# Classification of Inflammation Activity in Ulcerative Colitis by Confocal Laser Endomicroscopy

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OBJECTIVES: **The assessment of inflammation activity in ulcerative colitis (UC) includes endoscopy and histology. Confocal laser endomicroscopy (CLE) combines real-time endoscopy and histology. This study was aimed at evaluating the application of CLE in the assessment of inflammation activity in UC.**

METHODS: **In total, 73 consecutive patients with UC who visited Qilu Hospital for colonoscopy surveillance underwent CLE. Inflammation activity was first assessed by the colonoscopy Baron score, then by CLE with a 4-grade classification of crypt architecture, as well as by analysis of microvascular alterations and fluorescein leakage. Targeted biopsy samples were taken for histological analysis. Stored CLE images were subjected to post-CLE objective assessment.**

RESULTS: **Both assessment of crypt architecture and fluorescein leakage with CLE showed good correlations with histological results (Spearman's rho, both $P<0.001$). CLE seemed to be more accurate than conventional white-light endoscopy for evaluating macroscopical normal mucosa. More than half of the patients with normal mucosa seen on conventional white-light endoscopy showed acute inflammation on histology, whereas no patients with normal mucosa or with chronic inflammation seen on CLE showed acute inflammation on histology. Assessment of microvascular alterations by CLE showed good correlation with histological findings ($P<0.001$). On post-CLE objective assessment, subjective architectural classifications were supported by the number of crypts per image ($P<0.001$) but not fluorescein leakage results by gray scale ($P=0.194$).**

CONCLUSIONS: **CLE is reliable for real-time assessment of inflammation activity in UC. Crypt architecture, microvascular alterations, and fluorescein leakage are promising markers in CLE evaluation.**

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## INTRODUCTION

The diagnosis of ulcerative colitis (UC) depends on the integrated evaluation of clinical features and laboratory findings assessment, radiology, endoscopy, and histology (1). In the integrated evaluation, histological findings have an important role in predicting relapse, as well as in management. The histological assessment of inflammation in UC includes the investigation of acute inflammatory cell infiltrates (polymorphonuclear cells in the lamina propria), crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrates (round cells in the lamina propria), and crypt architectural irregularities. Patients with acute inflammatory infiltrates seen on histological assessment are more likely to experience relapse than are those without infiltrates, but chronic inflammatory cell infiltrates and crypt architectural irregularities are not associated with relapse (2). Furthermore, some studies suggest that severity of inflammation is a risk factor for colorectal neoplasia in UC (3).

Histological assessment of UC is inseparable from colonoscopic investigation and biopsy in current clinical practice. The assessment of inflammation activity by conventional colonoscopy is inaccurate in the prediction of acute inflammation in some cases, especially for those seeming to be in remission as evaluated by conventional colonoscopy. Studies have investigated other white-light endoscopy procedures such as magnification colonoscopy (4) and high-magnification chromoscopic colonoscopy (5); results have shown that these procedures had a better relation with histology than did conventional colonoscopy. However, the classification depends highly on pit alterations of colonic mucosa, but evaluating the infil-

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tration cell type by white-light colonoscopy, the gold standard for differentiating chronic and acute inflammation, is impossible.

Confocal laser endomicrosopy (CLE) combines white-light endoscopy and confocal laser microscopy and allows for real-time endoscopy and histological diagnosis of gastrointestinal diseases. The principle of endomicroscopy is based on an uneven distribution of fluorescein in tissues, which allows for the visualization of tissue structure after laser excitation. Recently, the use of CLE in the diagnosis of UC was reported. Kiesslich *et al.* (6) and Watanabe *et al.* (7) reported on real-time inflammation activity assessment by CLE. The inflammation activity assessment includes crypt architecture, cellular infiltration, and vessel architecture (6).

