Mucosal Integrity Testing Can Detect Differences in the Rectums of Patients with Inflammatory Bowel Disease Compared to Controls: A Pilot Study

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Abstract

Background—While the pathogenesis of inflammatory bowel disease (IBD) is incompletely understood, disruption of epithelial integrity is suspected to play a prominent role in disease initiation and progression. Currently, there is no convenient way to measure this in vivo.

Aims—Our aim is to determine whether a mucosal integrity (MI) testing device that has been used to measure MI in the esophagus can also be used to measure barrier function in the colon during colonoscopy.

Methods—Mucosal integrity testing was measured in patients with IBD (n = 17) and controls (n = 7) during colonoscopy. During the procedure, an MI catheter was passed down the working channel of the colonoscope and placed along the mucosal wall to measure MI in the rectum, left, transverse, and right colon. In patients with IBD, MI measurements and biopsies were taken...
in areas which appeared inflamed when present. We then determined if there was a significant
difference in MI between patients with IBD and controls.

**Results**—MI was significantly higher in the rectum of patients with IBD (CD and UC combined)
versus control colons [767 (618–991) vs. 531 (418–604) ohms, \( P < 0.01 \)]. There were no
significant differences in MI among patients with IBD versus controls in the right, transverse,
or left colon. Within the IBD group, there were no significant differences in MI between
inflamed versus non-inflamed rectums. There was no correlation between quality of life scores
or endoscopic severity with MI, though this study was likely underpowered to detect these
differences.

**Conclusion**—Rectal MI is significantly higher in patients with IBD versus controls. Future
studies are needed to determine how this information can be used clinically.

**Keywords**
Inflammatory bowel disease; Mucosal integrity testing; Intestinal barrier function; Endoscopy

**Introduction**

While the pathogenesis of inflammatory bowel disease (IBD) is incompletely understood,
disruption of colonic epithelial integrity plays a prominent role in disease initiation and
progression [1-4]. However, there is currently no convenient and efficient way to measure
intestinal barrier function during endoscopy. Prior methods for testing intestinal permeability
all have significant limitations. The lactulose/mannitol ratio (LAMA) which utilizes a large
molecule (lactulose) that cannot pass through normal mucosa, measures small intestinal but
not colonic permeability [5]. Confocal endomicroscopy makes use of images taken during
colonoscopy to measure epithelial integrity [6-9]. Abnormalities captured by this technology
have been shown to correlate with ongoing symptoms of diarrhea in patients with IBD, even
after endoscopic healing [10]. Imaging modalities such as these, however, require injection
of a permeability probe during the procedure and significant, time-consuming image
analysis by an expert post procedurally. Mucosal integrity (MI) testing, which has been used
in the esophagus to detect decreased epithelial integrity characteristic of gastroesophageal
reflux disease (GERD) and eosinophilic esophagitis (EoE), provides an efficient, real-time
measure of epithelial resistance during endoscopy [11-13]. Thus, we reasoned that MI may
also be able to measure colonic mucosal integrity in IBD. As such, our aim was to determine
whether mucosal integrity testing could detect changes in epithelial integrity in IBD.

**Methods**

The study was performed in accordance with the Declaration of Helsinki, Good Clinical
Practice, and applicable regulatory requirements. Each patient signed a consent form
before undergoing any study-related procedures. The Vanderbilt Institutional Review Board
approved this clinical trial (IRB# 160534), and it was registered at [www.clinicaltrials.gov
(NCT03165058)](https://www.clinicaltrials.gov). All authors had access to the study data and reviewed and approved the
final manuscript.
**Study Design and Patient Population**

This is a prospective study in which patients were enrolled at the time of colonoscopy from August 2020 to October 2020. Inclusion criteria for patients with IBD included age over 18 and a prior histologic diagnosis of ulcerative colitis (UC) or Crohn’s Disease (CD). Patients who had undergone prior small bowel surgeries or ileocecal resections were included. Exclusion criteria for patients with IBD included patients on experimental IBD therapy, pregnant patients, patients with indeterminate colitis, and patients who had undergone colonic resection. Indication for colonoscopy in the IBD group was either disease surveillance or worsening symptoms. Control subjects were patients who were undergoing average risk colon cancer screening without a known history of IBD. One control patient had a colonoscopy performed for gastrointestinal symptoms and was found to have a normal examination endoscopically (i.e., no evidence of inflammatory bowel disease).

