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Intestinal Barrier Healing Is Superior to Endoscopic and Histologic Remission for Predicting Major Adverse Outcomes in Inflammatory Bowel Disease: The Prospective ERIca Trial

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See editorial on page 184.

BACKGROUND & AIMS: Endoscopic and histologic remission have emerged as key therapeutic goals in the management of inflammatory bowel diseases (IBD) that are associated with favorable long-term disease outcomes. Here, we prospectively compared the predictive value of barrier healing with endoscopic and histologic remission for predicting long-term disease behavior in a large cohort of patients with IBD in clinical remission. **METHODS:** At baseline, patients with IBD in clinical remission underwent ileocolonoscopy with assessment of intestinal barrier function by confocal endomicroscopy. Endoscopic and histologic disease activity, as well as barrier healing, was prospectively assessed along established scores. During subsequent follow-up, patients were closely monitored for clinical disease activity and the occurrence of major adverse outcomes (MAOs): disease flares, IBD-related hospitalization or surgery, and initiation or dose escalation of systemic steroids, immunosuppressants, small molecules, or biological therapy. **RESULTS:** The final analysis included 181 patients, 100 with Crohn's disease [CD] and 81 with ulcerative colitis (UC). During a mean follow-up of 35 (CD) and 25 (UC) months, 73% of patients with CD and 69% of patients with UC experienced at least 1 MAO. The probability of MAO-free survival was significantly higher in patients with IBD with endoscopic remission compared with endoscopically active disease. In addition, histologic remission predicted MAO-free survival in patients with UC but not CD. Barrier healing on endomicroscopy was superior to endoscopic and histologic remission for predicting MAO-free survival in both UC and CD. **CONCLUSIONS:** Barrier healing is associated with decreased risk of disease progression in patients with clinically remittent IBD, with superior predictive performance compared with endoscopic and histologic remission. Analysis of barrier function might be considered as a future treatment target in clinical trials. ClinicalTrials.gov number, NCT05157750.

Keywords: Inflammatory Bowel Diseases; Endoscopy; Histology; Intestinal Barrier; Confocal Laser Endomicroscopy.

As shown by recent meta-analyses, achieving endoscopic remission in patients with inflammatory bowel diseases (IBD) is associated with improved long-term outcome in Crohn's disease (CD)[1] and with long-term steroid-free clinical remission and colectomy-free survival in ulcerative colitis (UC).[2] Therefore, mucosal healing is a key therapeutic goal in IBD that is advocated by several guidelines for clinical practice and trial end points.[3-7]

The composite of symptom control and mucosal healing is commonly referred to a "deep remission,"[5,8,9] and a consensus report by the Selected Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) working group recommends both clinical and endoscopic remission as targets for routine clinical practice,[5] and most recently, both treatment targets have been confirmed in the STRIDE-II intiative.[6]

However, assessing endoscopic disease activity with white light endoscopy (WLE) cannot accurately assess histologic disease activity or detect persistent histologic inflammation in patients with mucosal healing.[10–12] Apart from mucosal healing, histologic healing is another emerging end point in patients with IBD that is frequently included as a secondary end point in clinical trials. Although histologic healing in UC is associated with better disease outcome compared with clinical

Abbreviations used in this paper: **CD, Crohn's disease; CDAI, Crohn's disease activity index; CLE, confocal laser endomicroscopy; CRP, C-reactive protein; IBD, inflammatory bowel diseases; IO-IBD, International Organization for the Study of Inflammatory Bowel Disease; MAO, major adverse outcome; MCS, Mayo Clinical Score; MES, Mayo Endoscopy Score; mRiley, modified Riley score; NHI, Nancy histological index; pCLE, probe-based CLE; RHI, Robarts histopathology index; SES-CD, simple endoscopic score for Crohn's disease; STRIDE, Selected Therapeutic Targets in Inflammatory Bowel Disease; UC, ulcerative colitis.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Achieving endoscopic and histologic remission in patients with inflammatory bowel diseases are key therapeutic goals that are associated with favorable long-term disease outcome. The relevance of functional healing of the intestinal barrier is less well understood.

NEW FINDINGS

When directly comparing endoscopic remission, histologic remission, and barrier healing in patients with inflammatory bowel diseases in this long-term prospective study, we found that barrier healing was highly accurate for predicting the further course of disease and outcompeted endoscopic and histologic remission for predicting a survival free of major adverse outcomes.

LIMITATIONS

Although large in size with multiannual follow-up of the included patients, this was a single-center study conducted at a tertiary referral center.

IMPACT

Barrier healing is associated with decreased risk of disease progression of inflammatory bowel diseases, with superior predictive performance compared with endoscopic and histologic remission. Analysis of barrier function might be considered as a future treatment target.

remission or endoscopic remission, or both, as evidenced by several meta-analyses in the field,[13–15] data on the relevance of histologic healing in CD are limited to date. Furthermore, histologic scoring in UC is complex, with 26 different histopathologic scores, of which only 2 are validated,[16] and no score is completely representative or validated in patients with CD, thereby limiting determination and histology-based decision making in clinical practice.

Confocal laser endomicroscopy (CLE) is a high-resolution imaging technology that enables subsurface imaging of the mucosa in real time during ongoing endoscopy. Apart from the possibility to accurately grade inflammatory activity in patients with IBDs, CLE enables functional assessment of the integrity of the intestinal barrier, and pilot studies have shown that barrier dysfunction in patients with IBD correlates to clinical disease behavior and long-term disease outcome.[17–20] However, no trial to date has systematically and comparatively evaluated the relevance of endomicroscopic barrier healing on IBD disease outcomes in a large prospective trial. To directly compare the value of endoscopic remission, histologic remission, and barrier healing for predicting long-term disease behavior, we conducted the Endoscopic Remission, Histologic Remission and Barrier Healing for Predicting Disease Behaviour in IBD (ERIca) trial, a cross-sectional diagnostic study in which a large cohort of patients with IBD in clinical remission were prospectively included and closely monitored during long-term follow-up for >2 years.

Material and Methods

Ethics Approval and Informed Consent

The study was approved by the Friedrich-Alexander University Erlangen-Nuremberg Ethics Committee and by the Medical Faculty Institutional Review Board. All patients gave their written informed consent before study inclusion. The study was conducted in accordance to the ethical guidelines of the Declaration of Helsinki.

Study Design and Participants

The study was designed as a prospective observational study conducted at the Ludwig Demling Endoscopy Center of Excellence and the IBD outpatient department at the University Hospital of Erlangen. The study was registered at ClinicalTrials.gov with the following identifier: NCT05157750. All authors had access to the study data and reviewed and approved the final manuscript.

The study enrolled adult patients with an established IBD diagnosis for at least 12 months' duration presenting in clinical remission between January 2017 and December 2019. Written informed consent was obtained from all patients before the procedure. Patients with poor bowel preparation, total colectomy, concomitant β-blocker therapy (based on the statement by the Bundesinstitut für Arzneimittel und Medizinprodukte in Germany that the intravenous administration of fluorescein to patients on β-blocker therapy is relatively contraindicated), known allergy to fluorescein, or a planned change in IBD-related medication were excluded. Clinical disease activity for patients with UC and CD was determined using the partial Mayo clinical disease activity score (MCS) and the Crohn's disease activity index (CDAI),[21] with clinical remission defined as a CDAI <150[9,21] or a partial MCS <2 and no individual subscore >1,[21,22] respectively. Additionally, sociodemographic factors, current and past medication, and routine laboratory parameters were assessed at the time of colonoscopy.

After baseline ileocolonoscopy with CLE, patients under biological therapy were closely monitored in our IBD outpatient department every 4 to 8 weeks and those under conventional therapy were monitored every 8 weeks. At each visit, clinical disease activity using partial MCS and CDAI, respectively, routine laboratory parameters, and current and past medications were assessed. Further, at each visit, major adverse outcomes (MAOs) defined as (1) disease relapse, (2) IBD-related hospitalization, (3) IBD-related surgery, and (4) necessity for initiation or dose escalation of systemic steroids, immunosuppressants, small molecules, or biological therapy was recorded.

