Confocal laser endomicroscopy in ulcerative colitis: a longitudinal study of endomicroscopic changes and response to medical therapy (with videos)

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Background and Aims: Confocal laser endomicroscopy enables real-time in vivo microscopy during endoscopy and can predict relapse in patients with inflammatory bowel disease in remission. However, little is known about how endomicroscopic features change with time. The aim of this longitudinal study was to correlate colonic confocal laser endomicroscopy (CLE) in ulcerative colitis with histopathology and macroscopic appearance before and after intensification of medical treatment.

Methods: Twenty-two patients with ulcerative colitis in clinical relapse and 7 control subjects referred for colonoscopy were enrolled. The colonic mucosa was examined with high-definition colonoscopy, histopathology, and CLE at 4 colonic sites. Subsequently, patients requiring medical treatment escalation were referred for repeat endoscopy with CLE after 6 to 8 weeks.

Results: The baseline frequency of fluorescein leakage ($P < .001$), microerosions ($P < .001$), tortuosity of the crypts ($P = .001$), distortion of the crypts openings ($P = .001$), presence of inflammatory infiltrates ($P < .001$), and decreased crypt density ($P < .001$) were significantly higher in active ulcerative colitis compared with inactive ulcerative colitis and control subjects. A decrease in histopathologic score after medical treatment escalation was correlated with improvement in crypt tortuosity ($r_s = .35$, $P = .016$), distortion of crypt openings ($r_s = .30$, $P = .045$), and decreased crypt density ($r_s = .33$, $P = .026$) but not in other features.

Conclusions: CLE is an emerging endoscopic technique that reproducibly identifies mucosal changes in ulcerative colitis. With the exception of crypt changes, endomicroscopic features appear to improve slowly with time after medical treatment. (Clinical trial registration number: NCT01684514.) (Gastrointest Endosc 2016;84:279-86.)

(footnotes appear on last page of article)

Mucosal healing has become a hallmark in treatment of patients with ulcerative colitis because it predicts sustained remission.[1] Although the absence of ulcers is commonly accepted as an indicator of mucosal healing in Crohn's disease, this is less well defined for ulcerative colitis.[2] The endoscopic subscore of the Mayo Clinic Score, which is most frequently used for macroscopic assessment of ulcerative colitis, has not been validated for mucosal healing.[3] Nevertheless, a Mayo endoscopic subscore of 0 to 1 has been used to define mucosal healing and has been associated with an improved long-term clinical outcome and a reduced risk of colectomy in clinical trials.[4,5]

This video can be viewed directly from the GIE website or by using the QR code and your mobile device. Download a free QR code scanner by searching "QR Scanner" in your mobile device's app store.

Although mucosal healing is achieved, subtle changes not detectable with conventional endoscopy, such as a defective intestinal barrier function, may persist.[6] More importantly, the presence of an impaired mucosal barrier has been suggested to result in continuous bacterial translocation and subsequent abnormal activation of the immune system.[7,8] Re-establishment of an intact intestinal barrier is a complex cellular process that involves several immunologic factors as well as a proper homeostasis of the cells that form the epithelial lining.[9,10] Endoscopic mucosal healing may therefore represent a step in the process rather than the ultimate sign of complete healing of the gut.[1]

Despite improvements in conventional endoscopy with the introduction of high-definition colonoscopes and virtual chromoendoscopy, this method still has obvious

limitations when it comes to visualization of dynamic microscopic details and processes within the mucosa. The advent of confocal laser endomicroscopy (CLE) has enabled endoscopists to visualize these details in real time.[11] CLE is a novel method that allows in vivo microscopy during endoscopic examinations. The technique depends on a contrast agent, usually intravenously administered fluorescein. Currently, 2 systems are available: a probe-based system (pCLE) and an endoscope-based system (eCLE). pCLE consists of mini-probes fitting the working channel of any standard endoscope. The CLE technology has numerous possible indications, including inflammatory bowel disease (IBD), where it has been shown to depict structural changes, such as vascular alterations and abnormal crypt architecture.[12-15]

An additional key feature of CLE is that it can detect subtle barrier impairments by visualizing abnormal cell shedding and fluorescein leakage over the intestinal barrier.[8,16] Recently, we have shown that a defective barrier in the terminal ileum of patients with Crohn's disease is a risk factor for relapse even in patients in clinical and endoscopic remission.[17] This result is consistent with other studies and reveals an impaired barrier function in subgroups of patients with IBD.[6,8,18] Whether these findings can be reversed after medical treatment remains to be investigated. Furthermore, most trials using CLE in patients with IBD were performed with the eCLE system, which is currently not promoted by the manufacturer, and it is also unresolved whether the promising results of these studies are reproducible using pCLE equipment.