**Equipment**

Mucosal integrity testing is currently being utilized at Vanderbilt University Medical Center for the assessment of esophageal and small bowel epithelial integrity [11-17]. A previously described MI catheter was used to measure electrical resistance of the esophageal epithelium by direct mucosal contact (InSight; Sandhill Scientific, Inc). Data were viewed and analyzed on BioView Analysis software (Sandhill Scientific, Inc) [14].

**MI Testing Procedure**

During colonoscopy, an MI catheter was inserted through the biopsy channel of a colonoscope. Once inserted, the endoscopist gently rested the probe against the targeted colonic mucosa for five seconds. Measurements were taken at four sites: right colon, transverse colon, left colon, and rectum. Within each of these segments, measurements were preferentially taken at the site of what appeared to be the most severe inflammation as determined endoscopically. Care was taken to take measurements at sites with minimal fluid and maximum contact with colonic mucosa. The majority of the procedures were completed by a single proceduralist, although a total of 3 endoscopists participated. The primary author (M.B.) was present in all procedures and was responsible for data collection and management of probe hardware and software throughout the study.

**Endoscopic Assessment of Disease Activity**

Previously validated scoring systems were utilized for measuring endoscopic disease activity. The Mayo score was used to evaluate disease activity in UC and the simplified endoscopic activity score for Crohn’s disease (SES-CD) was used to endoscopically evaluate CD [18, 19]. The complete Mayo score typically utilizes stool frequency, rectal bleeding, physician global assessment, and endoscopic findings. In this case, we used only the endoscopic scoring system and applied a score of 0 to 3 to test subjects. Scores ranged from 0 (normal or inactive disease) to 3 (severe disease with spontaneous bleeding, ulceration). The SES-CD scoring system assesses the following variables in the ileum, right colon, transverse colon, left colon and rectum: presence and size of ulcers, extent of ulceration, extent of affected surface, and presence/type of stricture. Scores range from 0 (no disease) to 60 (severe disease).
Pathology

Biopsies were taken adjacent to the sites of mucosal integrity measurements. Histology was classified as normal, inactive/quiescent inflammation, mild inflammation, moderate inflammation, or severe inflammation based on expert GI pathologist review. We then assessed if MI values correlated with degree of inflammation on pathology. For this analysis, given the small sample size, we combined active disease (mild, moderate, severe) into one group and inactive disease into a second group (normal, inactive/quiescent).

Measuring Symptoms

Participants were asked on the day of endoscopy to rate their quality of life using the previously validated [20] short inflammatory bowel disease questionnaire (SIBDQ). The SIBDQ score is a tool used to measure physical, social, and emotional status in patients with IBD with scores ranging from 10 (low impact on quality of life) to 70 (high impact on quality of life). We assessed for correlation between patient’s SIBDQ scores and MI values.

Statistical Analysis

Continuous variables were summarized using the median and interquartile range, and categorical variables were summarized using percentages. To test differences between groups, we used the nonparametric Kruskal–Wallis test for continuous variables and Pearson’s Chi-squared test for categorical variables. We had 80% probability (power) to detect a 1.3 standard deviation difference in MI between UC/CD and Control, a 1.5 standard deviation difference between UC and CD patients. In supplementary analyses, simple linear regression was used to estimate the association between disease severity and quality of life (Mayo or SIBDQ score) and MI measurements. All analyses were conducted using a 2-sided significance level of 0.05 with the R statistics language [21].