Colonoscopy and Confocal Laser Endomicroscopy

All patients received bowel preparation with low-volume polyethylene glycol-based bowel lavage in a split dose regimen. Colonoscopy was performed using commercially available high-definition endoscopes and video processors (EC38-i10 and Optivista EPK-i7010, both Pentax Medical, Tokyo, Japan). Bowel preparation was assessed using the Boston Bowel Preparation Score (BBPS), with poor bowel preparation defined as a BBPS of <2 in any segment or a total BBPS of <6.

According to consensus statements, endoscopic remission or healing during WLE were defined as follows[5,23]: In UC, endoscopic remission was defined as a Mayo Endoscopy Score (MES) of $\leq$1 and endoscopic healing was defined as an MES of 0. In CD, endoscopic remission was assessed along 2 parameters: (1) absence/resolution of erosions and ulcerations, as a consensual definition of endoscopic remission by the IO-IBD[5,23] and (2) the simplified endoscopic index of severity (SES-CD) with an SES-CD of $<$3 for definition of endoscopic remission.[24,25]

For confocal imaging, a dedicated probe-based CLE (pCLE) imaging system, consisting of a portable laser station (Cellvizio) and confocal miniprobes (ColoFlex UHD, Mauna Kea Technologies, Paris, France), was used. pCLE imaging was performed in a standardized fashion in all patients: after reaching the terminal ileum, 5 mL fluorescein, 10%, was intravenously injected as a contrast agent, and imaging was initiated immediately after the injection. For this, the CLE probe was positioned under endoscopic guidance onto the mucosa of the terminal ileum, cecum, and rectosigmoid junction in patients with CD and in the cecum and at the rectosigmoid junction in patients with UC. In patients without endoscopic remission, areas with the highest degree of inflammation on WLE were additionally examined by pCLE.

On target tissue, low-powered blue laser light with a 488-nm wavelength was activated for tissue illumination. At each site, a CLE video of at least 2 minutes' duration was recorded, with an image acquisition rate of 8 frames/s. All pCLE images for each patient were stored on an external hard drive and were independently reviewed for presence of barrier dysfunction by 3 expert readers (T.R., J.B., and F.V.) masked to the clinical results of the patients.

Barrier dysfunction in the terminal ileum was assessed using the semiquantitative Watson score into 3 grades[17–19,26–28]: (I) intact epithelial barrier with no fluorescein leakage; (II) functional barrier defect with shedding of single epithelial cells and fluorescein leakage into the intestinal lumen; or (III) structural barrier defect with shedding of multiple epithelial cells, exposure of the lamina propria to the lumen, and fluorescein leakage into the lumen. Barrier dysfunction in the colon was assessed using a dichotomous distinction, as previously described[18,28–30]: intact epithelial barrier in the colon was characterized by a crypt opening that appeared as a dark center in the crypt. During colonic barrier dysfunction, fluorescein leaked into the crypt lumen; therefore, the lumen was brighter than the surrounding epithelium.[18,27,30,31] The integrity of the barrier was assessed in each image, and barrier dysfunction at the specific imaging site was defined as being present when $\geq$1 barrier defects were clearly visible on at least 3 consecutive images.

Histologic Analysis

From each patient, 2 samples for histopathology were obtained at the sites where CLE imaging was performed. In addition, in case macroscopic inflammation was present during WLE, areas with highest degree of inflammation on WLE were also biopsied, matching those areas that were also examined by CLE. In case of ulcerations, biopsies and CLE imaging were performed at the border of the ulcerations. Each biopsy sample had a registration number with the corresponding pCLE video sequence. All samples were scored by an experienced gastrointestinal pathologist (A.H.) masked to clinical and endoscopic patient data. For histopathologic scoring in UC, the Robarts histopathology index (RHI)[32] and the Nancy histological index (NHI)[33] were used as validated histology scores. Histologic disease remission was defined as an RHI of $\leq$3 without lamina propria or epithelial neutrophils or an NHI of $\leq$1.

In the absence of a validated score for grading of histologic inflammation in patients with CD, we used a modified Riley (mRiley) score, as previously described.[34] Apart from including the 6 histologic features for UC (acute inflammatory cell infiltrate, crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrate, crypt architectural irregularities), this score integrates typical histologic features observed in CD, namely, lymphocyte aggregates, granulomas, and number of eosinophils, thereby assessing a total of 9 histologic features. This mRiley score ranges from 0 to 27, with 0 to 4 corresponding to histologic remission, a score of $\geq$5 to histologically active disease, including 5 to 9 low activity, 10 to 18 moderate activity, and $\geq$19 high activity.[34] Supplementary Table 1 presents the composition of the mRiley score. The highest scores obtained during histopathologic scoring of all biopsy samples were used to define presence or absence of histologic remission.

End Points, Sample Size, and Statistical Analysis

The primary end point of this study was to comparatively assess the predictive values of barrier healing, endoscopic remission, and histologic remission for predicting occurrence of MAO in patients with IBD in clinical remission. As a secondary end point, the predictive value of endoscopic healing in UC (MES = 0) for predicting the further course of disease was calculated. As further secondary end point, the predictive values of the composite between endoscopic remission and histologic remission for predicting MAO in patients with IBD were assessed.

Demographic and other baseline characteristics were summarized by tabulating (relative) frequencies or providing descriptive statistics. Properties of predictive values of different dichotomized characteristics in predicting later occurrence of MAOs were derived. In this context, the positive predictive value quantifies the share of patients actually not experiencing MAOs during follow-up among those with a favorable status of the predictor. Conversely, the negative predictive value indicates the share of patients with occurrence of MAOs among all patients diagnosed with an unfavorable predictor status. To statistically test the different degrees of MAOs between patients with and without barrier healing, an extension of Fisher's exact test for a 2 $\times$ 4 contingency table was used. Statistical analyses were performed using R 4.0.x software (www.r-project.org). All statistical tests were considered explorative, and hence, no α adjustment was used. Moreover, time-to-event analysis by Kaplan-Meier estimates was used to examine the time to MAOs (or censoring at end of follow-up) in the 2 respective strata of the predictor variables.

Results

Study Inclusion and Flow of Participants

Between 2017 and 2019, 296 patients with IBD were screened for eligibility. The study excluded 94 patients who exhibited clinically active disease and 31 patients due to

Table 1. Clinical, Endoscopic, and Histologic Characteristics of the Patients With Ulcerative Colitis and Those With Crohn's Disease

