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Confocal laser endomicroscopy for prediction of disease relapse in ulcerative colitis: A pilot study

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Abstract

Background and Aims: Neoangiogenesis and increased endothelial permeability are observed as results of chronic intestinal inflammation. However, limited data on microvascular and crypt architecture during remission phases is available. The aim of this prospective investigator blinded cohort study was to assess crypt and microvascular architecture and function in ulcerative colitis by probe based confocal laser endomicroscopy; we also evaluated whether these findings may have the potential to predict disease relapse.
Methods: 19 ulcerative colitis patients in clinical and endoscopic remission and 19 controls were studied. A computer based image processing technique was applied to construct 20 mosaicing image sets from each subject. Remitting patients were sub-grouped into either inactive or quiescent disease according to histology.
Results: Pericrypt fluorescence ($p < 0.01$), crypt diameter ($p < 0.05$) but not intercrypt distance ($p = 0.07$) were significantly increased in ulcerative colitis patients compared to controls. Patients with inactive disease showed a significant increase in fluorescence leakage (median fluorescence (IQR), 3888 (3560–4240) vs. 2696 (2502–3390), $p < 0.01$), crypt diameter (median diameter (IQR), 92.5 (85.5–101) vs. 73 (70–77), $p < 0.05$) and intercrypt distance (median distance (IQR), 82.5 (70.5–91.2) vs. 66 (59.5–73.5), $p < 0.05$) compared to those with

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quiescent disease. A composite outcome score combining fluorescence leakage and crypt diameter was able to predict a disease flare during a 12 month follow-up period ($p < 0.01$).
Conclusions: In vivo intramucosal changes detected by confocal endomicroscopy in ulcerative colitis remittent patients can predict disease relapse. This observation may have further implications for disease management and medical treatment.

1. Introduction

In ulcerative colitis (UC) structural changes and alterations in the lamina propria infiltrate allow a distinction between active, inactive and quiescent disease.[1] Although the histologic pattern usually correlates with the endoscopic appearance, microscopic features of activity may persist in endoscopic remission. Several studies have found that the correlation between endoscopic findings and histology is worse in endoscopically uninflamed mucosa.[2] This issue is clinically relevant since the persistence of microscopic lesions in the absence of signs of endoscopic inflammation may indicate increased likelihood of relapse.[3–6] Microvasculature anatomical remodeling and changes in the vascular endothelial cell phenotype including leakiness, increased leucocyte recruitment and procoagulant activity are present in biopsies from patients with active inflammatory bowel diseases (IBDs).[7–9] Whether these changes are reversible during clinical remission phases of ulcerative colitis (UC) and colonic Crohn's disease (CD) is still under debate. Confocal laser endomicroscopy (CLE) is a novel imaging technique that allows characterization of the microvascular network of the mucosal layer in the gastrointestinal tract during ongoing endoscopy in real time. Two CE and FDA certified CLE systems are currently available. One is integrated into the tip of a high-resolution endoscope (iCLE; Pentax, Tokyo, Japan) and one is probe-based, thereby capable of passage through the working channel of a standard endoscope (pCLE, Cellvizio, Mauna Kea Technologies, Paris, France). In IBD patients, CLE has successfully been used to diagnose in vivo inflammatory changes and to detect intraepithelial dysplasia during surveillance colonoscopy in UC.[10,11] Recently, a novel classification of UC activity was proposed based on confocal endomicroscopy findings, including crypt architecture and microvascular alterations.[12,13] Moreover, one recent study included consecutive patients with and without Crohn's disease.[14] A significantly higher proportion of patients with active Crohn's had increased colonic crypt tortuosity, enlarged crypt lumen, microerosions, augmented vascularization, and increased cellular infiltrates within the lamina propria. In quiescent Crohn's, a significant increase in crypt and goblet cell number was detected compared with controls. Based on these findings the authors proposed a Crohn's Disease Endomicroscopic Activity Score (CDEAS) for assessing Crohn's disease activity in vivo which was able to predict inflammation even in the course of macroscopically uneventful mucosa.

The aim of our study was to assess the microvascular and crypt architectural pattern of the intestinal mucosa in patients with longstanding ulcerative colitis in clinical and endoscopic remission. Second study objective was to evaluate whether these findings are predictive of disease relapse within a 12-month period.