Results

Demographics

Patient demographics are shown in Table 1 (Crohn’s and UC patients separated) and Table 2 (patients with IBD as one group). MI testing was performed in 24 patients (9 with ulcerative colitis [UC], 8 with Crohn’s Disease [CD] and 7 controls) during colonoscopy. The Crohn’s disease patients were significantly younger than UC and controls. The IBD group had a higher rate of past GI surgical history. The average number of years since diagnosis for UC and CD was 7 and 11, respectively. Table 3 depicts the location and incidence of inflammation severity at a given segment of colon. There were no procedural complications in either the test groups or the control group.

MI Measurements

There was no significant difference in mucosal integrity in UC vs. Crohn’s disease (Table 4). Thus, we combined the two groups for comparisons with the control group. MI was significantly higher in the rectum of patients with IBD versus control colons (Table 5, 767 [618–991] vs. 531 [418–604] ohms, P < 0.01). There was not a statistically significant difference in MI between IBD and controls in the right, transverse, or left colon (Tables 4,
5). There was not a significant difference in rectal MI between patients with active (mild, moderate, severe) versus inactive (normal, inactive/quiet) inflammation by histology (Fig. 1). There was no significant correlation between rectal mucosal integrity and SIBDQ (Supplementary Fig. 1), Mayo Scores (Supplementary Fig. 2) in the UC patients or SES-CD scores in CD patients.

Discussion

Our results demonstrate that rectal MI is significantly higher in patients with IBD versus controls. This difference was not seen in the right, transverse, or left colon. Additionally, mucosal integrity values did not correlate with severity of inflammation, symptoms, or endoscopic inflammation. Since MI is lower in esophageal disease (e.g. GERD or EoE) where there is a loss of epithelial integrity, our initial hypothesis was that MI would be lower in patients with IBD for the same reason. However, this was not the case in our study as patients with IBD had significantly higher MI in the rectum compared with controls. There are multiple factors which could explain these results. It is likely that there are varying degrees of mucosal layer thickness and intraluminal fluid between subjects, depending on both colonic preparation quality and degree of inflammation, which could have varying effects on mucosal integrity testing. Differences in mucosal type (squamous vs. columnar) may also partially explain the differences in previously described MI values measured in the esophagus. This pattern is similar to prior findings which showed a significantly higher mucosal integrity in the duodenum of patients with active celiac disease versus control subjects [16]. It is possible that increased edema, mucosal thickness, or increased mucus production in the small intestine in celiac disease or colon in IBD can lead to higher MI values.

Further studies at the cellular and subcellular level are needed to further understand potential physiologic mechanisms for this observed MI difference in the rectum and to understand why this difference was not seen in other portions of the colon. Mucosal healing in IBD is a complex process with cellular, intracellular, and intercellular components at play simultaneously. Disruption in epithelial architecture, the extracellular matrix, and cytokine signaling is a function of degree and extent of inflammation, which is variable throughout the colon in patients with IBD [22, 23]. Thus, it is possible that a probe such as this is measuring a focal area of mucosal integrity rather than measuring barrier function of an entire segment of mucosa. A novel balloon MI catheter may decrease measurement variability by incorporating both radial and axial sensors mounted on a balloon to measure mucosal integrity along a longer segment of colonic mucosa, taking multiple measurements simultaneously [24]. Further research is needed using this new device.

Our study elucidated a significant difference in the rectums of patients with IBD versus control patients that was not significant in other colonic segments. Notably, there are differences in MI within the control group between the right side of the colon and the left side of the colon, suggesting that there are differences in the epithelium throughout the colon even in the absence of disease. One possible reason for this difference might be that the rectum is more susceptible to edema, resulting in variation in water and mucus.
content which might affect MI. However, it is unclear as to what the true etiology of these
differences, and future studies are needed to understand these differences.

Our study has multiple strengths. First, the mechanism for obtaining mucosal integrity
measurements is procedurally simple and easily employed during routine colonoscopy for
endoscopists of varying skill levels. This technology requires only a few added seconds per
procedure and adds no risk to the patient. Developing an easily utilized technology such as
this for a significantly prevalent condition such as IBD would prove extremely beneficial.
Additionally, our study was able to assess multiple commonly employed markers of disease
activity: symptomatology, endoscopic appearance, and histology.