Variables	Ulcerative colitis (n = 81)	Crohn's disease (n = 100)
Clinical characteristics		
Age, *y*	39 (18–69)	37 (19–68)
Sex		
Male	39 (48)	58 (58)
Female	42 (52)	42 (42)
Body mass index, *kg/m²*	25.6 (17.2–39.2)	26.8 (16–51.9)
Disease duration, *y*	10 ± 7.9	12.5± 11.9
Extent of disease		
Proctitis	6 (7.4)	
Left–sided colitis	39 (48.1)	
Pancolitis	36 (44.4)	
Ileum		29 (29)
Colon		8 (8)
Ileocolitis		42 (42)
Upper gastrointestinal + ileum		7 (7)
Upper gastrointestinal + colon		2 (2)
Upper gastrointestinal + ileocolitis		12 (12)
Extraintestinal manifestations	19 (23.5)	31 (31)
Primary sclerosing cholangitis	2 (2.5)	
Medication		
Mesalamine derivates		
Mesalazine	12 (14.8)	5 (5)
Sulfasalazine		2 (2)
Corticosteroids		
Budesonide		1 (1)
Budesonide (with colonic delivery)	2 (2.5)	
Prednisolone	4 (4.9)	3 (3)
Prednisolone dose, *mg*	10 ± 4	5 ± 4.3
Immunomodulator		
6-Mercaptopurin	1 (1.2)	1 (1)
Azathioprine	4 (4.9)	8 (8)
Biological therapy		
Anti-tumor necrosis factor	28 (34.6)	43 (43)
Vedolizumab	11 (13.6)	5 (5)
Tofacitinib	3 (3.7)	0 (0)
Ustekinumab	2 (2.5)	17 (17)
Combination therapy	8 (9.9)	5 (5)
No medication	6 (7.4)	10 (10)
Laboratory parameters		
Leukocyte count, 10^9/L	7.9 ± 3.2	8 ± 2.8
Hematocrit, %	41.5 ± 4.1	42.3 ± 3.8
Endoscopic and histopathologic data		
MES		
≤1	43 (53.1)	
>1	38 (46.9)	
Barrier function		
Colon		
Barrier healing present	21 (25.9)	27 (27)
Ileum		
Barrier healing present		25 (25)
Histopathology scoring		
RHI ≤ 3	44 (54.3)	
RHI > 3	37 (45.7)	
NHI <1	42 (51.9)	
NHI ≥1	39 (48.1)	
Modified Riley score <5		58 (58)
Modified Riley score ≥5		42 (42)
Erosions or ulcerations		
Absent		66 (66.0)
Present		34 (34.0)
SES-CD		
<3		50 (50.0)
≥3		50 (50.0)
Follow-up, *mon*	25 ± 11.9	35 ± 6.9
MAO during follow-up[a]		
No MAO	25 (30.9)	37 (37)
Occurrence of MAOs		
≤2 months	29 (35.8)	23 (23)
>2 to ≤4 months	12 (14.8)	12 (12)
>4 to ≤6 months	8 (9.9)	6 (6)
>6 to ≤8 months	5 (6.2)	7 (7)
>8 to ≤10 months	2 (2.5)	5 (5)
> 10 to ≤12 months	0 (0)	4 (4)
>12 months	0 (0)	6 (6)

NOTE. Continuous data are presented as n (%) and categorical data as mean ± standard deviation or mean (range).
[a]MAOs: disease flare; necessity for initiation or escalation of systemic steroids, immunosuppressants, small molecules, or biological therapy.

poor bowel preparation (n = 18), concomitant β-blocker therapy (n = 7), unwillingness to participate in the study (n = 6), or a planned change in pharmacotherapy (n = 5). Therefore, 181 patients with IBD (CD, n = 100; UC, n = 81) were finally eligible and included in the study. A flowchart of the included patients according to the Standards for Reporting of Diagnostic Accuracy Studies guidelines[35] is presented in Supplementary Figure 1.

The study included patients with clinical remission, as determined by established clinical activity scores (CDAI <150 or partial MCS <2 with no individual subscore >1).[5,21,22] Most patients were treated with biological therapies, and only few patients had mesalamine therapy or low doses of corticosteroids (Table 1).

Clinical Characteristics and Rates of Endoscopic and Histologic Remission and Barrier Healing in Patients With Ulcerative Colitis

The 81 patients with UC had nonmissing, valid information regarding the occurrence of MAOs during follow-up. Data on endoscopic remission and healing and histologic remission were available for all patients. In 1 patient with

UC, electronic backup of CLE images failed; hence, no data on barrier function in the colon were available for this patient. Clinical, endoscopic, and histologic characteristics of the patient cohort with UC are summarized in Table 1.

As presented in Table 1, from the 81 patients with UC, 43 (53.1%) had endoscopic remission on WLE at study inclusion. Histologic healing, as defined by RHI and NHI, was present in 54.3% and 51.9%, respectively, during endoscopic evaluation. As assessed by the RHI, 36 patients (44.4%) with UC exhibited endoscopic and histologic healing. In contrast, an intact barrier function, as defined by lack of fluorescein leakage into the crypt lumen,[18,27,30,31] was observed in the colon in only 21 patients (25.9%) during baseline evaluation with WLE and CLE.

Clinical, endoscopic, and histologic characteristics of UC patients with and without an intact barrier are summarized in Supplementary Table 2. Of the 21 patients with UC with colonic barrier healing, 8 (38.1%) exhibited an MES of 0, whereas 13 (61.9%) had an MES of 1. All patients (n = 8) with barrier healing and an MES of 0 also exhibited histologic remission. Clinical, endoscopic, and histologic characteristics of patients with UC with combined colonic barrier healing, endoscopic, and histologic remission compared with those without are summarized in Supplementary Table 3.

In additional studies, we assessed whether the presence of barrier healing was associated with changes of serum parameters. We determined C-reactive protein (CRP), albumin, and zonulin levels and noted that the serum levels did not significantly differ between patients with UC with intact colonic barrier compared with those with colonic barrier dysfunction (Supplementary Figure 2). Furthermore, serum zonulin levels were decreased in patients with endoscopic or histologic remission, whereas albumin and CRP levels were unchanged between UC patients with endoscopic or histologic remission compared with those without (Supplementary Figure 3*A*).

Follow-up and Occurrence of Major Adverse Outcomes in Patients With Ulcerative Colitis

Mean follow-up in patients with UC was 25 months (Table 1). In 25 patients with UC, no MAOs occurred in the course of follow-up, whereas in the remaining 56 patients, MAOs were noted, with a mean deviation lag of 3.2 (standard deviation, 52.5 months; range, 1–10 months) as follows: MAO I in 16, MAO II in 3, MAO III in 7, and MAO IV in 30.

Rates of the occurrence of MAOs in patients with endoscopic and histologic remission and in patients with barrier healing are summarized in Supplementary Table 4. As shown, of the 43 patients with endoscopic remission at study inclusion, 21 experienced MAOs; hence the rate of MAOs in patients with UC was 48.8%. Time-to-event analysis using Kaplan-Meier estimates showed that the probability of remaining free of MAOs during follow-up was significantly higher in patients with UC with endoscopic remission than in patients with endoscopically active disease ($P < .0001$) (Figure 1*A*). When a more stringent definition of endoscopic healing was applied, considering only patients with an MES of 0, 17 patients with UC exhibited mucosal healing. Of these 17 patients with mucosal healing on WLE, 6 experienced MAOs during the course of follow-up, thereby the rate of MAOs in patients with MES of 0 was 35.3% (Supplementary Table 4). Correspondingly, the probability of remaining without an MAO during follow-up was significantly higher in patients with UC with endoscopic healing compared with those with an MES of >0 ($P = .0018$) (Figure 1*B*).

From the 44 patients with UC with histologic remission, as defined by the RHI, 23 developed MAOs during follow-up (RHI MAO rate: 52.3%) (Supplementary Table 4), whereas MAOs occurred during the course of follow-up in 21 of 42 patients with histologic remission, as defined by the NHI (NHI MAO rate: 50%) (Supplementary Table 4).

On Kaplan-Meier analysis, patients with histologic remission, as defined by RHI or NHI, had a significantly higher likelihood of remaining without MAOs during follow-up compared with patients with UC with histologically active disease (both $P < .0001$) (Figure 1*C* and *D*). From those 36 patients with combined histologic (as defined by the RHI) and endoscopic remission, 16 experienced MAOs during study follow-up (MAO rate, 44.4%) (Supplementary Table 4), and likewise, those patients with combined endoscopic and histologic remission had a significantly better course of disease remaining free of MAO on Kaplan-Meier estimates ($P < .0001$) (Supplementary Figure 4*A*). Of the 17 patients with combined histologic remission (as defined by the RHI) and endoscopic healing (as defined by an MES of 0), 5 developed MAOs during follow-up, leading to an MAO rate of 29.4% (Supplementary Table 4), and correspondingly, patients with combined endoscopic healing and histologic remission had a significantly higher likelihood for remaining free of MAOs on Kaplan-Meier estimates ($P = .00039$) (Supplementary Figure 4*B*).

