Comparison of Endoscopic Brush And Net Catheters As Sampling Tools To Analyze Lower Rectum Intestinal Mucus Samples of Patients With Ulcerative Colitis

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Abstract

Background and aims: The pathophysiology of ulcerative colitis remains unclear, but early lesions on the colorectal mucosal surface may play an important role in its etiology. Intestinal mucus samples, including inner and outer layers, are collected by net or brush catheters, but the quality of the samples obtained by each method has not been fully investigated. The aim of this study was to compare the microbiome and protein content of intestinal mucus collected by net and brush catheters during colonoscopy.

Methods: Intestinal mucus samples from the lower rectum of four patients with ulcerative colitis were collected using a net catheter, a brush catheter, and intestinal fluid suction. Microbiome and protein content were analyzed using 16S rRNA gene sequencing and mass spectrometry.

Results: The patients demonstrated significant differences in microbiome alpha diversity \((p < 0.05)\), but this difference was not observed between the sampling methods. Net catheter samples demonstrated higher total protein concentrations than brush catheter samples. Mucus-associated proteins (Mucin-2, Mucin-5B, Mucin-13, and IgGFc-binding protein) were more abundantly collected by nets in three patients with active ulcerative colitis, but more abundant by brushes in patients with inactive ulcerative colitis. Bidobacterium and some oral bacteria were similarly associated with ulcerative colitis activity.

Conclusions: Brush catheters are more likely to collect the intestinal mucus of inner layer, whereas net catheters are more likely to collect larger samples that include the outer mucus layer, as well as intestinal fluid. Two sampling methods with different types of work on the mucosa may lead different results among patients with mucosal vulnerabilities.

Introduction

The incidence of ulcerative colitis (UC) has continuously increased previously, but its pathophysiology has yet to be elucidated. Studies have proposed that the intestinal immune abnormalities in inflammatory bowel disease may be related to genetic and environmental factors [1, 2]. In particular, mental stress and overconsumption of fatty foods induce sympathetic hyperactivity, which reduces surface intestinal mucus. Reduced intestinal mucus results in increased antigen-presentation from diet and bacteria and a lower anti-inflammatory response [3]. Decreased parasympathetic activity has also been shown to contribute to a collapse of the intestinal mucosal immune system [3]. The intestinal mucosal system, as well as the gut microbiome, is currently considered as the main mediator behind UC [4, 5].

Clinical studies have examined the gut microbiome through fecal samples [6, 7]. However, it is difficult to identify which part of the large intestine influences the microbiome data from fecal samples. Moreover, large inter-individual differences in gut microbiome data make it difficult to identify specific UC-causing organisms [8]. The gut microbiome can be examined by intestinal mucus sampling during gastrointestinal endoscopy [9], wherein a sample of intestinal mucus is extracted with a brush or net catheter. The brush catheter is used more frequently [10], but its collecting tip is small, which makes acquiring an adequate amount of samples more difficult. In contrast, the net catheter utilizes a loop that is approximately 2.5 cm in size. In clinical practice, it is used to retrieve resected colorectal polyps, but the intestinal mucus attached to the loop may be analyzed separately. Net catheters may be more useful for collecting larger amounts of intestinal mucus.

The mucus layer of the large intestine has inner and outer layers [11]. The inner layer is thicker and more resistant to bacteria, whereas the outer layer is less dense and more susceptible to bacterial colonization [12]. Brush sampling is more likely to provide information about the inner layer because it samples the mucosa perpendicular to the intestinal wall, whereas net sampling takes samples parallel to the intestinal wall.

The aim of this study was to collect samples of intestinal mucus obtained during colonoscopy and analyze these based on whether they were collected by a brush or net catheter. Microbiome and protein data were analyzed through 16S rRNA gene sequencing and mass spectrometry, respectively.