In this study, aiming to test the correlation of CLE-obtained features with histological findings in UC, we proposed a simplified classification system of four grades of crypt architecture, as our group had applied the classification of pit patterns by CLE in a histological diagnosis of gastric diseases and found it reliable (8). The real-time conventional colonoscopy Baron score and CLE assessment were also compared. The validity of microvascular alterations in assessment was also tested. Furthermore, we introduced a new marker, fluorescein leakage in crypt lumen (FLIL) seen in CLE images, which could indicate increased colonic permeability, and tested its validity in predicting acute inflammation.

## METHODS

### Patients

From 1 June 2008, to 30 April 2009, we recruited consecutive patients previously diagnosed as having UC, who visited the outpatient department of Qilu Hospital for colonoscopy surveillance. After being informed about the purpose of the study, those who were willing to choose CLE instead of conventional colonoscopy were included in the study. Those who had a history of allergy to fluorescein sodium, cirrhosis, renal dysfunction, acute gastrointestinal bleeding, jaundice, or age <18 or >80 years, or were pregnant or breastfeeding, unwilling to participate, or unable to provide informed consent were excluded. All patients gave their written informed consent to participate in this study. The study was approved by the local ethics committee (the Clinical Ethical Committee, Qilu Hospital, Shandong University). Finally, 73 patients (31 women) with documented UC were included. The mean (±s.d.) age of patients was 50.36±14.10 years. The median duration of UC was 12 months (range: 2–48 months).

The primary end point was the correlation between CLE and histological findings of inflammatory activity in UC. The secondary end point was the accuracy of CLE compared with conventional white-light colonoscopy in evaluating acute inflammation in UC.

### CLE assessment

***Real-time subjective CLE assessment***. Bowel preparation before CLE did not differ from that before conventional colonoscopy. The CLE device used was an EC3870K (Pentax, Tokyo, Japan). All patients were given intravenous injections of 1 ml of 2% fluorescein sodium (Baiyunshan Mingxing Pharmaceutical, Guangzhou, China) for an allergy test before the procedures. After successful intubation into the terminal ileum, 6 ml of 10% fluorescein sodium was intravenously injected. The CLE procedures did not differ from those of conventional colonoscopy, except for the additional evaluation of mucosal inflammation in the distal colon, including the sigmoid colon and rectum, by the Baron score (9), which is widely accepted for endoscopic evaluation of inflammation activity in UC. All CLE procedures were performed by two experienced endoscopists (TY and XMG) who were familiar with the endoscopic and CLE features of colonic mucosal inflammation. Each endoscopist had performed more than 100 CLE procedures before the study.

The endoscopists first evaluated the endoscopic features of the distal colon by white-light colonoscopy. Inflammation activity was assessed by the Baron score (9). After Baron endoscopy scores for inflammation were recorded, the distal tip of the endoscope was placed gently on the observed mucosa with the endomicroscopy mode turned on. At least 2–3 Z-stacks of images (scanning from the superficial to the deep layer of targeted mucosa) were obtained, and the endoscopists evaluated the crypt architecture simultaneously. Crypt architecture was classified into four types. Types A and B are considered as normal and chronic inflammation, respectively, and types C and D indicate acute inflammation. Crypt changes in grade B are irregular arrangement of colonic crypts with normal size and shape, and enlarged spaces between crypts. These changes indicate chronic inflammation in which chronic inflammatory cells infiltrate into the lamina propria, but the epithelium is generally intact. Type C has enlarged crypt opening and more irregular crypt arrangement than type B. The enlarged diameter of the crypt opening and distances between crypts are important features that identify acute inflammation in UC, which indicates a more severe inflammatory infiltration in the lamina propria and exudation into the crypt lumen (10,11). Details of the crypt architecture classification system are given in **Table 1**.

Microvascular alterations were evaluated by real-time CLE assessment of inflammation activity as well. Microvascular alterations were divided into three degrees: none, mild-to-moderate, and severe, as described by previous research (6). In addition to crypt architecture and microvascular alterations, we introduced a new marker, FLIL, to define the acute inflammation seen on CLE. In CLE images of normal colonic mucosa, the lumen of the crypt is free of fluorescein and appears as a dark center in the crypt; however, in inflamed mucosa, fluorescein leaks into the crypt lumen; therefore, the lumen is brighter than the surrounding epithelium.