In contrast to the aforementioned results, only 4 of 21 patients with UC with barrier healing in the colon developed MAOs during follow-up, all of which were MAO IV (Supplementary Table 5); hence, the MAO rate in patients with colonic barrier healing was 19.1% (Supplementary Table 4). Consistent with this, those patients with UC with barrier healing in the colon had a significantly more favorable course of disease as shown by Kaplan-Meier analysis ($P < .0001$) (Figure 2). Conversely, in most patients experiencing an MAO, no barrier healing was present, and the distribution of individual MAOs did not differ significantly between UC patients with and without barrier healing ($P = .2$)

Clinical Characteristics and Rates of Endoscopic and Histologic Remission and Barrier Healing in Patients With Crohn's Disease

The 100 patients with CD had nonmissing, valid information regarding the occurrence of MAOs during follow-up. Further, data on endoscopic and histologic remission were available for all patients. Because of technical defects in image recording, data on barrier function in the terminal ileum were not available in 2 patients with CD. Clinical,

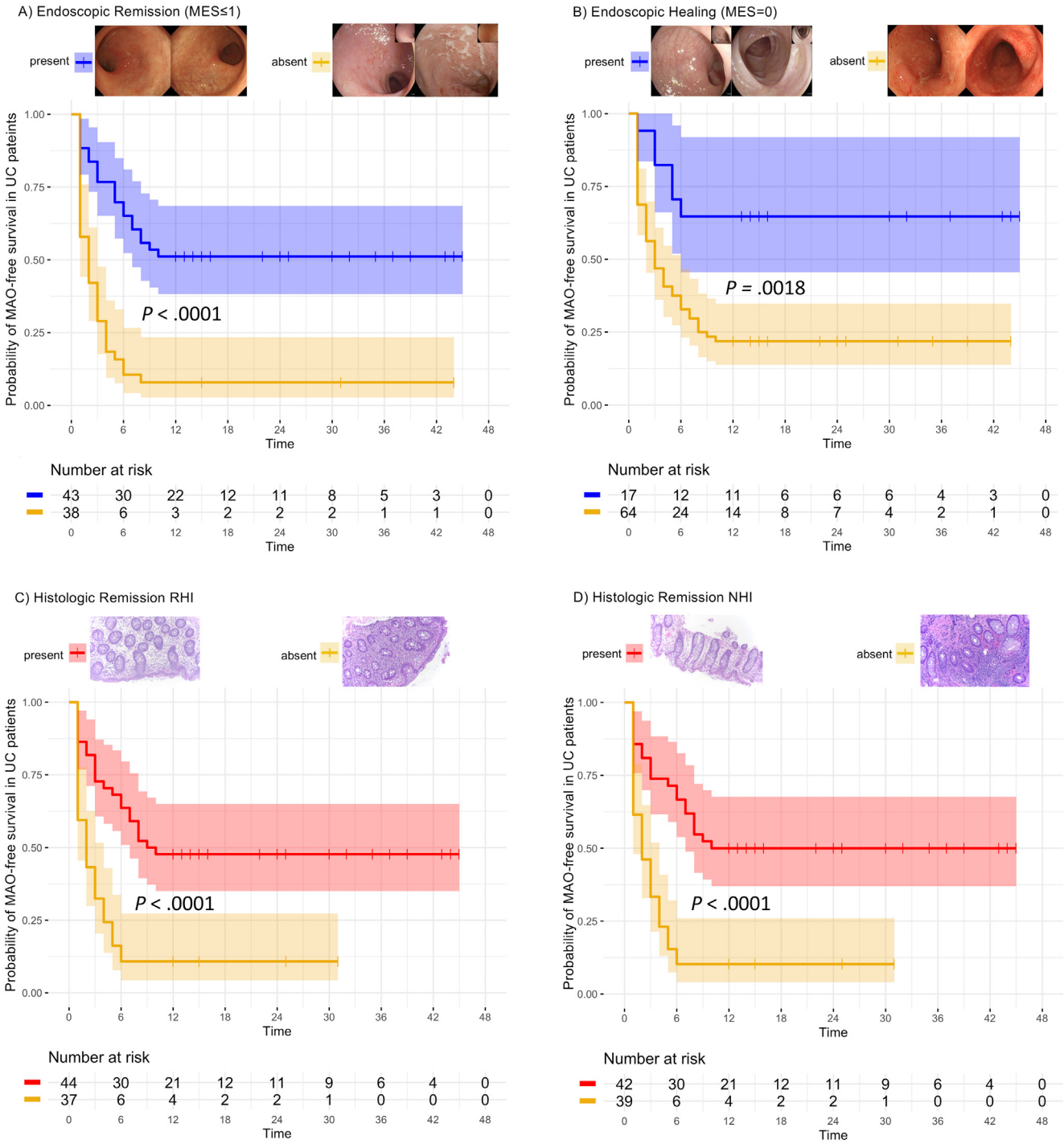

Figure 1. Time-to-event analysis for the occurrence of major adverse outcomes in patients with UC with endoscopic remission, endoscopic healing, and histologic remission. (*A*) Patients with UC with endoscopic remission (MES of ≤1) had a significantly higher probability of remaining free of MAOs compared with patients with endoscopically active disease. (*B*) In patients with UC with endoscopic healing (MES of 0), the probability of remaining without MAOs during follow-up was significantly higher compared with those with a MES of >0. (*C*) Patients with UC with histologic remission, defined by an RHI of ≤3, had a significantly higher likelihood of remaining without MAOs during follow-up compared with patients with histologically active disease according to the RHI. (*D*) In patients with UC histologic remission, as determined by an NHI of ≤1, the probability of remaining without a MAO during follow up was significantly higher compared with those with an NHI of >1. The shaded areas indicate the 95% confidence interval. (*C* and *D*: hematoxylin and eosin staining, original magnification ×20.)

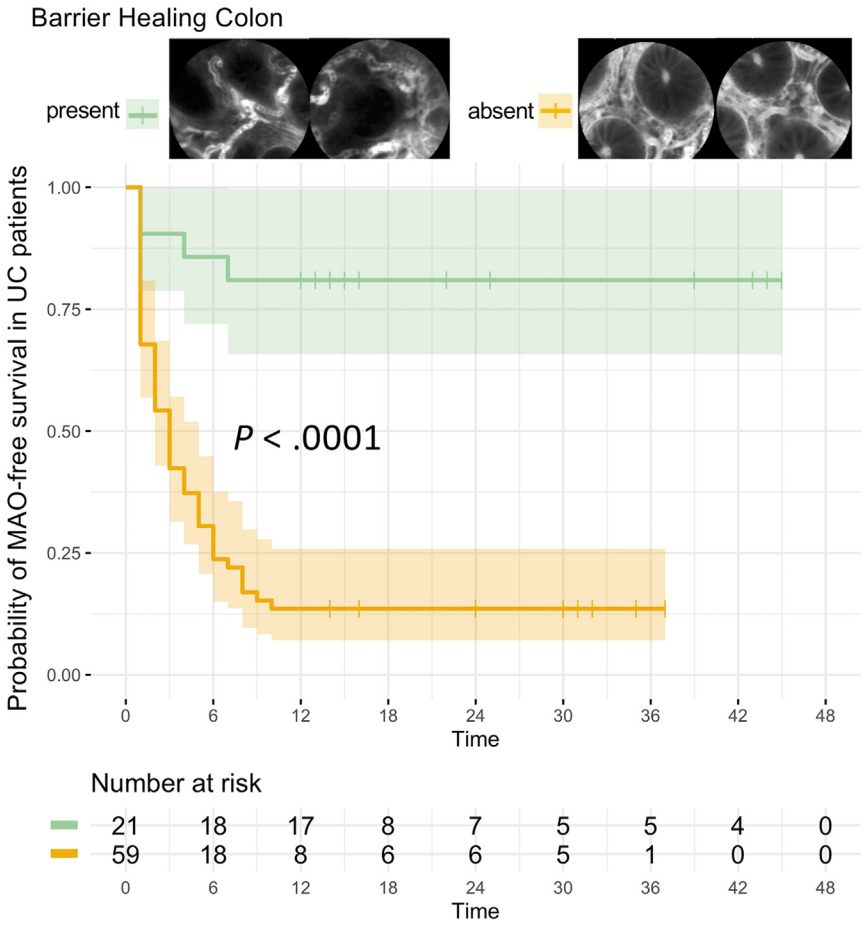

Figure 2. Time-to-event analysis for the occurrence of MAOs in patients with UC with barrier healing. Patients with an intact colonic barrier had a significantly higher probability of remaining without MAOs during follow-up compared with patients with barrier dysfunction in the colon. The shaded areas indicate the 95% confidence interval.

endoscopic, and histologic characteristics of the patient cohort with CD are summarized in Table 1.