Materials And Methods

This was a single-center study that examined four patients with UC. We collected lower rectum intestinal mucus samples through colonoscopy. Three samples were taken from each patient (Fig. 1). Intestinal mucus from the anterior and right rectal walls were collected using net (Roth Net®, US Endoscopy, OH, USA) and brush catheters (Colonoscope Cytology Brush®, Cook Medical, NC, USA),
respectively, whereas intestinal fluid on the left rectal wall was collected by suction. Each catheter was divided in half, and each half was used for microbiome and protein analysis, respectively. Microbiome data was sequenced using MiSeq, and the results were compared with linear discriminant analysis effect size (LEfSe) taxonomic comparison [13]. Human proteins were identified and analyzed using mass spectrometry.

This study was approved by the Research Ethics Committee of the Nagoya University Hospital (protocol number 2015–0420, August 30, 2016). Written informed consent was obtained from all patients prior to their enrollment in accordance with the Declaration of Helsinki. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN ID: 000020269). All clinical and stool sample information was anonymized before a database was constructed. The data of this research was based on the Nagoya gut microbiome database [14].

**Sample collection and 16S rRNA gene sequencing**

Gene sequencing was performed based on previously published methodology [15]. Samples were immediately stored at −80°C. Isolated DNA were amplified using universal primers (forward: 5’-TCGTCGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3’ and reverse: 5’-GTCTCGTGAGACATTGCTAAGAGACACCTCAGGGTACACTTCAGACAG-3’) to target the V3–4 regions of the bacterial 16S rRNA. Sequencing data were obtained using the MiSeq Reagent Kit v3 (Illumina, San Diego, California, USA), with 2 × 300 reads and 600 cycles for microbial analysis. We followed the instruction tutorials and used QIIME2 [16] and DADA2; GreenGenes was used as a reference. The details are shown in the supplementary information 1.

**Mass spectrometry**

The samples were lysed using the Minute Total Protein Extraction Kit (Funakoshi, Tokyo, Japan). The supematants were collected after centrifugation, and the protein concentrations of the supematants were determined by bicinchoninic protein assays to determine the total amount of proteins in the samples. After reduction and alkylation, the proteins were digested with trypsin for 16 h at 37°C. The peptides were analyzed by liquid chromatography mass spectrometry (LC MS) using an Orbitrap Fusionmass spectrometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) coupled to an UltiMate3000 RSLC nano LC system (Dionex Co., Amsterdam, The Netherlands) using a nano high-performance LC capillary column, 150 mm × 75 µm i.d. (Nikkyo Technos Co., Japan) and a nano-electrospray ion source. Reversed-phase chromatography was performed with a linear gradient (0 min, 5% B; 100 min, 40% B) with solvents A (2% acetonitrile with 0.1% formic acid) and B (95% acetonitrile with 0.1% formic acid) at an estimated flow rate of 300 nL/min. A precursor scan was performed using a 400–1,600 mass-to-charge ratio (m/z) prior to MS/MS analysis. Tandem MS was performed by isolation at 0.8 Th with the quadrupole, high-energy collisional dissociation fragmentation at a normalized collision energy of 30%, and rapid scan MS analysis was performed in the ion trap. Only precursors with charge states of 2–6 were sampled for MS2. The dynamic exclusion duration was set to 15 sec with a 10 ppm tolerance. The instrument was operated in the top speed mode with 3 sec cycles.

**Statistical analysis**

Categorical variables were compared using the chi-square or Fisher’s exact test, whereas continuous variables were compared using the Mann-Whitney U test. Data were analyzed using IBM SPSS Statistics version 24 (SPSS Inc., Chicago, IL, USA).

Microbiome data were visualized and statistically analyzed using MicrobiomeAnalyst [17]. The alpha diversities of the observed species were calculated using the Shannon-Weiner and Simpson indices. The analysis was performed with the default settings, but the filtering and taxonomic levels were set as the feature level. Low and variance filters were not used because they take minor bacteria. Beta diversity was calculated using an analysis of similarities, a non-parametric statistical test, and the taxonomic level was selected as the feature and genus level. The alpha diversities of the bacteria at the phylum level were compared using the Kruskal-Wallis H test, whereas the alpha diversities of the bacteria at the genus level were compared using LEfSe in the default setting.

