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Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing

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Title: **Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing**

(Short Title: Intestinal permeability defects despite IBD mucosal healing)

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Abbreviations:

CD - Crohn's disease

CDAI - Crohn's disease activity index

CDEIS - Crohn's disease endoscopic index score

CDO - cell drop out

CJE - cell junction enhancement

CLS - Confocal Leak Score

CRP - C-reactive protein

eCLE - endoscope based confocal laser endomicroscopy

ESR - erythrocyte sedimentation rate

FL - fluorescein leak

IBD - inflammatory bowel disease

IBS - irritable bowel syndrome

IQR - inter-quartile range

pMayo - partial Mayo

ROC – receiver operating characteristic

TNF - tumor necrosis factor

UC - ulcerative colitis

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JC: study concept and design, performing CLE procedures, acquisition and interpretation of data, statistical analysis, drafting of manuscript, final approval of manuscript

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ABSTRACT

Background & Aims: Many patients with inflammatory bowel diseases (IBD) have ongoing bowel symptoms of diarrhea or abdominal pain despite mucosal healing. We investigated whether impaired intestinal permeability contributes to these symptoms.

Methods: We performed a prospective study of intestinal permeability, measured by endoscopic confocal laser endomicroscopy in 110 consecutive subjects (31 with ulcerative colitis [UC], 57 with Crohn's disease [CD], and 22 healthy individuals [controls]) in Sydney Australia from May 2009 and September 2015. Symptomatic CD was defined by a CD Activity Index score of 150 or more and symptomatic UC by a partial Mayo score of 2 or more. Mucosal healing was defined as CD Endoscopic Index of Severity of 0 in CD or Mayo endoscopic sub-score of 0–1 for patients with UC. Intestinal permeability was quantified by the Confocal Leak Score (CLS, range: 0=no impaired permeability to 100=complete loss of barrier function). The primary endpoint was intestinal permeability in patients with symptomatic IBD in mucosal healing vs patients with asymptomatic IBD in mucosal healing. We determined the sensitivity and specificity of CLS in determining symptoms based on receiver operating characteristic (ROC) analysis.

Results: Ongoing bowel symptoms were present in 16.3% of patients with IBD and mucosal healing (15.4% of patients with CD and 17.4% with UC). Patients with symptomatic IBD had a significantly higher median CLS (19.0) than patients with asymptomatic IBD (7.3, $P<.001$) or controls (5.9, $P<.001$). There were no significant differences between patients with IBD in remission vs controls ($P=.261$). The median CLS was significantly higher in patients with symptomatic than asymptomatic CD (17.7 vs 8.1, $P=.009$) and patients with symptomatic than asymptomatic UC (22.2 vs

6.9, *P*=.021). A CLS of 13.1 or more identified ongoing bowel symptoms in patients with IBD and mucosal healing with 95.2% sensitivity and 97.6% specificity; the ROC area under curve value was 0.88. Based on this cutoff, 36.2% of patients with IBD in mucosal healing have increased intestinal permeability. On regression analysis, every increase in CLS of 1.9 correlated with an additional diarrheal motion per day (*P*=.008).

Conclusions: In a prospective study of intestinal permeability in patients with IBD and mucosal healing, we associated impaired intestinal permeability with ongoing bowel symptoms ; increases in permeability correlated with increased severity of diarrhea. Resolution of mucosal permeability beyond mucosal healing might improve outcomes of patients with IDB. ANZCTR.org.au: ACTRN12613001248752