**Table 1. Classification of crypt architecture by confocal laser endomicroscopy (CLE) assessment in ulcerative colitis**

| CLE crypt architecture | Description |
|---|---|
| A | Regular arrangement and size of crypts |
| B | Irregular arrangement of crypts, enlarged spaces between crypts |
| C | Dilation of crypt openings, more irregular arrangement of crypts, and enlarged spaces between crypts as compared to type B |
| D | Crypt destruction and/or crypt abscess |

Images of observed mucosa were stored digitally on laser discs for further evaluation. In the end, a targeted biopsy was performed for histological analysis.

***Objective post-CLE analysis***. Objective post-CLE analysis involved an evaluation of all Z-stacks of images of the observed mucosa for the number of crypts per CLE image (CPCI) and fluorescein density of CLE images (FDCI). CPCI was considered as the objective variable to show the reliability of the objective real-time crypt architecture analysis, as was FDCI for FLIL. Because the crypt number per unit area of mucosa decreases with advanced inflammation, it can be used to measure architectural alterations. As the CLE image is visible as a different gray scale by uneven fluorescein distribution in tissues, FDCI was represented as the mean gray scale of CLE images, for a value from 0 to 255 (0, all black; 255, all white). Measurements of CPCI and FDCI were presented as mean±s.d. of all Z-stacks of images of the observed mucosa. CPCI was counted manually and FDCI was measured using the image-processing software, MATLAB 6.5 (MathWorks, Natik).

### Histology

Biopsy specimens were fixed with 10% formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin for histopathological examination. Three pathologists (CJZ, WQH, and HC) from two independent hospitals evaluated the slices for histological assessment of inflammation in UC, according to the Geboes Index (12). The scale includes six grades: structural (architectural changes), chronic inflammatory infiltrates, lamina propria neutrophils and eosinophils, neutrophils in epithelium, crypt destruction, and erosion or ulceration. Each grade is divided into 4–5 subgroups (12). One of the pathologists has a background in specialized gastrointestinal pathology (CJZ), and the others are general pathologists. All pathologists were blinded to clinical and endoscopical information. Each pathologist evaluated the slices independently. The time needed to finish the reading was limited to one day. The final grade was the mean of three independent assessments. The final grades were then divided into two groups: grades ≤3.0 and >3.0, as grade 3.1 indicates neutrophils in epithelium, a hallmark of acute inflammation.

### Statistical analysis

Correlations between conventional colonoscopy and histological inflammation findings, and between CLE and histological inflammation findings were calculated by the Spearman rank correlation coefficient. A $P<0.01$ was considered to be statistically significant. Correlations between objective numerical variables and subjective CLE categorical variables, such as CDCI vs. crypt architecture and FDCI vs. fluorescein leakage were calculated by one-way ANOVA. FLIL association with histological findings was evaluated by the $\chi^2$-test. $P<0.05$ was considered to be statistically significant. Kappa (κ) values were used to estimate interobserver variations among pathologists. Agreement was considered poor (κ<0.4), good (κ 0.4–0.75), or excellent (κ>0.75). All statistical calculations involved the use of SPSS v13.0 (SPSS, Chicago, IL).

## RESULTS

### White-light colonoscopy and histology findings

The mean time to complete CLE procedures was about 30 min and was not significantly different from that for conventional colonoscopy in our unit. This finding was in agreement with that by Kiesslich *et al.* (13). No severe side effects or complications occurred; the most common side effect was transient yellow skin, and no allergic reactions were found.

Histological findings by all three pathologists were well correlated (Spearman's rho, 0.833, 0.851, and 0.893, respectively; $P<0.001$). With the Geboes Index divided into grade ≤3 and grade >3, the interobserver agreement among pathologists was excellent (kappa values 0.897, 0.901, and 0.905).