Protein data were processed using Proteome Discoverer 1.4 (Thermo Fisher Scientific) and identified with the MASCOT search engine version 2.6.0 (Matrix Science Inc., Boston, Massachusetts, USA). The peptides and proteins in the samples were identified against the human protein database in UniProt (release 2020_03), with precursor mass and fragment ion mass tolerances fixed at 10 ppm and 0.8 Da, respectively. Fixed modification was set with cysteine carbamidomethylation, and variable modifications were set for the oxidation of methionine. Two missed cleavages with trypsin were used.
Results

Patient data

The clinical backgrounds of the four patients with UC are shown in Table 1. We obtained 1,530,112 demultiplexed sequence counts (paired-end reads) with 948 features identified. The median sequencing depth was 86,207.5, and the minimum frequency was 55,730 (Table 2). Both sequencing and feature data showed more variability when analyzed among patients than among the methods (supplementary information 2). However, all samples had sufficient sequence counts and quality for the analysis.

Alpha diversity and relative bacterial abundance

The alpha diversity of each patient is shown in the Table 2. The alpha diversity was significantly different among patients ($p < 0.05$), but there was no significant difference in the alpha diversities between the sampling methods (supplementary information 3 and 4). This difference can be clearly observed in the principal coordinates analysis plot because the samples formed clusters for each patient. This finding suggested that the microbiome data were more influenced by the patient than the method (supplementary information 5).

The percentages of phylum-level bacteria in each sample are shown in Fig. 2a. The microbiome was classified into nine phyla, and *bacteroidetes*, *fimbicutes*, and *proteobacteria* were the most dominant. The major dominance differed among patients but not among methods. There was no significant difference in the phylum-level bacteria detected among the sampling methods (supplementary information 6).

The percentages of genus-level bacteria in each sample are shown in Fig. 2b. The relative abundance of each bacteria was different among patients and sampling methods. However, the heat map suggested that the differences were more significant among patients (Fig. 1c). The results from the LEfSe demonstrated significant bacterial differences among patients but not among methods (supplementary information 7).

Bacterial data by sampling method

Each sampling method demonstrated a similar microbiome for each patient, but the microbiome data showed variability among the patients. At the genus level, data from the brush and net catheter, brush catheter and intestinal uid, and net catheter and intestinal fluid groups matched 85.9%, 80.75%, and 84.93% of the time, respectively.

While the types of bacteria varied greatly among the patients, some bacteria consistently demonstrated a higher relative abundance when sampled with the net catheter compared to the brush catheter. Statistical comparison of each bacterium at the genus level is difficult because we only have four patients. Therefore, instead of comparing the mean of each bacteria, we searched for bacteria that showed the same trend in all patients. In four patients with widely varying relative abundance of bacteria, we searched for bacteria whose net sample was greater than the brush sampling in all patients. Five (*Rothia*, *Granulicatella*, *Streptococcus*, *Butyricicoccus*, and *Bulleidia*) genus were more abundant in the net catheter samples than the brush catheter samples (supplementary information 8). The relative abundance of these bacteria was higher in the intestinal fluid samples than in the net catheter samples. As such, intestinal fluid samples collected the most significant and representative amount of bacteria, followed by net catheters and brush catheters, respectively. This also suggested that net catheters may be collecting samples closer to the mucosal surface than brush catheters. In contrast no bacteria were detected in all patients with higher relative abundance in the brush than net.

Mass spectrometry

Specimens were collected from the anterior and right walls of the rectal mucosa using brush and net catheters, respectively. Patient characteristics are shown in Table 1. We identified the proteins in the net and brush catheter samples using mass spectrometry. Significantly higher protein concentrations were detected in the net catheter samples than the brush catheter samples (Fig. 3a). We used these sample concentrations to identify the total number of proteins in each patient. The medium number of identified proteins was 1183 (range 815–1537) for brush samples and 1104 (range 689–1423) for net samples Analysis of mucus samples collected from ex vivo colon biopsies by tandem mass spectrometry has identified 29 core proteins (Sjoerd et al). Consistent with this report, MUC2 mucin, the main constituent of intestinal mucus, and other mucins, such as MUC5 and MUC13A, IgGFc binding protein (FCGBP), were also detected and demonstrated similar concentrations regardless of the sampling method.
Inflammatory protein markers (protein S100-A8, protein S100-A9) and a neurotrophic protein (myeloperoxidase) were also detected, but their expression levels were similar between the net and brush catheter samples (Fig. 3b). Some proteins were only identified in the net catheter samples; these are shown in Table 3.

