study accession number PRJNA192210 (National Center for Biotechnology Information BioProject database).

**Ethics statement**

This study was approved by and conducted in accordance with the Research Ethics Board of Mount Sinai Hospital (Toronto, Canada).

**RESULTS**

**Description of the cohort**

A total of 233 patients were included in the analysis. The mean age was 47.9 (median = 48.6 ± 12.5) years, with 50% male (Table 1). At the phylum level of taxonomy (mean ± SD% relative abundance), Firmicutes (74.5 ± 22.3%), Proteobacteria (13.4 ± 20.5%), Bacteroidetes (7.1 ± 8.3%), and Actinobacteria (2.4 ± 7.3%) were the most abundant. At the genus level, Ruminococcus (26.5 ± 24.6%), an undefined Enterobacteriaceae (8.5 ± 17.3%), and an undefined Lachnospiraceae (8.4 ± 10.4%) were the most abundant.

**Clinical symptoms are associated with microbial composition in individuals with inflammation**

A total of 99 individuals were classified as endoscopically active. Within this subset of the cohort, we documented whether clinical symptoms were associated with the composition of the microbiota. The clinical output addressed was total stool frequency (over a 24-hour period), the individual PAS stool frequency score, PAS rectal bleeding score, PAS fecal urgency score, and overall presence or absence of clinical activity (total PAS score >3). We found a decrease in the relative abundance of Bacteroidetes when stool frequency was increased, and this was the strongest association found (coefficient estimate = −0.041 ± 0.014, q value = 0.03) (Figures 1 and 2, and see Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A40). Dissimilarity analysis of the microbial composition using weighted UniFrac distance showed that the general microbial composition was different when comparing individuals with a high vs low stool frequency, although not significantly in this population, the general microbial composition was different when comparing individuals with a high vs low stool frequency, although not significantly in this population (P < 0.067). At a lower taxonomic level, we found that this association was driven by the Bacteroidaceae family (coefficient estimate = −0.046 ± 0.016, q value = 0.14) and notably by the Bacteroides genus (coefficient estimate = −0.046 ± 0.016, q value = 0.10). Another undefined Chitinophagaceae genus was associated with increased stool frequency (coefficient estimate = 3.41 ± 0.83, q value = 0.01). Several other nominal associations were observed with a 1.2 × 10^4 < P < 0.05. However, no taxa were found to be associated with the rectal bleed PAS score, PAS stool frequency score, fecal urgency PAS score, and presence of symptoms/overall clinical activity score after correction for multiple testing (see Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A40).

**Individuals without endoscopic activity and with clinical activity are associated with microbial composition**

A subset of the cohort comprising 134 individuals had no objective endoscopic activity. Similar to individuals with active inflammation, we found that the general microbial composition was different when comparing individuals with a high vs low stool frequency (P < 0.047) (Figure 2). The total stool frequency association with Bacteroidetes was replicated with the same direction as in the noninflamed cohort, however, with a smaller effect size (coefficient estimate = −0.026 ± 0.010, q value = 0.08) (Figure 1 and see Table 2, Supplementary Digital Content 2, http://links.lww.com/CTG/A41). The PAS stool frequency score

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was associated with a decrease in the Bacteroidetes phylum (coefficient estimate = $-0.0147 \pm 0.004$, q value = 0.016). At the family level of taxonomy, Bacteroidaceae and an undefined Clostridiales contributed to the PAS stool frequency score, but did not survive correction for multiple testing (coefficient estimate $= -0.017 \pm 0.006$, q value $= 0.1$ and coefficient estimate $= -0.080 \pm 0.027$, q value $= 0.1$, respectively). No taxa were associated with rectal bleeding, fecal urgency, and symptomatic score after correction for multiple testing $7.5 \times 10^{-3} < P < 0.05$.

Stool frequency is associated with function of the gut microbiota in the presence of inflammation