Our study has multiple limitations to consider as well. The small sample size limits our
ability to answer several questions, such as whether there is a difference between Crohn’s
and Ulcerative Colitis, whether or not mucosal integrity testing correlates with symptoms
or endoscopic mucosal healing, or whether different drug therapies can alter MI values
irrespective of disease activity. Unfortunately, the specific probe model used in this study
was discontinued mid-study, limiting ability to obtain larger sample size. Determining
whether there was a correlation between symptoms and mucosal integrity would be helpful
clinically. For example, if larger studies revealed a potential correlation between IBD
symptoms or quality of life and MI, developing a reliable and easily performed MI testing
maneuver could provide valuable clinical data regarding the inflammatory status of an IBD
patient. Furthermore, symptoms in IBD can be unrelated to level of inflammation and thus
further assessing for inflammation with tools like fecal calprotectin and CRP may help
further delineate level of inflammation, symptoms, and MI differences.

Furthermore, our results revealed a significant age difference between our control patients
and patients with IBD. This raises the possibility that younger age may result in higher
MI values in the rectum rather than presence of disease. The correlation between age and
mucosal integrity with all groups combined did reveal a moderate and negative association
between rectal mucosal integrity and age. However, when looking at each group (UC,
Crohn’s, controls) individually, there is no significant correlation between rectal MIT and
age. Thus, while it is possible that higher rectal MIT in UC and Crohn’s is at least partially
due to age differences, our sample size is not big enough to make this conclusion. Notably,
our group’s prior studies in the esophagus have not shown any significant correlation in MIT
values with age [11, 12, 14-17]. Additionally, this correlation was not significant in other
colonic segments. Future studies are required to understand the relationship between age and
colonic MI.

In conclusion, while this device can detect a difference in impedance measurements in the
rectum of patients with IBD as compared with controls, future studies are needed to answer
whether this novel technology has clinical utility and whether it can be used to measure
disease activity.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.
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Fig. 1.
Rectal MI is not significantly different in patients with IBD with active versus inactive disease ($P = 0.81$). Severity of histologic disease activity in the rectum did not correlate with rectal MI values. Active disease included mild, moderate, and severe disease, while inactive disease included inactive/quiescent disease and normal
Table 1
Demographics for all patients by group (Controls, UC, CD) and medication use

|                                | UC    | CD    | Control | P value |
|--------------------------------|-------|-------|---------|---------|
| Age (median)                   | 48 (45.57) | 32 (26.37) | 62 (56.67) | 0.0011  
| Gender                         |       |       |         | 0.882   |
| Male (n)                       | 4     | 4     | 4       |         |
| Female (n)                     | 5     | 4     | 3       |         |
| Average # years since diagnosis| 7     | 11    |         | 0.613   |
| History of previous bowel surgery (%) | 0   | 40    | 0       | 0.04    |
| Indication for endoscopy (%)   |       |       |         |         |
| CRC screening (%)              |       |       | 86      | < 0.001 |
| Disease surveillance (%)       | 56    | 38    |         |         |
| Worsening symptoms (%)         | 44    | 62    | 14      |         |
| Number of procedural complications | 0 | 0     | 0       |         |
| Average Symptoms of IBD questionnaire (SIBDQ) score (range 10-70) | 55   | 48    |         | 0.173   |
| Average SES-CD score           | 65 (4.0, 11.2) | | | |
| Mayo score of 0                | 11% (1/9) | | | |
| Mayo score of 1                | 78% (7/9) | | | |
| Mayo score of 2                | 11% (1/9) | | | |
| Mayo score of 3                | 0% (0/9) | | | |
| 5′/ASA (%)                     | 78    | 38    |         |         |
| Topical steroids (%)           | 33    | 12    |         |         |
| Azathioprine (%)               | 0     | 12    |         |         |
| Methotrexate (%)               | 11    | 0     |         |         |
| Biologic therapy (%)           | 33    | 62    |         |         |
| Systemic steroids (%)          | 11    | 12    |         |         |

Continuous variables are represented as median (lower quartile, upper quartile). n is the number of non-missing values. Tests used:

1/ Kruskal-Wallis test

2/ Chi-square test
### Table 2

Demographics for all patients when combining patients with UC and CD

|                                | BBD (UC + Crohn’s) | Control | P value |
|--------------------------------|---------------------|---------|---------|
| Age (median)                   | 40 (27, 48)         | 62 (56,67) | 0.007<sup>1</sup> |
| Gender                         |                     |         | 0.65<sup>2</sup> |
| Male (n)                       | 8                   | 4       |         |
| Female (n)                     | 9                   | 3       |         |
| Average # years since diagnosis| 8                   | NA      |         |
| History of previous bowel surgery (%) | 14                   | 0       |         |
| Indication for colonoscopy (%) |                     |         | < 0.001<sup>2</sup> |
| CRC screening (%)              | 0                   | 86      |         |
| Disease surveillance (%)       | 47                  | 0       |         |
| Worsening symptoms (%)         | 53                  | 14      |         |

Continuous variables are represented as median (lower quartile, upper quartile). n is the number of non-missing values. Tests used:

<sup>1</sup> Kruskal-Wallis test

<sup>2</sup> Pearson test
### Table 3

**Histologic Inflammation severity based on colonic location**

|       | Right colon | Transverse | Left colon | Rectum |
|-------|-------------|------------|------------|--------|
| **UC** |             |            |            |        |
| Normal| 56% (5/9)   | 33% (3/9)  | 11% (1/9)  | 11% (1/9) |
| Mild  | 22% (2/9)   | 11% (1/9)  | 11% (1/9)  | 22% (2/9) |
| Moderate | 0% (0/9) | 11% (1/9) | 22% (2/9) | 11% (1/9) |
| Severe| 0% (0/9) | 0% (0/9) | 11% (1/9) | 11% (1/9) |
| Inactive | 22% (2/9) | 44% (4/9) | 44% (4/9) | 44% (4/9) |
| **CD** |             |            |            |        |
| Normal| 63% (5/8) | 50% (4/8) | 63% (5/8) | 63% (5/8) |
| Mild  | 13% (1/8) | 13% (1/8) | 0% (0/8) | 0% (0/8) |
| Moderate | 13% (1/8) | 25% (2/8) | 13% (1/8) | 25% (2/8) |
| Severe| 13% (1/8) | 0% (0/8) | 13% (1/8) | 0% (0/8) |
| Inactive | 0% (0/8) | 0% (0/8) | 13% (1/8) | 13% (1/8) |
### Table 4

Mucosal integrity (ohms) values in UC, CD versus control colons

|                | UC N = 9   | CD N = 8   | Control N = 7 | P value |
|----------------|------------|------------|---------------|---------|
| MI right colon | 1014 (805, 1347) | 1206 (1066, 1294) | 849 (716, 1250) | 0.45 |
| MI transverse colon | 753 (670, 1019) | 974 (844, 1180) | 783 (752, 880) | 0.33 |
| MI left colon  | 673 (549, 749) | 694 (538, 795) | 699 (594, 740) | 1.00   |
| MI rectum      | 785 (529, 991) | 759 (704, 838) | 531 (418, 604) | 0.034  |

Continuous variables are represented as median (lower quartile, upper quartile). N is the number of non-missing values. Test used: Wilcoxon test.
## Table 5

Mucosal integrity (ohms) values in UC, CD versus control colons (UC + CD combined)

|                      | IBD (UC + CD) N = 17 | Control N = 7 | P value |
|----------------------|-----------------------|---------------|---------|
| MI right colon       | 1139 (864, 1347)      | 849 (716, 1250) | 0.29    |
| MI transverse colon  | 891 (705, 1123)       | 783 (752, 880) | 0.73    |
| MI left colon        | 673 (547, 763)        | 699 (594, 740) | 0.97    |
| MI rectum            | 767 (618, 991)        | 531 (418, 604) | 0.009   |

Continuous variables are represented as median (lower quartile, upper quartile). N is the number of non-missing values. Tests used: Wilcoxon test.