**Relationship between microbiome and mass spectrometry for each patient**

We obtained samples from four patients. Of these, only patient No. 2 had a Clinical Activity Index (CAI) of 0, which means no active ulcerative colitis. The CAI was higher in patient No. 1, 4, 3, and 2, in that order. Mucin-2, Mucin-5B, Mucin-13 and IgGFc-binding protein (FCGBP) associated with intestinal mucosa of each patient are shown in Fig. 4a. Patients No.1, No.3, No.4 with active UC had higher abundance of these proteins collected in the net than in the brush. On the other hand, in the inactive UC patient No.2, more of these proteins were collected with the brush. Nine bacteria that showed a similar trend are shown in supplementary information 9. This means that only patient 2 showed a different trend when comparing brushes and nets. The bacteria with the highest relative abundance are shown in Fig. 4b. A enough amount of *Bifidobacterium* was detected in patients No.1, No.3 and No.4, but the percentage detected in patient No.2 was very low. The genus *Lachnospira*, a butyrate-producing bacterium, was higher in the brushes of patient No. 2 than in the nets, and the other patients had the opposite. On the other hand, oral bacteria *Staphylococcus* and *Dialister* were collected in brushes of active UC patients No.1, No.3 and No.4 more than the net.

**Discussion**

Recent studies have demonstrated that the microbiome of patients with UC differ from healthy controls [18] because the mucosal inflammation in UC changes the mucosa-associated microbiota (MAM). Ulceration of the colonic epithelium further aggravates this condition, which perpetuates the cycle. Nishino et al. [19] reported that the relative abundance of *Firmicutes* and *Proteobacteria* in intestinal surface mucus was different among patients with UC and Crohn's disease, and normal controls. Fecal microbiota transplantation (FMT), probiotics, prebiotics, or synbiotics have been shown to be effective for UC [20, 21]; however, the pathogenesis of UC must be clarified before new therapeutic microbiome-based strategies are introduced.

The gut microbiome differs depending on the location in the large intestine. As such, we only collected samples from a single site, the lower rectum. Our endoscopic samples demonstrated a large amount of human DNA but little bacterial DNA [22]. Among the sampling techniques, brush catheter sampling has been reported to provide more bacterial DNA than biopsies, even if the amount of DNA obtained through brush catheter sampling is lower. We also expected that the brush catheter would acquire a smaller sample of proteins, which would make protein analysis difficult.

The colonic mucus layer has inner and outer layers. Both layers have the same protein profile; however, the inner mucus layer is denser and does not contain any bacteria or bacterial metabolites [11]. Therefore, the microbiome data is not expected to change, even if a larger proportion of the inner layer is sampled. In contrast, there is a difference in the microbiome of the mucus layer and intestinal fluid [23]. Each of our sampling techniques may have collected different proportions of mucus and intestinal fluid content, which would explain the difference in the microbiome data identified in our study. While both brushes scrape the mucus layer to collect a sample, the net catheter scrapes over a larger area, which may catch more intestinal fluid and mucus than the brush catheter.

Lavelle et al. also analyzed whether biopsy, brush, and laser capture microdissection affected the MAM data in five patients with UC and four controls [24]. Similar to our results, Lavelle et al. detected large inter-patient variabilities. While they were not able to identify definite UC-causing bacteria, their data did show higher relative abundance of *Coriobacteriaceae, Bacteroidaceae, Ruminococcaceae* [25], and *Family XIII Incertae sedis* in the mucosa of patients with US than in controls.