As presented in Table 1, from 100 included patients with CD, 66 (66%) had endoscopic remission on WLE, as defined by the absence of erosions or ulcerations, or both, at study inclusion. When the SES-CD with an SES-CD of <3 was used for the definition of endoscopic remission,[24,25] 50 patients (50%) with CD exhibited endoscopic remission during baseline endoscopy.

Histologic remission, as defined by a mRiley score as previously reported,[34] was present in 58 patients (58%) with CD during the endoscopic evaluation. The combination of endoscopic and histologic healing was observed in 49 patients with CD. In contrast, an intact barrier on endomicroscopy without fluorescein leakage was observed in only 25 of 100 patients (25%) with CD in the terminal ileum and in 27 patients (27%) in the colon during the baseline evaluation with WLE and CLE. Clinical, endoscopic, and histologic characteristics of patients with CD with ileal barrier healing compared with those without barrier healing are summarized in Supplementary Table 6. Supplementary Table 7 summarizes characteristics of those patients with CD with combined ileal barrier healing and endoscopic and histologic remission compared with those without.

Moreover, we determined CRP, albumin, and zonulin levels in patients with CD and noted that their serum levels did not significantly differ between patients with intact ileal and colonic barrier on endomicroscopy compared with those with ileal or colonic barrier dysfunction (Supplementary Figure 2). Furthermore, apart from a decreased serum expression of zonulin in patients with CD with endoscopic remission, serum albumin and CRP levels were not significantly different between CD with endoscopic or histologic remission compared with those without, respectively (Supplementary Figure 3*B*).

Follow-up and Occurrence of Major Clinical Events in Patients With Crohn's Disease

Mean follow-up in patients with CD was 35 months. No MAOs occurred in 37 patients during the follow up, whereas MAOS were noted in the remaining 63 patients with a mean lag of 6 months (standard deviation, 6.9 months; range, 1–38 months) as follows: MAO I in 12, MAO II in 4, MAO III in 12, and MAO IV in 35.

Rates of the occurrence of MAO in patients with endoscopic and histologic remission and in patients with barrier healing are summarized in Supplementary Table 8. Of the 66 patients with endoscopic remission at study inclusion, defined by the absence of erosions or ulcerations, 37 experienced MAOs; hence the rate of MAOs in patients with CD in endoscopic remission was 56.9%. When the SES-CD was used to define endoscopic remission, 25 of 50 patients with an SES-CD of <3 experienced MAO; therefore, the MAO rate for SES-CD was 50% (Supplementary Table 8).

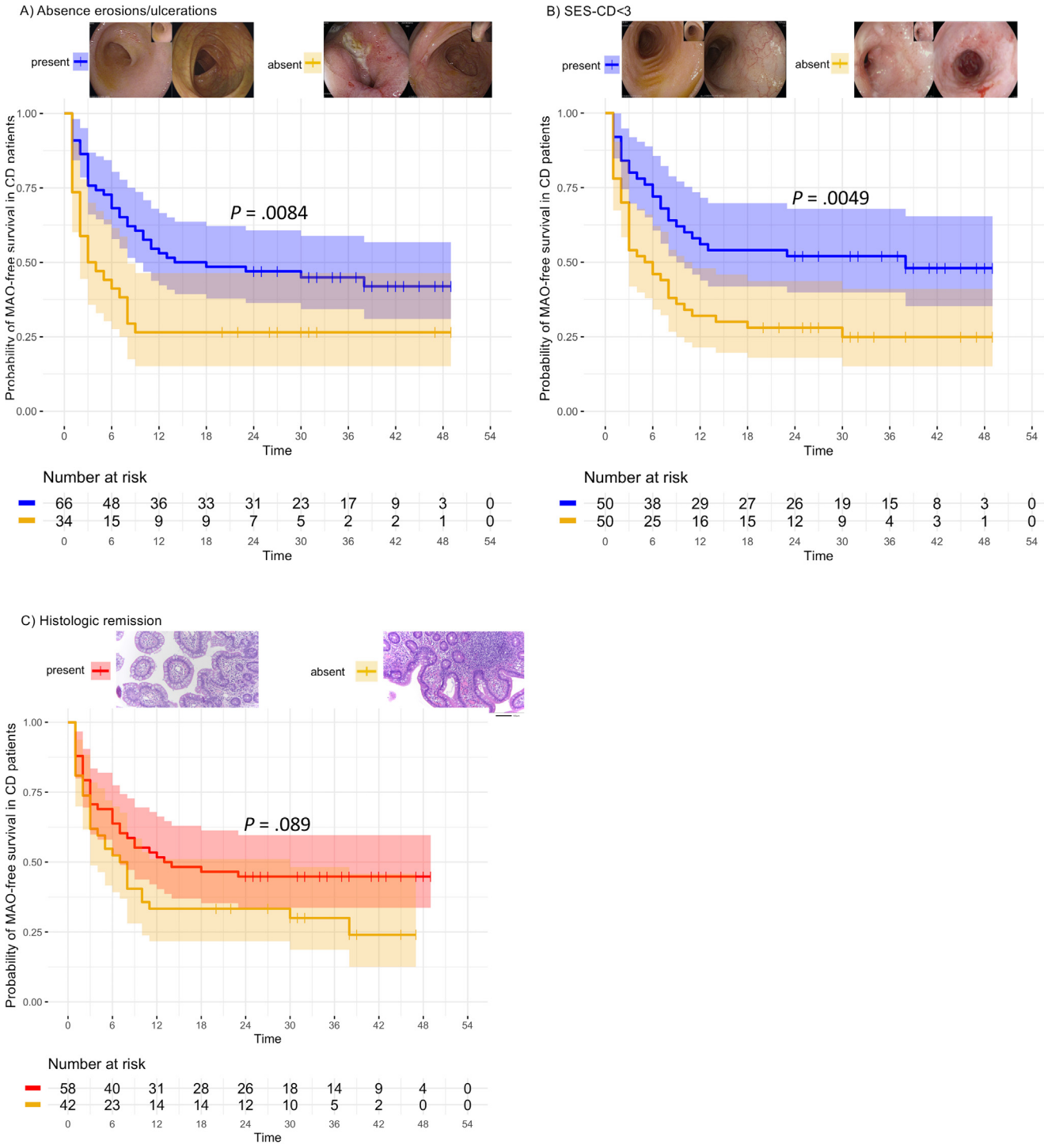

Figure 3. Time-to-event analysis for the occurrence of MAOs in patients with CD with endoscopic remission and histologic remission. (*A*) Patients with endoscopic remission, as defined by the absence of erosions and/or ulcerations, had a significantly higher probability of remaining free of MAOs compared with those patients with endoscopically active disease. (*B*) In patients with CD with endoscopic remission, as defined by an SES-CD of <3, the probability of remaining without MAOs during follow-up was significantly higher compared with those with an SES of ≥ 3. (*C*) Patients with CD with histologic remission, defined by an mRiley score of ≤ 4, had a significantly higher likelihood of remaining without MAOs during follow-up compared with patients with histologically active disease. The shaded areas indicate the 95% confidence interval. (*C*: hematoxylin and eosin staining, original magnification $\times 20$.)

Time-to-event analysis using Kaplan-Meier estimates showed that the probability of remaining free of MAOs during follow-up was significantly higher in patients with CD with endoscopic remission compared with patients with endoscopically active disease ($P = .0084$) (Figure 3*A*), with slight superiority of assessing endoscopic remission with the SES-CD ($P = .0049$) (Figure 3*B*) compared with the definition based on absence of erosions or ulcerations. Of the 58 patients with CD with histologic remission, as defined by an mRiley score of <5,[34] 32 developed MAOs during follow-up; hence, the MAO rate in patients with CD with histologic remission was 55.2% (Supplementary Table 8). Kaplan-Meier analysis showed no significant differences in the probability of remaining without MAOs during follow-up in patients with CD with and without histologic remission ($P = .089$) (Figure 3*C*).