KEY WORDS: CLE; intestinal barrier function; small intestine; leaky gut

INTRODUCTION

Inflammatory bowel diseases (IBD) are characterized by recurrent intestinal inflammation and broadly classified as Crohn's Disease (CD) and ulcerative colitis (UC). While the exact pathogenic mechanisms remain unknown, IBD is thought to arise from the complex interplay of environmental, genetic, immunological and intestinal microbial factors.[1,2] Current medical therapies aim to modulate the intestinal immune system, but up to 35% of IBD patients remain symptomatic with abdominal pain and altered bowel habit, symptoms that overlap with irritable bowel syndrome (IBS).[3] The exact cause of these ongoing bowel symptoms in IBD subjects despite mucosal healing remain uncertain and may reflect dietary intolerance, sub-clinical inflammation, or true concurrent IBS.[4] Despite the induction of mucosal healing with or without normalization of fecal calprotectin, ongoing bowel symptoms of abdominal pain and/ or diarrhea remain present in 18-29% of IBD subjects.[5,6] This disconnect has so far not been explained. Impaired epithelial barrier function has been considered to be important in the pathogenesis of IBD, IBS and food hypersensitivities.[7-9] Intestinal permeability may be visible using real-time confocal laser endomicroscopy (CLE),[9-11] which combines a confocal laser microscope into the tip of a flexible colonoscope. Whether impaired epithelial barrier function and increased intestinal permeability underlies ongoing symptoms in IBD has not been adequately addressed. Indeed restoration of functional homeostasis of the mucosal barrier may be critical in symptom resolution in IBD or induction of long term remission.

The primary aim of this study therefore was to examine the association of small intestinal permeability as detected by CLE and ongoing symptomatic IBD patients

who have achieved mucosal healing. Our secondary aim was to examine the association between symptom severity and small intestinal permeability as detected by CLE.

METHODS

Design

This was a prospective study on IBD subjects versus age- and sex-matched controls. IBD subjects that demonstrated endoscopic mucosal healing were assessed by imaging of their intestinal mucosal barrier to determine whether impaired intestinal permeability correlated with bowel symptoms. The two patient reportable outcomes of interest were number of diarrheal stools passed per day and abdominal pain. The study was approved by Human Research Ethics Committee of Sydney South Western Area Health Service (Reference number 14/327). All authors had access to the study data and reviewed and approved the final manuscript.

Participants

Between May 2009 and September 2015, consecutive patients with established IBD for at least 12 months' duration, who required a colonoscopy for clinical indications, were enrolled for endoscope-based confocal laser endomicroscopy (eCLE) from the IBD clinics of Bankstown-Lidcombe Hospital and Concord Hospital, Sydney, Australia. Age- and sex-matched controls were recruited in parallel and were typically those referred for screening colonoscopy, had minor symptoms such as bloating or iron deficiency. Controls were excluded if they presented with regular diarrhea or abdominal pain or were subsequently diagnosed with a gastrointestinal disease after the eCLE. All subjects were aged 18-70 years old and provided written

informed consent. Predetermined exclusion criteria were known IBS, celiac disease, intestinal resection surgery (apart from limited ileal resection for CD), pregnancy or breast-feeding, renal disease, diabetes mellitus, decompensated liver disease, regular use of non-steroidal anti-inflammatory drugs (NSAID) or known allergy to fluorescein.

Data Collection

Clinical data collected from IBD subjects included disease phenotype, extent of disease, duration of disease, smoking status and NSAID-use. Symptoms prior to the eCLE bowel preparation were recorded in a diary. The Crohn's Disease Activity Index (CDAI, symptomatic defined as ≥150) was used for CD and partial Mayo score (symptomatic defined as ≥2) for UC. Symptoms were further subclassified as diarrhea or abdominal pain. Diarrhea was recorded as the daily loose stool frequency. Abdominal pain severity was graded from 0-3 corresponding with nil, mild, moderate to severe, and averaged over 7 days. Serum inflammatory markers of erythrocyte sedimentation rate (ESR, upper normal limit 16mmHg) and C-reactive protein (CRP, upper normal limit 6mg/L) were recorded. Endoscopic activity was measured by the Crohn's Disease Endoscopic Index of Severity (CDEIS) for CD and the Mayo endoscopic sub-score for UC. Mucosal healing was defined as a CDEIS of 0 for CD or Mayo endoscopic sub-score of 0-1 for UC. Histological biopsies also had to demonstrate quiescent disease for those meeting the definition of mucosal healing, which was defined as absence of epithelial breach (ulceration, erosions) and inflammation (cryptitis, crypt abscess, neutrophilic infiltration). Non-acute inflammatory changes (crypt distortion, branching) were not excluded.[12,13]. Fecal culture was performed to exclude infection where relevant.