2. Material and methods

2.1. Patients

From June 2010 to March 2011, 19 adult UC patients referred to our IBD unit and 19 subjects undergoing colonoscopy for non IBD-related indications (post-polypectomy follow up, colorectal cancer screening) were studied (Table 1). All patients were informed of the aims and the methodology of the study and gave their written consent. Ethical approval was obtained by the local institutional review board and the study was performed according to the Declaration of Helsinki. Patients with a previous history of allergy to fluorescein sodium, renal dysfunction, liver cirrhosis, jaundice or chronic respiratory disorder were excluded. Only UC patients in clinical and endoscopic remission for at least 12 weeks were included. Clinical and endoscopic remission was defined according to the Clinical Activity Index (CAI) and Mayo UC Endoscopic Score of Severity, respectively[15] Relapse was defined as a CAI ≥ 3 and abnormal mucosa at endoscopy. These patients were offered to undergo colonoscopy in order to assess mucosal healing. After obtaining informed consent, all patients underwent white-light ileocolonoscopy under either conscious or deep sedation using midazolam and pethidine or propofol respectively.

2.2. CLE assessment

After cecal intubation, 5 ml of fluorescein sodium 10% was administered intravenously as fluorescent contrast agent. Accordingly, the CLE-probe (ColoFlex UHD, Cellvizio, Mauna Kea Technologies, Paris, France) was introduced through the working channel of the endoscope and gently pushed against the mucosal wall. Confocal images were obtained from every

Table 1 Clinical characteristics of the study population.

	RCU	Controls	p value
Age, median years (IQR)	52 (50–57)	52.5 (49.7–56)	NS
Gender (M/F)	15/4	15/4	
BMI, median (IQR)	24 (16)	24.5 (21–26)	NS
Years of disease, median (IQR)	7.4 (5–9)		
Treatment			
5-ASA oral 2, 4 g/die	17		
Aza 2.5 mg/kg/die	1		
Infliximab 5 mg/kg/8 weeks	1		

NS, not significant.

colonic segment (i.e. cecum, ascending colon, transverse colon, descending colon, sigmoid colon, rectum) and digitally stored. Subsequent confocal diagnosis biopsies from the corresponding areas were collected. In case of macroscopically uneventful mucosa random biopsies were performed (both, optical and physical). In case of any circumscript lesions targeted biopsies were performed. Biopsy specimens were put in separate tubes. All CLE procedures were performed by one experienced endoscopist who had performed more than 100 CLE procedures previously to this study. All investigators were blinded to clinical and histological data.

Colonic mucosal images obtained by CLE correspond to an *en face* vision of the wall of the GI tract. In normal colonic mucosa, the glands have a normal shape and are regular in their distribution; they are roundish and the crypt luminal openings appear as black holes surrounded by epithelial cells radially oriented; enterocytes which are bright-gray cells and goblet cells which are black cells (due to their mucin content). The lamina propria, surrounding the crypts, has some inherent capacity for autofluorescence, and appears as a gray matrix containing single aggregates or scattered immune cells, along with capillaries which form a honey-comb network around each crypt.[16–19]

2.3. Post-CLE analysis

In order to avoid motion artifacts and gain better orientation of the imaged area, an image reconstruction algorithm using a video mosaicing technique (Mauna Kea Technologies, Paris, France) was used in a standardized fashion.[20] Briefly, this system combines all real time pictures acquired in a high quality wide-field image, allowing an evaluation of a mucosal area with an increased field of view (4 × 2 mm). Twenty mosaicing reconstructions, which each included 10 crypts, providing a total of 200 crypts/patient were analyzed using a dedicated software algorithm (CellvizioViewer, Mauna Kea Technologies, Paris, France). Crypt distribution along the mucosal surface, crypt diameter, intercryptic distance and crypt openings were considered; opening crypt shape, the presence of branching crypts, goblet cell density and the presence/absence of inflammatory cells in the lamina propria were also evaluated using the modified Miami classification.[13] During inflammation there is a physiological increase in endothelial vascular permeability with consequent extravasation of fluorescein into avascular areas.[21] The fluorescence intensity, related to the fluorescein leakage in a region of interest, was measured by using a special dedicated software (Mauna Kea Technologies, Paris, France)[22] (Fig. 1). A post-CLE analysis was performed by a single clinical researcher who was blinded to clinical and endoscopic findings.