Of the 49 patients with CD with combined histologic remission and endoscopic healing, 25 experienced MAOs during study follow-up, for an MAO rate of 51% (Supplementary Table 8). Kaplan-Meier analysis revealed a significantly higher probability of remaining free of MAOs in patients with endoscopic and histologic remission compared with those without ($P = .0022$, Supplementary Figure 5). Considering only patients with combined histologic remission and endoscopic ileal remission, as defined by an SES-CD subscore of 0 in the ileum, 16 of 35 patients developed MAOs during follow-up, leading to an MAO rate of 45.7% (Supplementary Table 8).

In contrast to the aforementioned results, no MAOs occurred during follow-up in the 25 patients with CD with barrier healing in the terminal ileum; hence, the MAO rate in patients with barrier healing in the ileum was 0% (Supplementary Table 8). Of the 27 patients with colonic barrier healing, 8 exhibited MAOs during follow-up, most of which were MAO III and MAO IV (Supplementary Table 9). Hence, the MAO rate for patients with colonic barrier healing was 29.6% (Supplementary Table 8). Consistent with this, patients with CD with barrier healing in the terminal ileum or in the colon had a significantly higher probability for MAO-free course of disease compared with patients with CD without barrier healing ($P < .0001$ and $P = .00017$, respectively) (Figure 4). Conversely, in most patients with CD experiencing an MAO, no barrier healing was present, and the occurrence of individual MAOs did not differ significantly between patients with CD with vs without barrier healing ($P = .2$).

As shown in comparative Kaplan-Meier estimates for endoscopic remission, histologic remission, and barrier healing, barrier healing in the terminal ileum and the colon were superior compared with endoscopic and histologic remission, or the combination of the latter, for predicting an MAO-free course of disease during long term follow-up in both UC and CD patients (Supplementary Figure 6).

Diagnostic Performances of Endoscopic Healing, Histologic Healing, and Barrier Healing for the Prediction of the Course of Disease

Based on the observed low MAO rates in CD and UC patients with intact barrier function and the high probabilities for remaining without MAOs during follow-up, we sought to directly compare the diagnostic performances of endoscopic healing, histologic healing, and barrier healing for the prediction of the further course of disease.

In UC, endoscopic remission, as defined by an MES of ≤ 1, had an overall accuracy of 70.4% for predicting an MAO-free course of disease, with positive and negative predictive values of 51.2% and 92.1%, respectively (Table 2). When a more stringent definition of mucosal healing was applied, considering only patients with an MES of 0, overall accuracy for predicting a MAO-free course of disease was increased, with an accuracy of 75.3% and positive and negative predictive values of 64.7% and 78.1%, respectively (Table 2).

Histologic remission, as defined by the RHI, had an accuracy of 66.7%, with negative and positive prediction for the occurrence of MAOs during follow-up of 47.7% and 89.2%, respectively. With an overall accuracy of 69.1% and positive and negative predictive values of 47.7% and 89.2%, respectively, assessment of histologic remission as defined by the NHI score was comparably accurate in prediction of a MAO-free course of disease (Table 2).

When the combination of endoscopic remission, as defined by an MES of ≤ 1, and histologic remission, as assessed by the RHI was used, overall accuracy for predicting the occurrence of MAOs was increased to 74.1%, with positive and negative predictive values of 55.6% and 88.9%, respectively (Supplementary Table 10). When a more stringent definition for endoscopic remission was used, including only patients with an MES of 0 (ie, endoscopic healing) and histologic remission as assessed by the RHI, overall accuracy for predicting the occurrence of MAOs was further increased to 77.8% (Supplementary Table 10).

In contrast, diagnostic performances of barrier integrity as a marker for the prediction of long-term disease behavior were markedly increased. As such, barrier healing in the colon had an overall accuracy of 85%, with a positive and negative predictive values of 81% and 86.4%, respectively. Diagnostic performances of endoscopic healing, histologic healing, and barrier healing for assessing the occurrence of MAO in patients with UC are summarized in Table 2.

In CD, endoscopic remission, defined by the absence of erosions or ulcerations, exhibited an overall accuracy of 54% for predicting MAOs during the course of follow-up, with a positive predictive value of 43.9% and negative predictive value of 73.5% (Table 2). Using the SES-CD slightly improved diagnostic accuracy: for an SES-CD of <3, overall accuracy for predicting MAOs was increased to 62%, with a positive and negative predictive values of 50% and 74%, respectively (Table 2).

Histologic remission, as defined by a mRiley score of <5,[34] had an accuracy of 56%, with positive and negative predictive values of 44.8% and 71.4%, respectively (Table 2). When the combination of endoscopic and histologic remission was used, diagnostic performance was only slightly increased compared with histology alone (Supplementary Table 11).

Similar to the results observed in UC, diagnostic performances of barrier healing were superior to endoscopic and histologic remission for predicting the occurrence of

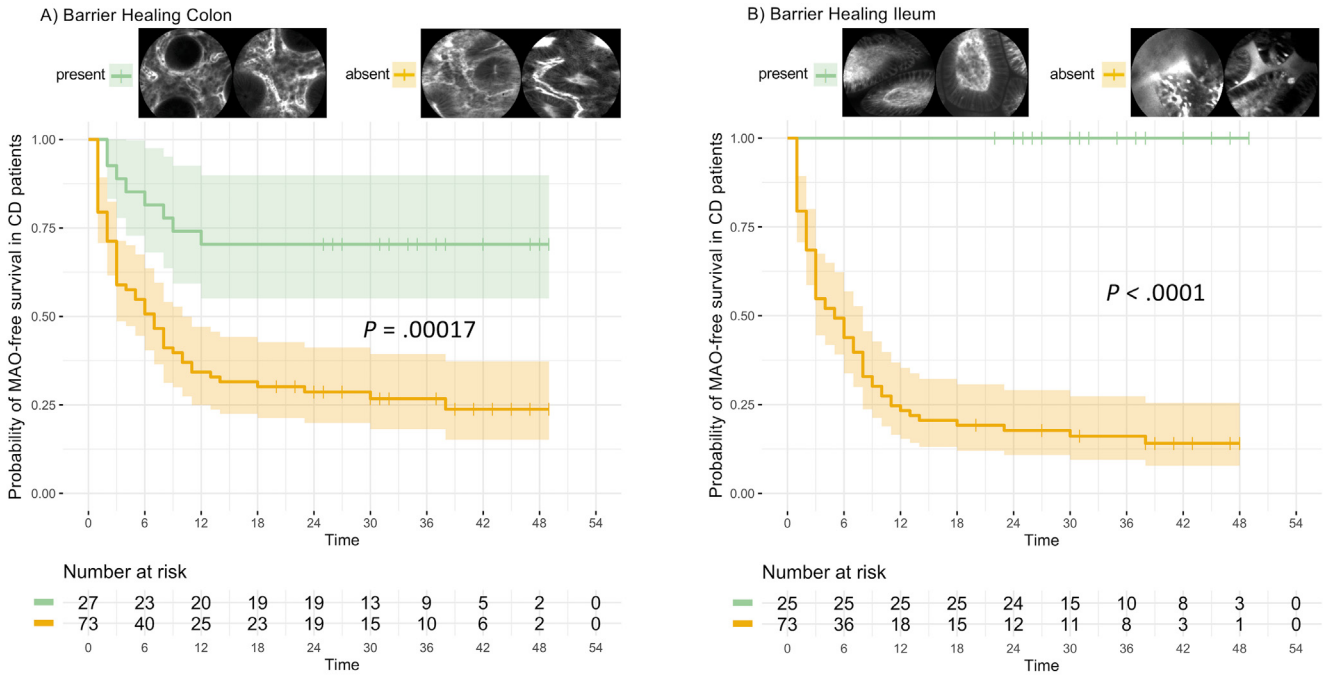

Figure 4. Time-to-event analysis for the occurrence of MAOs in patients with CD with barrier healing. (*A*) Patients with CD with an intact colonic barrier had a significantly higher probability of remaining without MAOs during follow-up compared with patients with barrier dysfunction in the colon. (*B*) In patients with CD with barrier healing in the terminal ileum, the probability of remaining without MAOs during follow-up was significantly higher compared with those with a barrier defect in the terminal ileum, as defined by the semiquantitative Watson grading.[18,19] The shaded areas indicate the 95% confidence interval.