Endoscope-based confocal laser endomicroscopy

Subjects underwent eCLE (Pentax EC-3870FK, Tokyo, Japan) under conscious sedation with propofol delivered by an anesthesiologist. eCLE provides 1,000-fold magnification of the intestinal mucosa and has a lateral resolution of 0.7µm, adjustable to a depth from 0-250µm at 7µm increments. The ileum was selected to measure intestinal permeability as all subjects required full colonoscopy-ileoscopy in the assessment for mucosal healing, and eCLE can tangentially image multiple ileal villi simultaneously to grade the severity of fluorescein leakage (**Figure 1**). Our and others have previously demonstrated fluorescein leakage to be well-visualized in the ileum of both CD and UC subjects, and was independent to disease phenotype.[10,11] Inflamed segments were avoided so local inflammation does not influence the assessment of intestinal permeability. Care was taken to minimize suction and endoscope trauma. To reduce sampling errors, 5 macroscopically normal (non-inflamed) segments of the terminal ileum were selected for eCLE with a minimum of 50 confocal images obtained at each site and stored in a digital database. At each of the 5 terminal ileum sites, incremental imaging from the surface down towards the lamina propria was performed to capture a variety of mucosal depths. A total of 5mL of intravenous fluorescein sodium (10%, AC Pharm, Australia) was administered, at increments of 1 ml for each terminal ileal site. Incremental dosing allowed for a more sustained and standardized level of intravascular fluorescein especially in subjects that had high intraluminal leakage of the contrast. Hyoscine butylbromide 10mg (Buscopan, Australia) given intravenously at the discretion of the endoscopist to minimize peristalsis affecting CLE imaging. Forceps biopsies were taken from the paired ileal sites imaged following eCLE imaging to confirm absence of histological

inflammatory activity. The endoscopists were fully trained in the performance and interpretation of eCLE procedures and have demonstrated inter-individual agreement on the interpretation of eCLE images.[11,14,15]

Confocal laser endomicroscopy features of intestinal permeability

Three predefined features had been previously characterized on eCLE to represent loss of epithelial barrier integrity and they were used for this study. These same features were induced and visible with time-lapse two-photon confocal microscopy in a mouse model of impaired intestinal permeability following intra-peritoneal injection of 5μg of tumor-necrosis factor alpha (TNF-alpha) and 10μM intravenous fluorescein.[16] Temporary leaky gut was induced by TNF-alpha and features were equivalent to those selected for eCLE studies in humans. These features had been validated to be easily learnt with excellent intra- and inter-individual agreement.[11,14] The derivation of these eCLE features as well as their learning curve, inter-observer and intra-observer agreements have recently been described.[15] In brief these features are cell junction enhancement (CJE, representing fluorescein enhancement between epithelial cells secondary to loss of tight junction protein integrity); fluorescein leak (FL, an efflux of fluorescein from the submucosal space into the gut lumen through epithelial breaks) and cell drop out (CDO, actively shedding enterocyte(s) with epithelial gap(s) often accompanied with FL). (**Figure 1**)

To quantify the severity of barrier dysfunction, a new quantitative numerical score was developed, named the Confocal Leak Score (CLS). Previous grading of barrier dysfunction via CLE have included the categorical Watson grade and quantitatively by counts of epithelial gaps.[10,17] The rationale for devising a novel scoring system

was the advantage of CLS in providing a continuous grade of severity assessment, rather than a categorical variable between normal or abnormal, given that epithelial barrier dysfunction is likely to be a continuum of severity.[15] In addition, whilst calculations of epithelial gap densities allows quantitative analysis, it is prone to selection bias given a handful rather than a volumetric block of CLE images, which is the case in CLS, are available for comprehensive analyses and final grading. CLS was also easy to determine. In order to calculate the CLS, all stored eCLE images for each patient were analyzed independently by a researcher blinded to both patient disease category and endoscopic findings from stored images after the procedure. Images were excluded from analysis either if less than one third of the image contained villi, or was blurred secondary to movement artifact. The presence or absence of impaired intestinal permeability was assessed in each image, defined as the findings of one or more features of CJE, FL or CDO or none of the 3 features respectively. To minimize bias, identical features on serial images could only be counted up to a maximum of 5 times to be included for the total CLS calculation. The final CLS is derived from the number of images with one or more of the features, divided by the total number of terminal ileal confocal images per patient multiplied by 100 giving a score of 0 (absence of barrier dysfunction) to 100 (complete barrier dysfunction). Use of CLS has been prospectively validated as a measure of epithelial barrier dysfunction identifying physiological leak in non-IBD controls, reversal of CLS with treatment in IBD patients and importantly, prognostic implications of clinical relapse free remission associated with low leak.[18] This method of deriving a quantitative scale of disease severity using eCLE has also been previously demonstrated for celiac disease. The Confocal Celiac Score demonstrated excellent correlation with the histological Marsh classification.[19]