2.4. Histology

Biopsy samples were fixed in formalin and embedded in paraffin, then stained with hematoxylin–eosin. Samples were sectioned transversely in order to allow direct comparison between confocal images and histology; a specialized gastrointestinal pathologist analyzed the sections blinded to endomicroscopic information and disease status. Histological features were analyzed according to the Riley Index and disease activity was defined as follows: active disease, neutrophiles in conjunction with epithelial cell damage; inactive chronic disease, architectural changes (irregular surface and crypt abnormalities) and increase in lamina propria mononuclear

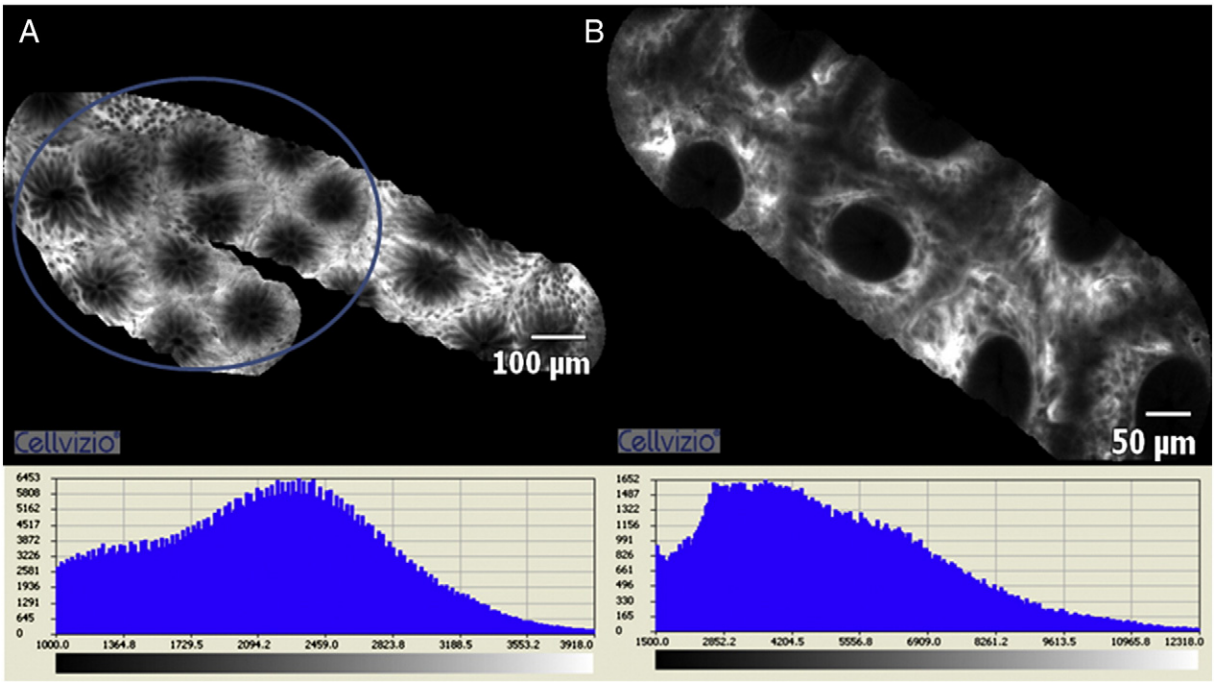

Figure 1 Mosaicing images of colonic mucosa from a healthy subject (A) and a UC patient with inactive disease (B) and corresponding histograms. The horizontal axis represents the dynamic range and the vertical axis the total number of pixel with the given value.

cells; quiescent disease, architectural changes without alteration in the intensity and composition of the lamina propria cellular infiltrate.[1,23]

2.5. Statistical analysis

Descriptive statistics are reported as proportions for categorical data or median (and interquartile range, IQR) for continuous variables. χ^2 test (or Fisher's exact test when applicable) or Mann Whitney U non-parametric test was used to compare groups for categorical or continuous variables respectively and to test their association. A p-value less than 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed and the area under the ROC curve (AUC) was calculated by the trapezoidal rule. Optimal cut-off values for crypt diameter and pericryptal fluorescence were selected to maximize sensitivity, specificity and diagnostic accuracy. The probability of disease flare was estimated according to the method of Kaplan and Meier, and analyzed using the Log-Rank test. All analyses were performed with the statistical package SPSS 18.0 (SPPS, Inc., Chicago, IL).

3. Results

The demographic and clinical characteristics of the study population are summarized in Table 1. No significant differences in age, sex or BMI were observed between UC patients and controls. The mean dose of sodium fluorescein administered to each patient was 3.86 ± 0.85 ml without statistical difference between groups (p = 0.6); confocal images were captured during the 15 minute period following fluorescein sodium administration. Four subjects (10.5%) showed a transitory yellowing of the skin as previously reported,[24–26] but no other adverse events have been recorded up to 30 days following the procedure. Endoscopy revealed a normal mucosal pattern in all patients (Mayo UC Endoscopic Score of Severity = 0). Among UC patients, histology demonstrated quiescent disease in 7 (36.8%), and inactive disease in 12 (63.2%) (Fig. 2).