Our sample also showed a great variation among patients. Therefore, it was difficult to compare between patients in our study as well. However, comparison of brush and net proteins may suggest an association with activity. Abnormalities in mucus production have been reported in active UC. Abnormalities of the mucus system have been described in active UC. Reduction of MUC2 and FCGBP in the colonic mucus occurs prior to the onset of Reduction of MUC2 and FCGBP in the colonic mucus occurs prior to the onset of inflammation of UC and has been suggested to be related to the pathogenesis of UC. Mucins, component of mucus are classified into two different types: transmembrane mucins and gel-forming mucins. MUC2 and MUC5B are gel-forming MUC2 and MUC5B are gel-forming secreted mucins and MUC13 is transmembrane mucin. Both types of mucins are secreted by the goblet cells. In samples from patients with active ulcerative colitis, more than twice as much protein associated with mucus was detected in the net. On the other hand, brush samples collected more protein associated with mucus in patient 2, who had less active disease. This may indicate that
the relatively weak horizontal force of the net may cause the mucus to peel off easier. In other words, these differences indicate mucosal weakness, which may be associated with higher symptom scores.

Nishino et al. [19] showed that Bifidobacterium levels were higher in UC patients than in healthy controls using brush samples. Lavelle A [24] also showed that UC patients had higher Bifidobacterium than controls and also showed higher abundance in Mucus than in Lumen. This is in agreement with our finding that only highly active patients had higher relative abundance of Bifidobacterium. The lower abundance of Lachnospira, a butyrate-producing bacterium collected by netting in less active UC patients than in brushes may indicate that the bacterium has settled in a deeper mucosal layer. On the other hand, in active UC patients, Staphylococcus Dialister, a resident oral bacterium, was collected more abundantly by brushes than nets, which may indicate that the bacterium resides in the deep mucosal layer.

Studies have proposed that patients with UC have different microbiomes compared to healthy controls, but whether this is the cause or result of the disease process remains unknown. Various bacteria have been also been identified as the possible triggers for UC, but these have yet to be proven. FMT has been suggested as an effective therapy for UC because it increases the production of short-chain fatty acids, particularly butyric acid [26]. Butyric acid decreases intestinal permeability and maintains the integrity of the intestinal epithelium, which reduces overall disease severity [27]. Our analysis was able to identify genus Butyricicoccus bacteria, a butyric acid bacterium, in our samples. Rothia and Streptococcus are commonly found in the oral cavity and are expected to be present near the mucosal lumen surface. Net catheter sampling has high potentials for future research because it can collect more bacteria.

Our study also demonstrated that net and brush catheter sampling collected the same types and amounts of mucosal proteins and inflammatory markers, except for leucine-rich alpha-2-glycoprotein (LRG) [28], which was detected in the brush catheter samples alone. As mentioned previously, the brush catheter collects samples perpendicular to the intestinal mucosa and is more likely to obtain portions of the inner mucus layer. This presumes that LRG may be more predominant in the inner layer rather than the mucosal surface.

This study has several limitations. First, our sample size was small. Second, we tried to perform a multi-omics analysis; however, there were significant differences in the microbiomes among the patients, which made the analysis impossible. Lastly, we did not analyze the proteins that were only detected in small amounts.

In conclusion, our study demonstrated the bacterial and protein content of intestinal mucosal samples taken with net and brush catheters among patients with UC. Brush catheters were more likely to acquire samples from the inner mucus layer, whereas net catheters were more likely to collect larger samples that include the outer mucus layer and intestinal fluid.

Abbreviations

UC: ulcerative colitis
LEfSe: linear discriminant analysis effect size
LC MS: liquid chromatography mass spectroscopy
MAM: mucosa-associated microbiome
FMT: fecal microbiome transplantation

Declarations

- **Ethics approval and consent to participate**

This study was approved by the Research Ethics Committee of the Nagoya University Hospital (protocol number 2015-0420, August 30, 2016). Written informed consent was obtained from all patients prior to their enrollment in accordance with the Declaration of Helsinki. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN ID: 000020269).