To determine the consistency between the 2 eCLE methods of measuring intestinal permeability, we compared the CLS against the Watson grades (I, II and III in order of increasing severity).[10] A set of 30 high-quality images were randomly selected and graded by the Watson grade and the CLS. Paired testing for discordance, non-parametric correlation (after transforming the categorical Watson grade to a continuous scale) and construct validation testing were conducted to determine their relationship.

Sample size calculation

Sample size calculation was based on 21-39% of IBD patients in remission having IBS-like symptoms.[5,20] For purposes of calculation, standard deviation was estimated at three quarters of the inter-quartile range (IQR).[21] Assuming a 1.5-fold increase in permeability comparing symptomatic- versus asymptomatic subjects in mucosal healing with a two-sided significance of 0.05 and a power of 80%, a total of 32 subjects will be required at an enrolment ratio of 3 asymptomatic to every 1 symptomatic IBD subjects. This ratio was determined by a one-in-three to one-in-four ratio of IBD patients in remission that have ongoing bowel symptoms.[3]

Statistical analysis

Non-parametric continuous variables were described as medians and IQR and compared with the Mann Whitney U test. All comparisons between CLS were calculated as medians. Categorical variables were described as counts (n=) or percentages and compared using the Chi-square test. Correlation of CLS was performed with Spearman correlation and described as the rho (*r*) coefficient. Linear

regression was calculated for continuous dependent variables, and logistic regression for categorical dependent variables was performed using the backward model and expressed as odds ratios (OR) and 95% confidence intervals (CI). Receiver operating characteristic (ROC) curve and its area under curve was used to designate a CLS cutoff that is sensitive and specific to the presence of ongoing bowel symptoms in the setting of a normal mucosa. McNemar's test, Spearman correlation and the Kruskal Wallis tests were conducted to test the relationship between the Watson grades[10] with the CLS. *P* values of <.05 were considered statistically significant. Statistical analyses were performed on SPSS 20.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

A total of 110 patients were recruited to undergo eCLE and comprised of 22 controls, 31 with UC and 57 with CD. Of the 88 IBD subjects, 2 were excluded from the final analysis due to incomplete colonoscopy from a CD stricture preventing terminal ileal intubation (n=1) and from a new co-diagnosis of celiac disease (n=1) in a UC subject. From the remaining 86 IBD patients, 49 (26 CD and 23 UC) had mucosal healing as previously defined and were included for final analysis. The prevalence of patients suffering from ongoing bowel symptoms despite mucosal healing was 16.3% (8/49) in IBD, 15.4% (4/26) in CD and 17.4% (4/23) in UC (**Figure 2**).

Among the IBD subjects in mucosal healing, there were no statistically significant differences between the symptomatic and asymptomatic groups with respect to sex, age, median disease duration, smoking status, CRP, ESR, NSAID use, IBD medication use, median images analyzed per patient and total endoscopic procedure

time. A trend was seen with a greater percentage of asymptomatic IBD subjects taking immunomodulators (61.0% versus 25.0%, *P*=.06) and/ or anti-TNF-alpha agents (13.0% versus 0%, *P*=.06) when compared to the symptomatic IBD subjects. The baseline demographics, clinical features and use of medications are outlined in **Table 1**.