Of the specimens without acute inflammatory infiltrates into the epithelium, most (21 out of 23) had a Baron score of 0 or 1 by white-light colonoscopy, and of those with acute inflammation, 19 out of 50 were scored 0 or 1. Conventional endoscopy results and relation to histology results are shown in **Table 2**. A Baron score of 2 or 3 showed a good correlation with histology results (31 out of 33 with a histology score >3). In total, the Baron score and Geboes Index showed a statistically significant correlation (Spearman's $\rho=0.401$; $P<0.001$).

### CLE and histology findings

***Subjective CLE real-time analysis***. Of the specimens without acute inflammatory infiltrates into the epithelium, 21 out of 23 showed features of normal or chronic inflammation on CLE (**Table 3**; CLE grades A and B). Of those with acute infiltration,

**Table 2. Conventional endoscopy by histology findings**

| Conventional colonoscopy | Histology (Geboes Index) | | Total number of patients (%) |
|---|---|---|---|
| | ≤3 (%) | >3 (%) | |
| *Baron score* | | | |
| 0 | 12 (43) | 16 (57) | 28 (100) |
| 1 | 9 (75) | 3 (25) | 12 (100) |
| 2 | 1 (6) | 15 (94) | 16 (100) |
| 3 | 1 (6) | 16 (94) | 17 (100) |
| Total number of patients | 23 (32) | 50 (68) | 73(100) |

**Table 3. Confocal laser endomicroscopy (CLE) crypt architecture assessment in relation to histology findings**

| CLE crypt architecture | Histology (Geboes Index) | | Total number of patients (%) |
|---|---|---|---|
| | ≤3 (%) | >3 (%) | |
| CLE A | 7 (100) | 0 (0) | 7 (100) |
| CLE B | 14 (100) | 0 (0) | 14 (100) |
| CLE C | 2 (7) | 27 (93) | 29 (100) |
| CLE D | 0 (0) | 23 (100) | 23 (100) |
| Total number of patients | 23 (32) | 50 (68) | 73 (100) |

100% (50 out of 50) were of CLE grades C or D. CLE-assessed crypt architecture and its relation to histology results are in **Table 3**. Real-time CLE crypt architecture and Geboes Index findings showed a strong correlation (Spearman's $\rho = 0.738$; $P < 0.001$). CLE seemed to be more accurate, in comparison with histology findings, than conventional colonoscopy ($P = 0.005$). CLE images representing the four types of crypt architecture are in **Figure 1a–d**. CLE-assessed microvascular alterations and Geboes Index findings were strongly correlated (Spearman's $\rho = 0.617$, $P < 0.001$). CLE microvascular alterations and their relation to histology results are in **Table 4**.

FLIL findings correlated well with histological evidence of inflammation activity ($P < 0.001$) (**Table 5**). FLIL does not seem to be time dependent because it was not detected in normal mucosa even when the fluorescein density began to attenuate, whereas in inflamed mucosa, it was revealed instantly after intravenous injection. Characteristics of fluorescein leakage are in **Figure 1c** and **d**.

***Objective post-CLE analysis of CLE images***. A total of 168 Z-stacks of CLE images corresponding to the 73 observed mucosae were collected. The total number of images was 6,012 (35.7 images per Z-stack). Only a small proportion (180 images, 3%) was ranked as being of poor quality and were not evaluated.

The mean CPCI decreased significantly with increasing crypt architecture classification ($F = 75.381$, $P < 0.001$), with significantly different mean CPCI among crypt architectures (**Figure 2**). The mean CPCI was inversely and moderately correlated with histological findings (Spearman's $\rho = -0.683$, $P < 0.001$) (**Figure 3**).