- **Consent for publication**

Not applicable
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Conflicts of interest

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Conception and design: Nakamura M, Maeda K, and Yamamoto K; analysis and interpretation of the data: Nakamura M, Yamamura T, Kakushima N, Maeda K, Yamamoto K, Ishikawa E, Ishikawa T, Mizutani Y, Ohno E, Sawada T, Furukawa K, and Honda T; drafting of the article: Nakamura M, Maeda K, and Yamamoto K; critical revision of the article for important intellectual content: Iida T, Honda T, Ishigami M, and Kawashima H; and final approval of the article: Fujishiro M.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1. Demographic and baseline characteristics of patients
|                       |       |
|-----------------------|-------|
| Gender, (female: male) | 3/1   |
| Age (median, range)   | 46 (22–70) |
| Disease duration (month, range) | 900 (24–144) |
| Disease location, \(N\) |       |
| Extensive             | 3     |
| Left-sided            | 1     |
| Proctitis             | 0     |
| Treatment, \(N\)     |       |
| Oral 5-aminosalicyclic acid, \(N\) (%) | 4 (100) |
| Corticosteroids, \(N\) (%) | 1 (25) |
| Biologic agents, \(N\) (%) | 1 (25) |
| Immunomodulators, \(N\) (%) | 1 (25) |
| Calcineurin inhibitors, \(N\) (%) | 0 (0) |
| Topical Agents, \(N\) (%) | 0 (0) |
| C-reactive protein (mg/dL) (median, range) | 0.12 (0.01–0.39) |
| Albumin (g/dL) (median, range) | 4.3 (4.2–4.4) |
| Rachmilewitz Clinical Activity Index (median, range) | 5 (0–9) |
| UCEIS score (median, range) | 3 (2–4) |

* UCEIS, Ulcerative Colitis Endoscopy Index of Severity
Table 2
Demultiplexed sequence counts (paired-end reads) with 948 features identified by 16S rRNA gene sequencing

| Patient 1 | Brush catheter | Net catheter | Intestinal fluid |
|-----------|----------------|--------------|------------------|
| Sequence count | Feature Count | Simpson     | Chao1  | Shannon | Observed | Brush catheter | Net catheter | Intestinal fluid |
| 137,043   | 98,629         | 0.8690262   | 158    | 2.812758 | 158      | -            | 85.9        | 91.2             |
| 147,142   | 101,024        | 0.8807727   | 121    | 2.863048 | 121      | -            | -           | 85.3             |
| 169,781   | 118,025        | 0.8765724   | 118    | 2.852898 | 118      | -            | -           | -                |

| Patient 2 | Brush catheter | Net catheter | Intestinal fluid |
|-----------|----------------|--------------|------------------|
| Sequence count | Feature Count | Simpson     | Chao1  | Shannon | Observed | Brush catheter | Net catheter | Intestinal fluid |
| 76,634    | 56,846         | 0.8840762   | 94     | 2.629995 | 94       | -            | 80.6        | 71.9             |
| 116,490   | 85,041         | 0.9031833   | 108    | 2.83076  | 108      | -            | -           | 82.3             |
| 79,657    | 57,825         | 0.8997583   | 103    | 2.857096 | 103      | -            | -           | -                |

| Patient 3 | Brush catheter | Net catheter | Intestinal fluid |
|-----------|----------------|--------------|------------------|
| Sequence count | Feature Count | Simpson     | Chao1  | Shannon | Observed | Brush catheter | Net catheter | Intestinal fluid |
| 146,611   | 105,408        | 0.9195404   | 172    | 3.386226 | 172      | -            | 93.2        | 75.1             |
| 193,501   | 124,311        | 0.9407875   | 182    | 3.56386  | 182      | -            | -           | 78.5             |
| 139,854   | 86,681         | 0.9549685   | 167    | 3.653543 | 167      | -            | -           | -                |

| Patient 4 | Brush catheter | Net catheter | Intestinal fluid |
|-----------|----------------|--------------|------------------|
| Sequence count | Feature Count | Simpson     | Chao1  | Shannon | Observed | Brush catheter | Net catheter | Intestinal fluid |
| 97,864    | 55,733         | 0.9432242   | 231    | 3.740973 | 231      | -            | 83.9        | 84.8             |
| 136,630   | 85,734         | 0.9646357   | 271    | 4.085237 | 271      | -            | -           | 93.6             |
| 88,905    | 55,730         | 0.9635789   | 262    | 4.074341 | 262      | -            | -           | -                |