Intestinal permeability of symptomatic and asymptomatic IBD subjects who are in mucosal healing and healthy controls

The median CLS of the IBD cohort in mucosal healing (12.9, IQR: 6.7-19.4) was significantly higher than controls (5.9, IQR: 3.7-9.6, *P*=.001). Symptomatic patients had a significantly higher CLS (18.9, IQR: 14.4-33.8) than asymptomatic patients (7.3, IQR: 5.0-12.8, *P*<.001) and controls (*P*<.001) with no differences between control and asymptomatic IBD subjects (*P*=.26). When the IBD cohort is divided into the subtypes of CD and UC, median CLS of symptomatic patients remained significantly higher than asymptomatic patients. The median CLS of symptomatic versus asymptomatic patients with CD were 17.7 (IQR: 14.4-37.2) and 8.1 (IQR: 4.6-12.8, *P*=.009) and with UC they were 22.2 (IQR: 14.4-33.8) and 6.9 (IQR: 5.9-14.5, *P*=.021) respectively (**Figure 3**). No differences in CLS were seen between symptomatic CD versus UC patients, and asymptomatic CD versus UC patients (*P*>.05). No differences in CLS were seen between asymptomatic CD (8.1, IQR: 4.6-12.8) and UC (6.9, IQR: 5.9-14.5) subjects when compared to control (*P*=.526). The ROC area under curve of the CLS in determining symptoms in the setting of mucosal healing was 0.88 (95% CI: 0.79-0.98, *P*<0.001, **Supplementary Figure 1**). A CLS cutoff of ≥13.1 defined ongoing bowel symptoms in IBD with a sensitivity of 95.2%

and specificity of 97.6%. In this study 36.2% of IBD subjects in mucosal healing had intestinal permeability that exceeds this cutoff.

Inflammatory markers and intestinal permeability

In patients who are in mucosal healing, neither the median ESR (*P*=.64) or CRP (*P*=.97) differed significantly between symptomatic and asymptomatic subjects. Elevated ESR and/ or CRP did not predict for bowel symptoms (OR: 1.12, *P*=.89). Neither ESR (*r*=0.06, *P*=.75) nor CRP (*r*=-0.06, *P*=.70) correlated with CLS.

Correlation of severity of symptoms to intestinal permeability

The relationship between CLS and patient's symptomatic complaints of diarrheal stools per day, abdominal pain, age, sex, disease duration, smoking status, ESR, CRP, and medication use were assessed by linear regression. On simple linear regression, CLS predicted for diarrheal motions per day (R^2 of 0.139) with every CLS increase of 1.82 points predicting for each additional daily diarrheal motion (**Table 2**). On multiple linear regression CLS predicted for diarrheal motions/day and female sex were significant with a R^2 of 0.477. CLS increased by 1.87 for each additional daily diarrheal motion, and by 6.29 for female sex of IBD subjects when compared to males (**Table 3**).

Assessment of intestinal permeability using the Confocal Leak Score versus the Watson grade

We tested the CLS against the Watson grade[10] in the evaluation of intestinal permeability. There was consistency and low discordance in the assessment of intestinal permeability (McNemar's test *P*=.45). The two scores also correlated

significantly (r=0.40, P=.03). The median CLS for Watson grades I, II and III were 9.2 (IQR: 2.7-15.4), 15.3 (IQR: 9.2-28.7) and 15.4 (IQR: 15.4-33.1) respectively, but did not differ significantly between the three levels (Kruskal Wallis P=.09).

DISCUSSION

Increased intestinal permeability is increasingly recognized to be an underlying pathogenic factor not only in IBD but other gastrointestinal and non-gastrointestinal diseases.[22] This was the first study to characterize intestinal permeability by eCLE as a plausible explanation for ongoing bowel symptoms despite mucosal healing in IBD. Supportive of our hypothesis that intestinal permeability is associated with ongoing symptoms, CLS was significantly higher in symptomatic than asymptomatic IBD patients who are in mucosal healing. This was also the case when assessed separately for CD and UC. Age, IBD disease duration, smoking status, NSAID use, CRP and ESR, were not predictive of CLS on linear regression. Importantly, no significant differences were seen between asymptomatic IBD subjects versus healthy controls, indicating the full recovery of intestinal permeability is possible.