**Table 4. Confocal laser endomicroscopy (CLE) microvascular alteration assessment by histology findings**

| CLE microvascular alterations | Histology (Geboes Index) | | Total number of patients (%) |
|---|---|---|---|
| | ≤3 (%) | >3 (%) | |
| None | 19 (59) | 13 (41) | 32 (100) |
| Mild to moderate | 4 (33) | 8 (67) | 12 (100) |
| Severe | 0 (0) | 29 (100) | 29 (100) |
| Total number of patients | 23 (32) | 50 (68) | 73 (100) |

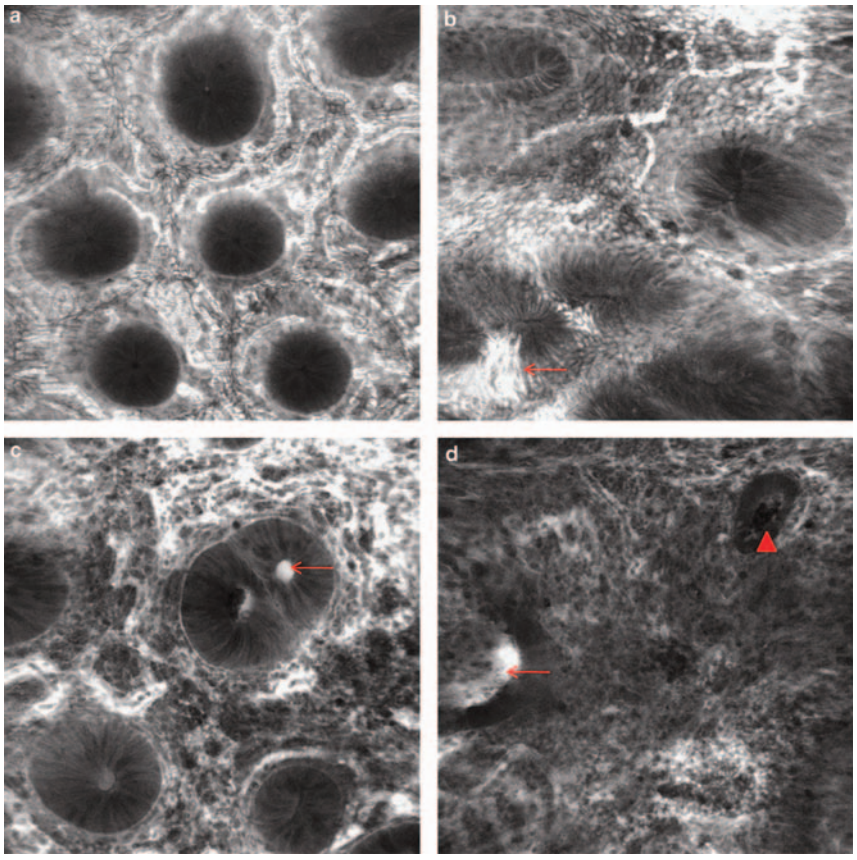


**Figure 1.** Confocal laser endomicroscopy images representing four types of crypt architecture and fluorescein leakage. (**a**) Regular arrangement of crypts surrounded by regular microvessels. Lumen of crypts shown as dark spots without fluorescein leakage. (**b**) Normal size and irregular arrangement of crypts. Lumen of crypts is still free of fluorescein, with fluorescein leakage into spaces among epithelial cells but not in the lumen (arrow). Sporadic crypt fusion can be seen. Spaces between some crypts are enlarged. (**c**) Decreased number of crypts in one image; some of the cryptal lumens are dilated and bright (arrow), which indicates fluorescein leakage, but the epithelium was still intact. (**d**) Most normal crypts were replaced by diffuse necrosis, and the remaining crypts were destroyed. Fluorescein leakage in cryptal lumens was more obvious than that in type C (arrow); crypt abscess can be seen (triangle).