The aims of this study were first to correlate colonic mucosal pCLE features with histopathology and evaluate the reproducibility of these findings in patients with ulcerative colitis. The second aim was to examine how pCLE findings change after intensified medical treatment and correlate these with endoscopic and histopathologic scores.

METHODS

Participants

Patients with ulcerative colitis and a suspicion of clinical relapse at Copenhagen University Hospital Herlev were prospectively enrolled in the study. Patients referred with nonspecific abdominal symptoms and normal colonoscopy and patients undergoing adenoma surveillance with no history of IBD or diarrhea served as control subjects. All patients gave their informed written consent. Patients below age 18 years, pregnant or breastfeeding women, and patients with an impaired renal function or known allergy to fluorescein were excluded from the study. The study was approved by the Regional Ethics Committee (17 September 2012; H-1-2012-089-94) and The Danish Data Protection Agency (13 September 2012; HEH.750.89-32) and was registered at clinicaltrial.gov (Clinical trial registration number: NCT01684514).

Endoscopy

Depending on clinical presentation, either colonoscopy or sigmoidoscopy was performed using a high-definition colonoscope (EC38-i10L; Pentax Medical, Tokyo, Japan). No distal cap was used. When colonoscopy was performed, a macroscopic assessment was performed at 4 predefined colorectal sites (cecum, splenic flexure, left side of the colon 40 cm from the anal verge, and rectum), whereas only 3 colorectal sites (splenic flexure, left side of the colon 40 cm from the anal verge, and rectum) were assessed at sigmoidoscopy. CLE was performed at the same sites, and subsequently a parallel set of biopsy specimens was obtained. Any adverse events were registered. The biopsy specimens were formalin-fixed and paraffin-embedded. From each biopsy specimen 3-μm sections were cut and stained with hematoxylin and eosin.

The Mayo Clinic endoscopic subscore was used for macroscopic assessment, and an experienced pathologist (L.B.R.) graded the biopsy specimens using the Geboes index.[3,19] The histopathologic assessment was completely blinded to age, sex, disease status, and CLE data. The Mayo Clinic endoscopic subscore grades the severity of ulcerative colitis from 0 to 3, 0 for no signs of inflammation, 1 for mild disease (erythema, decreased vascular pattern, and mild friability), 2 for moderate disease (marked erythema, absent vascular pattern, friability, and erosions), and 3 for severe disease (spontaneous bleeding and ulceration).[3] The Geboes index is a composite score that grades disease severity in ulcerative colitis from 0 to 5 points by assessing structural changes, chronic inflammation, lamina propria eosinophils, neutrophils, neutrophils in the epithelium, crypt destruction, and erosions or ulcers.[19,20] After the first examination, patients requiring medical treatment escalation were referred for follow-up endoscopy after 6 to 8 weeks with CLE, macroscopic assessment, and biopsy specimens at the same predefined colonic sites.

Confocal laser endoscopy

CLE was performed using the pCLE system (Mauna Kea Technologies, Paris, France), which can be applied through the working channel of a standard colonoscope. The mini-probes are dedicated for colonic use and have a depth of view of 55 to 65 μm. The field of view is 240 μm and the resolution is 1 μm. Five mL of 10% sodium fluorescein (Skanderborg Pharmacy, Skanderborg, Denmark) was administered intravenously just before CLE imaging was initiated. CLE imaging was continued until the microscopic structure of the mucosa was depicted. The CLE videos were saved and subsequently assessed by a highly experienced (>100 CLE procedures) endomicroscopist (A.S.) after blinding to patient details. To our knowledge there is no consensus on the grading of ulcerative colitis with CLE.