Bacterial functions were imputed using PICRUSt software in the cohort. A single strong association was found in individuals with inflammation, which remained significant after correction for multiple testing (see Table 3, Supplementary Digital Content 3, http://links.lww.com/CTG/A42). We found that COG3936 encoding for a protein involved in polysaccharide intercellular adhesin synthesis/biofilm formation was associated with increased stool frequency (coefficient estimate $= 0.79 \pm 0.18$, $P = 6.55 \times 10^{-3}$). Several other nominal associations were observed for the other symptoms. Rectal bleeding was nominally associated with 54 COGs, with the most significant being COG2232 encoding for a predicted ATP-dependent carboligase related to biotin carboxylase ($P = 3.7 \times 10^{-3}$). The patient stool frequency score was nominally associated with 240 COG functions, the most significant being COG1139 encoding for an uncharacterized conserved protein containing a ferredoxin-like domain ($P = 5.4 \times 10^{-4}$). Fecal urgency was nominally associated with 1,020 COGs, the most significant being COG0469 encoding pyruvate kinase ($P = 8.3 \times 10^{-4}$). Overall symptomatic activity (stool frequency $> 8$ bowel movements/24 hours, daily rectal bleeding, and daily urgency/incontinence) demonstrated nominal associations with 27 COG functions, the most significant being COG5304 encoding for an uncharacterized protein conserved in bacteria ($P = 8.0 \times 10^{-4}$). In the noninflamed individuals, none of the clinical symptoms evaluated were associated with bacterial function after correction for multiple testing. However, several nominal associations were observed. Stool frequency shows nominal association with 12 COG functions, the most significant being COG3896 encoding for chloramphenicol 3-O-phosphotransferase ($P = 1.3 \times 10^{-3}$). Rectal bleeding shows nominal associations with 59 COG functions, the most significant being COG0446 encoding for an uncharacterized nicotinamide adenine dinucleotide (flavin adenine dinucleotide)-dependent dehydrogenase ($P = 0.011$). The PAS stool frequency score shows nominal association with 13 COG functions, the most significant being the same as for stool frequency, i.e., COG3896 ($P = 0.016$). Fecal urgency from the PAS shows nominal association with 6 COG functions, the most significant being COG4071 encoding for an uncharacterized protein conserved in archaea ($P = 3.2 \times 10^{-3}$). Finally, the overall symptomatically active group shows a large number of nominal associations with 1,168 COG functions, the most significant being COG3069 encoding for a C4-dicarboxylate transporter ($P = 3.4 \times 10^{-3}$).

DISCUSSION

Patients with IPAA often develop symptoms, which negatively affect their quality of life. Some of these symptoms may be due to active endoscopic and histologic pouchitis. However, it is also important to understand why symptoms may arise in the absence of inflammation. Patient-reported symptoms account for a portion of the total PAS and PDAI scores. However, as has been
demonstrated previously, in the setting of an ileal pouch, endoscopic and histologic inflammation correlates poorly with clinical symptoms such as stool frequency and urgency (4). This absence of correlation indicates that other factors might contribute to the symptoms reported by pouch patients. In this study, we suggested that some of these reported symptoms might be associated with microbiome structure and function. Particularly, we found that the composition of the mucosa-associated microbiome assessed at the time of pouchoscopy was associated with their concurrent clinical symptoms.

Our data suggest an association of microbiome composition with stool frequency. More specifically, a decrease in Bacteroidetes relative abundance was observed in patients with the highest stool frequency. A previous study, in patients with irritable bowel syndrome, also found Bacteroides to be more abundant in those with loose stools (26). A different study, in 69 healthy subjects undergoing colonoscopy, found that stool frequency was associated with mucosa-associated microbiome composition (27). Specifically, they found that an unclassified Ruminococcaceae genus was negatively associated with stool frequency. This finding is consistent with the direction of the association in our data set (coefficient estimate $= -0.06 \pm 0.03$, $P = 0.06$ in individuals without inflammation and coefficient estimate $= -0.14 \pm 0.06$, $P = 0.02$ in individual with inflammation).

Figure 2. Principal Coordinates Analysis plot showing that stool frequency is associated with microbiome composition. The top panels represent the microbiota observed in individuals with no inflammation (F-statistic $= 2.29$, $P = 0.047$, 10,000 permutations). The bottom panels represent the microbiota observed in individuals with active inflammation (F-statistic $= 1.76$, $P = 0.067$, 10,000 permutations). Samples are colored in blue for individuals with less than 5 bowel movements a day and in red for individuals with more than 15 bowel movements a day. Dissimilarity is measured using the weighted UniFrac distance. Each circle represents a sample from a given individual.
inflammation). However, an association of the Bacteroides genus with stool frequency (27) was not replicated in our data, which may reflect the smaller sample size and divergent population in their study.

The association of Bacteroidetes with stool frequency has a stronger effect size in individuals with active inflammation in the pouch compared with noninflamed individuals. This could suggest a response from the host to bacterial components related to inflammation, resulting in increased stool frequency. Such an example exists as epidermal growth factor receptor is released in response to inflammation and results in increased stool frequency through a complex feedback mechanism allowing a “wash out” of toxins to occur, thus allowing for wound healing in the gut (28). This process can be enhanced or antagonized by many components of stool, and certainly, the microbiome or its components could result in crosstalk with many of the complex signaling pathways activated by inflammation, resulting in increased fluid transport and thus increased stool frequency.