MAOs during the course of follow-up. As such, barrier healing in the terminal ileum exhibited an overall accuracy of 88.7% for predicting MAOs in patients with CD, with positive and negative predictive values of 100% and 84.7%, respectively (Table 2). In the colon, barrier healing had an accuracy of 72.7%, with positive and negative predictive values of 70.4% and 73.6%, respectively. Diagnostic performances for endoscopic healing, histologic healing, and barrier healing for predicting MAO-free course of disease in CD patients are summarized in Table 2.

Discussion

Impaired barrier function and increased intestinal permeability are increasingly recognized as pivotal pathogenic factors in IBD.[36] Consistent with impaired barrier function in patients with IBD, evidence from basic science studies has revealed impairments in tight junction function and epithelial resistance in UC and CD patients,[37–39] and importantly, alterations in barrier function were found evenly distributed and independent of focal lesions, such as erosions or ulcerations, in patients with CD.[39] Recent studies also have successfully implemented CLE for dynamic structural and functional assessment of the intestinal barrier in vivo in patients with UC and in those with CD[18,19] and further substantiated the observation that impaired barrier function is indicative of relapsing disease behavior. Just recently, a prospective study in patients with IBD with endoscopic mucosal healing was able to associate increased intestinal permeability with persistence of clinical symptoms, finding that impaired barrier function, as evaluated by CLE, was significantly correlated with severity of diarrhea in UC and CD.[17] From these observations, the authors speculated that resolution of mucosal permeability beyond mucosal healing might improve outcomes of patients with IBD.[17]

Based on this evidence, we hypothesized that barrier healing, as assessed by dynamic monitoring of the intestinal barrier with CLE, might serve as an accurate parameter that can predict long-term disease behavior in patients with IBD. For this purpose, we conducted a large prospective study in which patients with IBD in clinical remission were included with subsequent close-meshed and multiannual follow-up, during which major clinical events were recorded. This study used established clinical scores, such as CDAI and MCS, rather than biochemical markers or therapeutic regimens to define clinical remission.[5,22] Patients had normal or only slightly elevated CRP levels, with absent or subclinical, mild systemic inflammation.

To gain broad insights into the diagnostic and predictive capabilities of barrier assessment in these patients, we comparatively assessed barrier healing against other established or emerging treatment end points such as endoscopic and histologic healing. Our data clearly show that barrier healing, especially when present in the terminal ileum, is a prognostic parameter that by far outcompetes endoscopic and histologic remission, or their combination, in forecasting the occurrence of major clinical events in both UC and CD patients.

In UC, endoscopic remission is a key therapeutic goal that, as corroborated by several studies in the field including

Table 2. Diagnostic Performances of Endoscopic Remission, Histologic Remission, and Barrier Healing for Predicting Major Adverse Outcomes in Ulcerative Colitis and Crohn's Disease Patients

Parameter	Accuracy % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Ulcerative colitis					
Endoscopic remission (MES <1)	70.4 (59.2–80)	88 (68.8–97.5)	62.5 (48.6–75.1)	51.2 (42 -60.2)	92.1 (79.8–97.2)
Endoscopic healing (MES = 0)	75.3 (64.5–84.2)	44 (24.4–65.1)	89.3 (78.1–96)	64.7 (43.3–81.5)	78.1 (71.4–83.7)
RHI remission[a]	66.7 (55.3–76.8)	84 (63.9–95.5)	58.9 (45–71.9)	47.7 (39–56.6)	89.2 (76.6–95.4)
NHI remission[b]	69.1 (57.9–78.9)	84 (63.9–95.5)	62.5 (48.6-75.1)	50 (40.6–59.4)	89.7 (77.7–95.7)
Barrier healing–colon	85 (75.3–92)	68 (46.5–85.1)	92.7 (82.4–98)	81 (61.4–91.9)	86.4 (78.2–91.9)
Crohn's disease					
Endoscopic remission[c]	54 (43.7–64.2)	76.3 (59.8–88.6)	40.3 (28.1–53.6)	43.9 (37.4–50.7)	73.5 (59.3–84.1)
SES-CD[d]	62 (51.8–71.5)	65.8 (48.7–80.4)	59.7 (46.5–72)	50 (40.6–59.4)	74 (63.6–82.2)
Histologic remission[e]	56 (45.7–65.9)	68.4 (51.4–82.5)	48.4 (35.5–61.4)	44.8 (37–52.9)	71.4 (59.4–81)
Barrier healing–colon	72.7 (62.9–81.2)	504 (33.4–66.6)	86.9 (75.8–94.2)	70.4 (53.7–83)	73.6 (66.7–9.6)
Barrier healing–ileum	88.7 (80.6–94.9)	69.4 (51.9–83.7)	100 (94.1– 100)	100	84.7 (77.2–90.1)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[a]Histologic remission according to the RHI.
[b]Histologic remission according to the NHI.
[c]Endoscopic remission was defined as absence of erosions and ulcerations.
[d]SES-CD <3 = endoscopic remission.
[e]Histologic remission was defined according to a mRiley score.

meta-analysis, is associated with favorable disease behavior such as long-term corticosteroid-free clinical remission and colectomy-free survival.[2,40] However, although an MES of ≤1 is generally accepted to indicate endoscopic remission, decisive differences exist between patients with an MES of 0 and 1, as most recently evidenced in a meta-analysis of 17 studies including 2608 patients with UC in clinical remission, which showed that patients with an MES of 0 had a 52% lower risk of clinical relapse compared with patients with an MES of 1.[15] To reflect these differences, we chose to analyze both endoscopic remission (MES of ≤1) as well as endoscopic healing (MES of 0) for the prediction of long-term disease outcome in our study. As shown in time-to-event analysis, endoscopic remission and endoscopic healing were both associated with a significantly more favorable course of disease over a mean follow-up period of 25 months. As expected, patients with endoscopic healing were less likely to experience major clinical events compared with patients with endoscopic remission, as shown in the Kaplan-Meier analysis.

A similar result was obtained in CD. Assessing endoscopic remission along an IO-IBD consensus definition that is based on the absence or resolution of erosions and ulcerations[5,23] and the SES-CD, we found a significantly more favorable course of disease over a mean follow-up period of 35 months in patients with endoscopic remission, with superiority of the SES-CD over the IO-IBD definition. Together, these data are consistent with various studies, including meta-analyses, showing that endoscopic remission or mucosal healing is associated with long-term clinical remission and reduced need for surgical intervention in active CD.[1,40,41]

Histologic remission represents an emerging end point in IBD, particularly in UC. Analyzing data from >1500 patients from 15 studies, a meta-analysis by Park et al[14] quantified a risk reduction for clinical relapse in patients with histologic remission of 52%, with superiority of histologic remission over clinical and endoscopic remission in predicting clinical outcomes. Similarly, a recent meta-analysis of 10 studies with patients with endoscopic healing showed that patients with UC who achieved histologic remission had a 63% lower risk of clinical relapse compared with patients with persistent histologic activity.[15]

In our study, histopathologic scoring was performed using the RHI and the NHI, 2 of the most commonly used scores, both of which are also validated. As shown in our study and consistent with data in the literature, histologic remission, as quantified by RHI and NHI, was associated with significantly lower risk of remaining with major clinical events in our cohort with UC compared with those patients who had histologically active disease. Our analysis on the diagnostic performances for predicting disease outcome showed both scores were comparably accurate in forecasting the further course of disease. Furthermore, time-to-event analysis showed that the combination of endoscopic remission and histologic remission, as assessed by the RHI, increased the predictive values compared with histology or endoscopy alone, although this increase was only incremental in our cohort.