| Average | Brush catheter | Net catheter | Intestinal fluid |
|---------|----------------|--------------|------------------|
| Sequence count | Feature Count | Simpson     | Chao1  | Shannon | Observed | Brush catheter | Net catheter | Intestinal fluid |
| 114,538  | 79,154         | 1            | 164    | 3       | 164      | -            | 85.9        | 80.75            |
| 148,441  | 99,028         | 1            | 171    | 3       | 171      | -            | -           | 84.925           |
| 119,549  | 79,565         | 1            | 163    | 3       | 163      | -            | -           | -                |
| Accession | Protein name                                                                 | MW   | Score     |
|-----------|------------------------------------------------------------------------------|------|-----------|
| P8217     | Chymotrypsin-like elastase family member 2A                                    | 85.4 | 8,965.87  |
| P09923    | Intestinal-type alkaline phosphatase                                           | 72.5 | 2,977.77  |
| P04118    | Colipase                                                                     | 35.4 | 2,679.83  |
| P08861    | Chymotrypsin-like elastase family member 3B                                    | 141.5| 2,611.26  |
| P13688    | Carcinoembryonic antigen-related cell adhesion molecule 1                      | 28.9 | 1,804.4   |
| P22748    | Carbonic anhydrase 4                                                          | 57.5 | 1,700.48  |
| P04054    | Phospholipase A2                                                              | 15.3 | 1,693.51  |
| Q9BYE9    | Cadherin-related family member 2                                               | 25.6 | 1,667.72  |
| P05107    | Integrin beta2                                                                | 28.9 | 1,371.29  |
| P35580    | Myosin-10                                                                    | 29.2 | 1,252.92  |
| P13797    | Plastin-3                                                                    | 191.5| 1,023.11  |
| A0A075B6K5| Immunoglobulin lambda variable 3–9                                             | 11.9 | 869.48    |
| P49913    | Cathelicidin antimicrobial peptide                                            | 60.1 | 851.99    |
| P14136    | Glial fibrillary acidic protein                                                | 63.1 | 840.2     |
| O43451    | Malate glucoamylase, intestinal                                                | 36.2 | 804.48    |
| Q00610    | Clathrin heavy chains 1                                                        | 50.1 | 784.71    |
| P08575    | Receptor-type tyrosine-protein phosphatase C                                  | 39.1 | 644.21    |
| P14923    | Junction plakoglobin                                                           | 16.1 | 536.23    |
| A0A075B6S6| Immunoglobulin kappa variable 2D-30                                           | 49.8 | 535.85    |
| Q09666    | Neuroblast differentiation-associated protein AHNAK                            | 78.8 | 474.64    |
| P51970    | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 8                 | 12.7 | 372.85    |
| O75830    | Serpin12                                                                      | 13.5 | 358.93    |
| P62834    | Ras-related protein Rap-1A                                                     | 13.2 | 357.57    |
| P08962    | CD63 antigen                                                                  | 12.6 | 320.95    |
| P29350    | Tyrosine-protein phosphatase non-receptor type 6                               | 12.5 | 305.96    |
| Q06210    | Glutamine-fructose-6-phosphate aminotransferase (isomerizing) 1               | 12.6 | 301.47    |
| Q6UX82    | Ly6/PLAUR domain-containing protein 8                                          | 12.3 | 293.9     |
| P19075    | Tetraspanin-8                                                                 | 12.4 | 290.21    |
| P26641    | Elongation factor 1-gamma 1                                                    | 84.7 | 290.03    |
| Q06323    | Proteasome activator complex subunit 1                                        | 56.8 | 278.75    |
| Q9UFN0    | Protein NipSnap homolog 3A                                                     | 81.7 | 262.03    |
| P19338    | Nucleolin                                                                      | 38.9 | 259.06    |
| O75131    | Copine-3                                                                       | 25.2 | 252.41    |
| O00584    | Ribonuclease T2                                                                | 209.7| 219.07    |