**Table 5. Fluorescein leakage into crypt lumen (FLIL) by histology findings**

| FLIL | Histology (Geboes Index) | | Total number of patients (%) |
|---|---|---|---|
| | ≤3 (%) | >3 (%) | |
| No | 18 (64) | 10 (36) | 28 (100) |
| Yes | 5 (11) | 40 (89) | 45 (100) |
| Total number of patients (%) | 23 (32) | 50 (68) | 73 (100) |

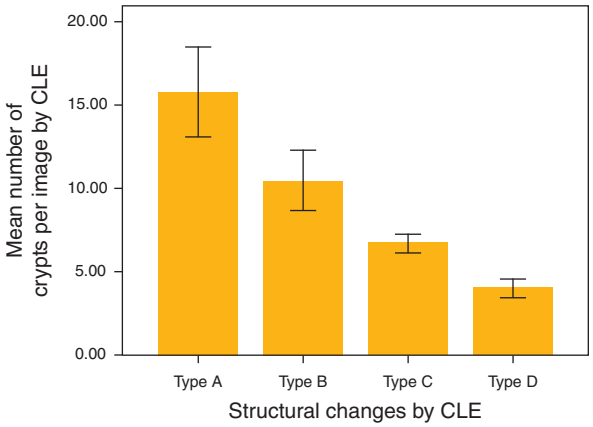


**Figure 2.** CPCI of each crypt architecture classification by CLE. The number of crypts per CLE image decreases with advance of crypt architecture changes. CLE, confocal laser endomicroscopy; CPCI, crypts per CLE image.

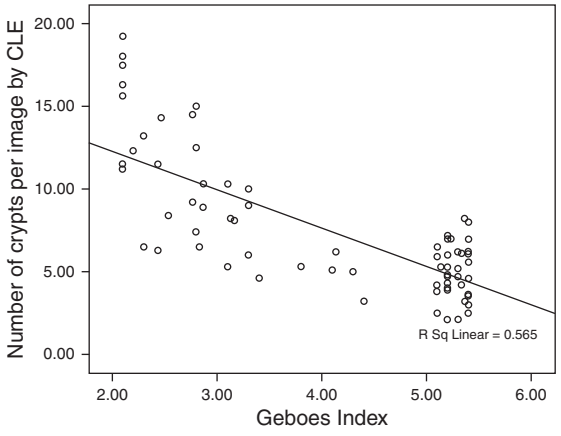


**Figure 3.** Correlation between CPCI and histological findings. Number of crypts per CLE image negatively correlated with Geboes Index. CLE, confocal laser endomicroscopy; CPCI, crypts per CLE image.

In contrast to findings for CPCI, images with and without FLIL did not significantly differ in FCDI ($P=0.247$). Moreover, FDCI and histological findings were not correlated (Spearman's $\rho=0.183$, $P=0.194$).

## DISCUSSION

Colonoscopy and histology are important procedures in the evaluation of inflammation activity and in predicting relapse and neoplasia in UC (1,3,14). Assessment of inflammation activity by conventional colonoscopy has been intensively studied, but the results have been controversial. Some studies suggest that conventional colonoscopy is not reliable for assessing acute inflammation and prelapse conditions (9). Results of advanced colonoscopy techniques such as high-magnification colonoscopy (4) and chromoendoscopy (5) show better correlation with histology findings than those of conventional colonoscopy. With more detailed observation, endoscopists could predict histology results more accurately. However, none of the colonoscopy techniques has the power of the histological level of observation, which usually requires at least a 200-fold magnification.

CLE is a newly developed endoscopy technique with 500- to 1,000-fold magnification. Kiesslich *et al.*, (6) reported on a 3-grade classification of inflammation activity by CLE, combining crypt architecture, cellular infiltrations, and vessel architecture. Our study aimed at comparing the findings on CLE used with a simplified 4-grade crypt-architecture classification with histological findings for UC and whether CLE is superior exclusively to white-light colonoscopy. Results show that the 4-grade crypt architecture classification correlates well with histological inflammation activity, and this classification is superior exclusively to white-light colonoscopy assessment.