We did not find any differences in predicting the long-term disease outcome between patients with CD with and without histologic remission. Certainly, assessing histologic remission is more complex in CD than in UC for several reasons. Firstly, no scoring system for assessing histologic disease activity in CD has been validated to date.

Secondly, due to the discontinuous character of the disease with frequently patchy and focal distribution of

inflammatory lesions, CD is heterogenous, thereby rendering CD more prone to sampling artifacts compared with UC. To circumvent these challenges at least partly, we took an approach where we obtained biopsy specimens not only from the site of CLE imaging, but also, if present, from those parts of the ileum or colon exhibiting the most severe inflammation on WLE. Clearly, in case histopathologic scoring was different between biopsy samples from a single patient, the highest score was used for further analysis. To address the lack of a validated scoring system for CD, we made use of a comprehensive score with 6 histologic features previously described by Riley et al[42] in combination with typical histologic findings observed in CD (ie, lymphocyte aggregates, granulomas, and eosinophils), so that a score with 9 features was used, as previously reported.[34] However, in light of the lack of differences in long-term outcome between patients with CD with and without histologic remission, as observed in our study, and also the relatively weak differences between patients with and without endoscopic remission, these results indicate that additional parameters are needed in CD for more accurate forecasting of future disease behavior.

Barrier healing on functional CLE imaging was superior in its ability to predict the further course of disease in both UC and CD, as shown by comparative analyses of the diagnostic performances of the various parameters assessed in this study. Especially in the terminal ileum, barrier healing had a considerably improved diagnostic performance compared with endoscopic and histologic remission: overall accuracy in UC and CD exceeded or closely reached the 90% threshold. Of note, barrier healing in the terminal ileum in CD further exhibited a perfect specificity and a perfect positive predictive value (both 100%). However, it has to be kept in mind that 83% of patients with CD included in this study had ileal involvement.

Against the background that data from clinical behavior, epidemiology, genetics, and the gut microbiota suggest that ileal and colonic CD should be regarded as at least 2 different subtypes of CD,[43] further studies need to clarify whether barrier function in the ileum can forecast disease behavior equally well in ileal and colonic CD. The higher diagnostic accuracy of ileal barrier healing over colonic barrier healing for forecasting the further course of disease in patients with CD is especially interesting against the background that the development of a penetrating disease phenotype is significantly higher in patients with ileal disease compared isolated colonic disease.[44] Furthermore, the cumulative probability of progression from Montreal classification phenotype B1 to B2 and B3 is substantially higher in patients with ileal disease (68%) than in those with colonic disease (23%).[44]

Isolated ileal involvement is also associated increased risk of developing an intestinal complication compared with patients with CD with isolated colonic involvement.[45] These data clearly indicate that ileal CD is more prone to occurrence of disease complications than isolated colonic CD, and it is tempting to speculate that integrity of the ileal barrier could be important to prevent ileal disease-associated complications in CD.

The molecular reasons for these differences between ileal and colonic disease are currently unclear but might be related to the local micromilieu. For instance, there are striking differences in the composition of the mucus layer between the ileum and the colon. The small intestine is covered by a single, removable mucus layer that is penetrable, with protection provided by antibacterial mediators, whereas there is a double mucus layer in the colon, in which the inner layer is impenetrable to bacteria.[46] Furthermore, ileum and colon are characterized by a distinct T-cell profile and cytokine signature, with a predominant T helper 1 cells profile in the colon and a mixed T helper 1 cell/17 cell profile in the ileum of patients with CD.[47,48] Clearly, further studies are needed to specifically investigate the molecular mechanisms that drive barrier integrity in the ileum and colon and their implications in determining the further course of disease.

Nevertheless, the high positive and negative predictive values for barrier healing in the ileum in both diseases might directly translate into clinical decision making. In this context, high positive prediction might help in risk-stratifying those patients in whom a complicated disease behavior will occur with high probability, whereas, based on the high negative prediction, assessment of barrier healing in the ileum at the same time might allow the identification of patients in which the occurrence of major clinical event is unlikely to occur. With this, we postulate that functional assessment of the integrity of the intestinal barrier with CLE is a powerful parameter that when used in addition to established or emerging parameters, such as endoscopic and histologic remission, may significantly extend the predictability of future disease behavior.

Consistent with results from our study, previous clinical studies observed an increased intestinal permeability as assessed by CLE imaging despite the lack of macroscopic inflammation in patients with IBD.[17–19,49] In this regard, it is noteworthy that impaired barrier dysfunction has been noted also in nonulcerated epithelia from patients with IBD with even distribution of barrier function alterations in patients with CD.[39] Furthermore, noninflamed ileum from patients with CD exhibits increased permeability to large proteins,[50] and even in histologically unaffected ileal tissue from patients with CD, increased epithelial uptake of protein antigens has been found to be mediated by tumor necrosis factor.[51]

The identification of surrogate markers for intestinal barrier healing on endomicroscopy requires further investigations. Here, we observed no correlation between barrier healing and serum levels of albumin, CRP, and zonulin. These findings suggested that the presence of intestinal barrier healing cannot be predicted by these protein serum markers.

Furthermore, the structural alterations of the intestinal barrier in patients with leakage and absent barrier healing need future analyses. Although the association between impaired barrier function and IBD was noted >30 years ago,[52–55] it is unclear to date whether the leakage mainly occurs through the tight junction, and if so, what tight junction alterations are present in patients with barrier

dysfunction. Most studies on the paracellular route of transport describe at least 2 populations of pores regulated by tight junctions: (1) the high-capacity charge-selective "pore" pathway allowing paracellular passage of small ions and (2) the low-capacity "leak" pathway permeable to large ions and molecules irrespective of charge.[56] At the molecular level, the first pore is mainly regulated by claudins and the latter by the tight junction proteins occludin and the zonula occludens family, and given these considerations, it seems clear that future studies implementing clinical tests assessing the integrity of the intestinal barrier in patients with IBD in combination with basic science or molecular approaches to evaluate barrier structure are highly warranted.

The current study has some limitations that need to be acknowledged. Firstly, although large in size with multiannual follow-up of the included patients with IBD, this study was conducted at a tertiary referral center with high levels of expertise in the care of patients with IBD and in endoscopic and microscopic imaging in these patients. Given this, especially the reading of the CLE images for presence or absence of barrier healing might be more difficult outside of expert centers. However, we used a highly reproducible semiquantitative scoring system to assess barrier dysfunction and barrier healing in patients with IBD,[17-19,49] and various studies in the field have demonstrated that successful use of CLE and image analysis, and especially the identification of barrier dysfunction, can be rapidly learned with high interobserver and intraobserver agreement and without the need of prior pathology training.[57–59]

In this context, we mention that probe-based CLE imaging is currently not widely spread and is mainly used at expert centers. Although probe-based CLE is compatible with any existing endoscopy setup with the possibility for rapid image acquisition after fluorescein injection, CLE clearly needs dedicated time during the procedure (in our study, ~6 minutes per procedure); however, with the development of algorithms for automated CLE image analyses, a reduction in the times needed for CLE images analysis can be expected and might facilitate transferability of this technology to less experienced centers in the future.[20,60]

Secondly, no allowance was made for multiple comparisons in this exploratory approach.

Finally, we did not include central reading for endoscopic or histopathologic scoring. However, the inclusion of IBD-experienced endoscopists in our expert center and 2 different masked pathologists, along with the use of 2 of the most commonly used scores in UC and the observed high concordance between RHI and NHI, might reflect that no significant bias was present despite the lack of central pathology reading.

Conclusion

In summary, our results show for the first time that barrier healing is highly predictive of the further course of disease in patients with clinically remittent IBD and that the predictive capabilities of barrier function might well exceed established or emerging parameters such as endoscopic and histologic remission. Therefore, CLE-based dynamic monitoring of the intestinal barrier during routine ileocolonoscopy might be a helpful tool in clinical practice for risk-stratifying patients with IBD and predicting complicated disease behavior. Finally, our findings suggest that analysis of barrier function might be considered as a future treatment target in clinical trials.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://dx.doi.org/10.1053/j.gastro.2022.10.014.

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