Confocal laser endomicroscopy can target the uninflamed terminal ileum and measure the severity of intestinal permeability independent to local inflammation. Neither fecal calprotectin nor the presence of low grade intestinal inflammation differentiates symptomatic versus asymptomatic IBD patients who are in mucosal healing.[5] Urinary lactulose-mannitol ratio (LMR) is an alternative non-invasive biomarker of intestinal permeability based on differential urinary excretion of orally-ingested sugars that have variable intestinal penetration. LMR, unfortunately, is unable to determine whether impaired intestinal permeability is the result of barrier

dysfunction or from active intestinal inflammation. eCLE not only can assess the entire ileocolon for active inflammation and presence of mucosal healing, but can direct functional imaging of the confocal laser microscope to an uninflamed segment of the bowel to determine intestinal permeability. We and others have previously identified the ileum to be the most suitable site in demonstrating increased permeability in both CD and UC.[10,11,16] Another method[16] of counting of individual epithelial gaps in between enterocytes on CLE images is laborious and biased according to which single image of the ileal villus was used. Another eCLE study used the categorical Watson grade to measure intestinal permeability.[10] We found the Watson grade to be concordant with the CLS in diagnosing the presence or absence of increased intestinal permeability but only the CLS assessed intestinal permeability severity as a continuous measurement. The CLS has been validated to be reproducible[15] and predicted the need for treatment escalation in IBD.[23] In this study the CLS verified that intestinal permeability to be a continuum of severity, which ranged from physiological cell turnover in controls (median CLS of 5.9) to more severe leakage in IBD (median CLS of 12.9). A CLS value of ≥13.1 correlated well with ongoing bowel symptoms despite mucosal healing in IBD. Based on this cutoff, a third of IBD subjects who are in mucosal healing had increased intestinal permeability.

Despite mucosal healing, failure to achieve clinical remission occurred in 15.4% of CD, 17.4% of UC and 16.3% of the overall IBD cohort. The prevalence of ongoing bowel symptoms was similar to the prevalence of IBS in the general population of 11% according to a recent meta-analysis.[24] A previous meta-analysis had estimated a prevalence of symptoms meeting the criteria for IBS in IBD in remission of 31% in

UC and 41% in CD.[3] Possible explanations for these differences are that remission in some studies was defined by clinical rather than endoscopic or histological endpoints. Clinical indices of CDAI and pMayo score lack sensitivity and specificity for endoscopic remission.[25-28] Another study identified the prevalence of ongoing bowel symptoms to be 18% of UC subjects based on the definition of deep remission.[5] Ongoing bowel symptoms are seen in some clinical trials where despite mucosal healing, patients were not in clinical remission.[6] For example, in the PURSUIT study of moderate-to-severe UC,[29] the mucosal healing rate at weeks 30 and 54 was 43.5% on 100mg golimumab despite only 28.6% achieving clinical remission during the same time periods. The 15% difference between mucosal healing and remission may be explained by ongoing impaired intestinal permeability.

Higher CLS in symptomatic patients in this study was not attributable to either intestinal or systemic inflammation. There was no correlation of CLS to either ESR or CRP. All subjects had mucosal healing and were in histological remission. A recent study identified increased tissue mRNA expression of TNF-alpha in patients with IBS-like symptoms with IBD, although actual TNF-alpha expression was not statistically different.[20] Increased serum cytokine levels correlated with IBS-like symptoms in UC subjects in deep remission.[5] On post-hoc analysis, the use of anti-TNF-alpha biological agents was not shown to improve intestinal permeability. However, the study was not powered to demonstrate the effects of individual therapeutic classes on intestinal permeability. There was a trend towards more asymptomatic IBD subjects that were on anti-TNF-alpha therapy than symptomatic subjects (13.0% versus 0%, *P*=.06) suggesting a link between anti-TNF-alpha and permeability. Previous studies have demonstrated improved barrier function upon

treatment with anti-TNF-alpha therapy,[30,31] although this study was the first in an entire population in mucosal healing. As a cross sectional study we did not explore the full reversibility of all subjects with impaired intestinal permeability. It is possible that subjects eventually enter clinical remission with healing of the mucosal barrier.

In our study, CLS correlated significantly with diarrheal motions per day but not to abdominal pain. Interestingly, female sex was also an independent predictor of a higher CLS. Higher levels of psychological stress and anxiety in symptomatic IBD patients in remission, as well as sex dependent increase of intestinal permeability with stress, may explain this finding.[32,33]

Limitations need to be acknowledged. Ongoing bowel symptoms in IBD patients with remission may be due to small bowel disease proximal to the terminal ileum. However, all symptomatic patients had abdominal imaging such as magnetic resonance enterography within the prior 12 months, stool examination to exclude infective causes, exclusion of celiac disease by serology and/ or duodenal biopsies, and prior intestinal resection was an exclusion criterion. Based on these assessments, we are confident that active proximal small bowel disease was excluded. Another limitation is the absence of a separate IBS cohort. Recent evidence suggests and supported by our findings that ongoing bowel symptoms are likely due to incomplete repair of mucosa rather than mechanisms responsible for IBS[20,25] The primary focus of this study was in assessing the role of intestinal permeability in symptomatic IBD patients in endoscopic remission, rather than the assessment of IBS. In addition symptoms were based on clinical indices of CDAI and the pMayo score which makes direct comparison to an IBS cohort inappropriate.