We found a considerable proportion (16 out of 50) of patients with a histological evidence of acute inflammation (Geboes Index >3) whose conditions were graded as normal (Baron score 0) on white-light colonoscopy alone. Therefore, conventional colonoscopy assessment of inflammation activity is not reliable for assessing macroscopic normal mucosa, although the findings correlate well with histological findings of severely inflamed mucosa with edema, erosion, ulcer, or spontaneous hemorrhage. However, for 50 patients with histological evidence of acute inflammation, only 31 (62%) had active disease as determined by conventional colonoscopy. CLE had the same accuracy as white-light colonoscopy in diagnosis of severe inflammation. However, in cases of subtle acute inflammation in macroscopic normal mucosa, CLE demonstrated higher sensitivity than white-light colonoscopy. This result supports the use of CLE in surveillance of patients with UC to identify those at high risk of relapse, especially those whose conditions seem normal on conventional colonoscopy.

Our 4-grade classification system for crypt architecture did not involve an analysis of cellular infiltration and vessel architecture, as recommended by other researchers (6). The assessment of cellular infiltration is difficult because of the difficulty in differentiating cell types by CLE. The 4-grade crypt architecture classification might be easy to learn, especially for endoscopists beginning to use CLE. Given the limitations of subjective assessment, we introduced an objective counting of CPCI, because the number of colonic crypts per mucosal area decreases with advanced inflammation activity (10). The good correlation among crypt architecture assessment, CPCI, and histology supported the simplified evaluation of inflammatory activity by CLE.

The imaging of microvessels is one of the unique capabilities of CLE. In this study, microvascular alterations seen on CLE correlated well with histological findings. However, for patients

with normal microvessels, almost one-half of patients (13 out of 32) showed acute inflammation on histology. Thus, microvascular alterations alone seen on CLE might not be a sensitive marker for differentiating between acute and chronic inflammation.

FLIL is a new marker for assessment in CLE procedures. Our findings of a correlation between FLIL and histological inflammation findings encourage further studies of this phenomenon. Increased colonic permeability is a reasonable explanation for evidence of FLIL, because fluorescein sodium and sucralose, a common marker for assay of colonic permeability (15–17), have a similar molecular mass (fluorescein sodium, 376.78; sucralose, 397.64). However, fluorescein density was poorly correlated with FLIL and histology findings on objective assessment. This observation could be explained by a poor choice of index, because fluorescein sodium decays over time, and image acquisition time points interfere with the gray-scale image. In addition, necrosis and goblet cell depletion in advanced inflammation (18) decrease the gray scale of the image, even if fluorescein leakage increases it.

In conclusion, crypt architecture, microvascular alteration, and fluorescein leakage are promising markers of inflammation activity evaluation of UC by CLE. Further studies with a larger group of patients are required to confirm these findings.

## CONFLICT OF INTEREST

**Guarantor of the article:** Yan-Qing Li, MD.

**Specific author contributions:** Yan-Qing Li: designed the study; Chang-Qing Li: reviewed relevant articles, performed data extraction of real-time and post-CLE, and wrote the paper; Xiang-Jun Xie: determined the inclusion and exclusion criteria for patients and applied the criteria by conventional colonoscopy in the study; Tao Yu and Xiao-Meng Gu: responsible for patient inclusion and exclusion and for real-time CLE assessment; Xiu-Li, Zuo: responsible for the statistics and revision of the paper; Cheng-Jun Zhou, Wei-Qing Huang, and Hua Chen: contributed to histological assessment.

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**Potential competing interests:** None.

## Study Highlights

### WHAT IS CURRENT KNOWLEDGE

- ✓ Classification of inflammation activity is important in ulcerative colitis.
- ✓ Conventional colonoscopy evaluation may not be reliable.

### WHAT IS NEW HERE

- ✓ Confocal laser endomicroscopy is reliable for evaluating inflammation activity in ulcerative colitis.
- ✓ Crypt architecture, fluorescein leakage, and microvascular alterations are promising markers in confocal laser endomicroscopy evaluation.

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