* MW, molecular weight
| Accession | Protein name                                              | MW     | Score  |
|-----------|-----------------------------------------------------------|--------|--------|
| Q8WZ42    | Titin                                                     | 46.6   | 206.99 |
| Q99798    | Aconitate hydratase, mitochondrial                        | 83.6   | 197.84 |
| P27216    | Annexin A13                                               | 228.9  | 193.28 |
| Q6UXH1    | Cystein-rich with EGF-like domain protein 2               | 20.1   | 160.39 |
| P30048    | Thioredoxin-dependent peroxide reductase, mitochondrial   | 628.7  | 153.36 |
| O75477    | Erin-1                                                    | 76.6   | 147.2  |
| P11310    | Medium-chain specific acyi-CoA dehydrogenase, mitochondrial| 48.5   | 146.29 |
| A0A087WSY6| Immunoglobulin kappa variable 3D-15                      | 16.3   | 138.52 |
| P61457    | Pterin-4-alpha-carbinolamine dehydratase                   | 70.8   | 125.63 |
| Q9H4A4    | Aminopeptidase B                                          | 28.7   | 124.13 |
| Q5TZA2    | Rootletin                                                 | 28.4   | 123.3  |
| Q5T0J7    | Testis-expressed sequence 35 protein                      | 12     | 118.9  |
| P01709    | Immunoglobulin lambda variable 2–8                       | 21     | 116.05 |
| P01706    | Immunoglobulin lambda variable 2–11                      | 147.4  | 115.43 |
| Q16881    | Thioredoxin reductase1                                    | 29.5   | 106.31 |
| A0A0A0MRZ8| Immunoglobulin kappa variable 3D-11                       | 228.4  | 105.43 |
| A0A0B4J1U7| Immunoglobulin heavy variable 6 – 1                       | 46.1   | 91.95  |
| P01742    | Immunoglobulin heavy variable 1–69                        | 26.5   | 87.15  |
| Q86YQ8    | Copine-8                                                  | 26     | 84.42  |
| Q15645    | Pachtene checkpoint protein 2homolog                      | 70.9   | 81.42  |
| Q16891    | MICOS complex subunit MIC60                               | 56.5   | 56.71  |
| Q05315    | Galectin-10                                               | 3,813.7| 56.69  |
| Q86TE4    | Leucine zipper protein 2                                   | 67.5   | 47.66  |

* MW, molecular weight

**Figures**
Figure 1

Endoscopic images of the lower rectum. This photograph shows the mucus in the lower rectum being collected by a net catheter, brush catheter, and intestinal suction.

Figure 2

16S rRNA gene sequencing a. This image shows the proportions of the microbiome at the phylum level. b. This image shows the proportions of the microbiome at the genus level. c. This image shows the heatmap of the microbiome at the genus level.
Figure 3

(a) Protein concentrations in the net and brush catheter samples
(b) Proteins collected by the net and brush catheter samples

Figure 4

(a) The amount of protein associated with the mucus layer obtained by brush or net is shown for each patient. Only patient 2 is a patient with no activity (CAI:0). These proteins were more collected in the brushes in patient 2 versus the nets in the other patients. In addition, the net collected twice as much as the brush. Because the vertical force on the mucus layer is weaker with the net than with the brush, the mucus layer may peel off more easily in active patients. b: Bifidobacterium in patient 2 is significantly less. In addition, more bacteria were collected on the brush than on the net. On the other hand, the oral bacteria Staphylococcus, Dialister was collected more on the brush than on the net in three patients. If brushes are collected from a deeper mucus layer than nets, this may indicate that there is less Bifidobacterium and more oral bacteria in the deeper mucus layer of active patients. CAI: Clinical activity index, UCEIS: Ulcerative Colitis Endoscopic Index of Severity B: Proteins collected by the net and brush catheter samples

Supplementary Files
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- supplementaryinformationall.pdf