This study supports the role of impaired intestinal permeability as a contributing factor towards ongoing bowel symptoms despite mucosal healing in IBD. In addition it demonstrated the role of CLE as an effective tool in the assessment of intestinal permeability beyond its traditional role of obtaining virtual histology.[34] Longitudinal and interventional studies are likely to be helpful to better understand intestinal barrier dysfunction and its potential for recovery. In conclusion, impaired intestinal permeability underlies ongoing bowel symptoms in IBD subjects who are in mucosal healing. The degree of impaired intestinal permeability was predictive of the severity of diarrhea. This supports that the ultimate treatment target of IBD should include not only mucosal healing but also recovery of the intestinal mucosal barrier function.

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Figure 1. Endoscopic confocal laser endomicroscopy features of terminal ileal intestinal permeability (a) control (b) cell junction enhancement (c) fluorescein leak (d) cell drop-out.

Figure 2. Patient recruitment flow diagram

Figure 3. Median Confocal Leak Score (CLS) in controls, asymptomatic and symptomatic patients with inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD).

Table 1. Baseline characteristics of inflammatory bowel diseases subjects who are in mucosal healing.

NSAID = non-steroidal anti-inflammatory drug; 5-ASA = 5-aminosalicylatic acid; TNF=tumor necrosis factor

*Clinical remission is defined as Crohns Disease Activity Index <150 for Crohn's disease or partial Mayo score <2

	Asymptomatic IBD subjects in clinical remission*	**Symptomatic IBD subjects not in clinical remission***	***P* value**
Images per case (n)	396	375	.30
Females (%)	58.5	75.0	.38
Age, y, median (IQR)	43 (31.5-62.0)	37 (30.3-48.3)	.55
Disease duration, y, median (IQR)	10.0 (6.5-13.0)	9.0 (5.5-15)	.57
Smokers (%)	14.6	12.5	.88
CRP, mg/L, median (IQR)	2.3 (1.2-6.1)	2.3 (0.3-15.4)	.97
ESR, mm/hr, median (IQR)	12.5 (5.0-26.2)	11.0 (5.0-21.5)	.65
NSAID use (%)	4.9	0	.52
Medications (%)			
Steroids	4.9	0	.53
5-ASAs	43.9	37.5	.74
Immunomodulator	61	25	.06
Anti-TNF agent	31.7	0	.06

Presence of ongoing bowel symptoms			
Diarrhea (%)	9.8	75.0	.001
Abdominal pain (%)	29.3	37.5	.64
Abdominal pain severity (mean ± SD)	0.32 ± 0.52	0.75 ± 1.16	.096

Table 2. Simple (univariate) linear regression of factors associated with Confocal Leak Score

NSAID = non-steroidal anti-inflammatory drug; CRP = C reactive protein; ESR = erythrocyte sedimentation rate; 5-ASA = 5-aminosalicylatic acid; TNF=tumor necrosis factor

Factor	**B Coefficient**	***P* value**
Female	5.98	.06
Age (yrs)	-0.02	.89
Disease duration (yrs)	0.16	.61
Diarrhea motions/ day (n)	1.82	.008
Abdominal pain	0.45	.85
CRP	0.06	.81
ESR	0.09	.51
Smoker	-4.09	.37
On NSAID	-3.43	.67
On Steroids	1.17	.88
On 5-ASA	-1.66	.61
On Immunomodulator	-1.52	.63
On anti-TNF agent	-6.46	.07

Table 3. Multiple (multivariate) linear regression of factors associated with the Confocal Leak Score

Factor	**B Coefficient**	***P* value**
Female	6.29	.036
Diarrhea motions per day	1.87	.005