One proposed mechanism to explain ongoing residual symptoms in patients with inflammatory bowel disease (IBD) is the possible overlap between IBD and irritable bowel syndrome (IBS) in a proportion of patients (29,30). Patients with IBD have an increased relative abundance of Enterobacteriaceae with lower abundance of Faecalibacterium, Clostridium, and Ruminococcus genera compared with asymptomatic individuals (31,32). We observed a similar direction of the association in our study when compared with clinically and endoscopically inactive patients which was not significant after correction for multiple testing. This suggest that despite the microbial composition is different in patients with IBS and IBD, the presence of specific taxa could play a role in persistent symptoms in healed Crohn’s disease, particularly patients who have persistent increased stool frequency.

Mechanistic studies on the association of stool frequency and microbiome remain limited. In this study, we found that COG3936, encoding for a protein involved in polysaccharide intercellular adhesin synthesis/biofilm formation, was associated positively with increased stool frequency. This might suggest that increased stool frequency promotes biofilm formation through specific bacteria that in turn colonize the digestive tract more easily, potentially promoting a positive feedback loop resulting in further increased stool frequency (33). However this is only an association, and we cannot infer a direct cause and effect between microbial differences and stool frequency.

Interestingly, it was shown that a fecal transplant from a patient with slow-transit constipation into germ-free mice subsequently reduced the stool frequency in host mice as compared to a fecal transplant from a healthy donor (34). This effect was observed in humans when a fecal transplant from a healthy donor to a slow-transit constipation subject significantly increased stool frequency of the constipated subject (34). This suggests that the microbiome can directly affect stool frequency in both humans and mice. A study by Jankipersadsing et al. (35) suggested that host genetic components might be contributing to stool frequency. In this study, they were able to identify 53 suggestive loci contributing to stool frequency. Specifically, this study showed that mexiletine, a drug able to rescue SCN5A expression, restored stool frequency in carrier of SCN5A defects (35). However this effect seems to be independent of the gut microbiota because mexiletine itself did not affect microbial composition and abundance in in vitro experiment (36).

In conclusion, in the setting of an ileal pouch, endoscopic and histologic inflammation correlates poorly with clinical symptoms such as stool frequency and urgency. We found that the composition of the mucosa-associated microbiome from pouch biopsies correlates with clinical symptoms, particularly stool frequency, independent of endoscopic disease activity. These data could explain why pouch patients can have frequent stool and seemingly active disease even in the absence of significant inflammation and also respond well to antibiotic or probiotic therapy. These data may help us to better rationalize treatment strategies for these patients and to inform future clinical trials for pouchitis. Traditional endoscopic and histologic outcome measures in other phenotypes of inflammatory bowel disease may not apply in the pelvic pouch setting. Future therapies directed at modulation of the microbiome may be a particularly important approach in the management of pouchitis, especially for treatment of clinical symptoms.

CONFLICTS OF INTEREST
Guarantor of the article: Mark S. Silverberg, MD, PhD.
Specific author contributions: Conceived and designed the experiments: W.T., O.K., and M.S.S. Performed the experiments: W.T., O.K., K. Borowski, K. Boland, A.T., and M.S.S. Performed statistical analysis: W.T. and K. Borowski. Contributed reagents/materials/analysis tools and significant patient recruitment: K. Borowski, O.K., Z.C.; K.C., and M.S.S. Wrote the manuscript: W.T., O.K., K. Borowski, Z.C., and M.S.S. Financial support: Funding sources and study sponsors had no role in the study design, collection, analysis, and interpretation of the data and in the writing of the report. This research was funded through a grant from the Crohn’s and Collitis Foundation of Canada, CCFC (now known as Crohn’s and Collitis Canada, CCC). W.T. is a recipient of a Postdoctoral Fellowship Research Award from the CIHR Fellowship/Canadian Association of Gastroenterology (CAG)/Ferring Pharmaceuticals Inc. and a fellowship from the Department of Medicine, Mount Sinai Hospital, Toronto. O.K. is a recipient of an Advanced Inflammatory Bowel Disease Clinical Fellowship Research Award from the CIHR Fellowship/Canadian Association of Gastroenterology (CAG)/AbbVie. M.S.S. is supported in part by the Gale and Graham Wright Chair in Digestive Diseases. Potential competing interests: There are no conflicts of interest.

Study Highlights

| WHAT IS KNOWN |
|----------------|
| ✓ Pouchitis can occur in 30%–50% of individuals after IPAA. |
| ✓ Microbiome composition is associated with health and disease. |

| WHAT IS NEW HERE |
|------------------|
| ✓ Clinical components of the pouch activity scoring systems are associated with microbiota. |
| ✓ Stool frequency is associated with mucosal microbiome composition and function. |

| TRANSLATIONAL IMPACT |
|-----------------------|
| ✓ Future therapies directed at modulation of the microbiome may be a particularly important approach in the management of pouchitis, especially for treatment of clinical symptoms. |
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INFLAMMATORY BOWEL DISEASE

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