3.1. Endomicroscopic assessment of vascular pattern

3.1.1. Microvascular Architecture

The vascular architecture in the normal colon (Fig. 3A) consisted of a network of capillaries in the lamina propria surrounding the crypts opening with a honeycomb appearance (Fig. 3B–C). Capillaries were highlighted brightly and blood cells were recognized as dark inclusions. In the 12 UC patients with histologically inactive disease and in 2 patients with histologically quiescent disease, vascular structures appeared tortuous and irregular with a significantly increased vascular density (Fig. 4A–B); the remaining 5 patients with histologically quiescent disease, showed a pattern similar to the control group.

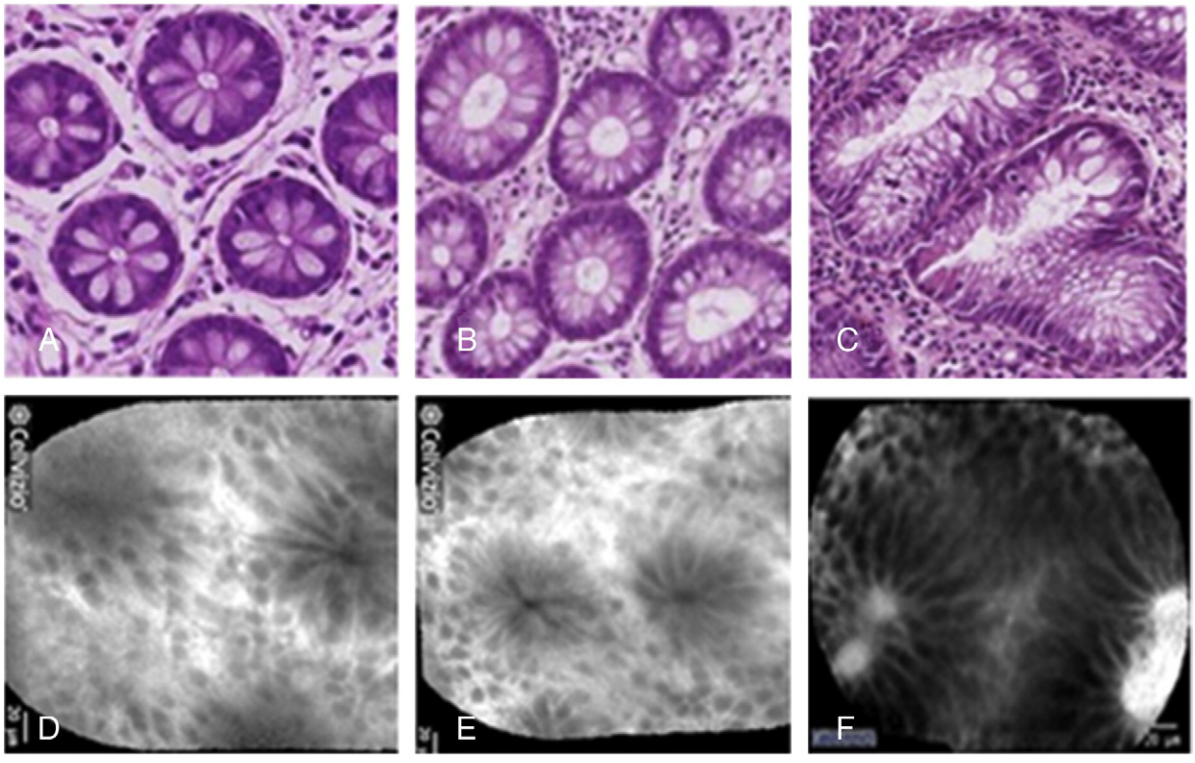

Figure 2 Histologic sections from a healthy subject (A) showing crypts with regular profile, evenly spaced; UC patient with quiescent disease (B), showing minimal architectural abnormality (i.e. variable crypt diameter and intercryptal distance); UC patient with inactive disease (C), architectural abnormalities (i.e. bifid crypts, increased crypt diameter), Paneth's cell metaplasia and mild increased in the lamina propria cellularity can be observed; D, E and F, corresponding confocal endomicroscopy images.

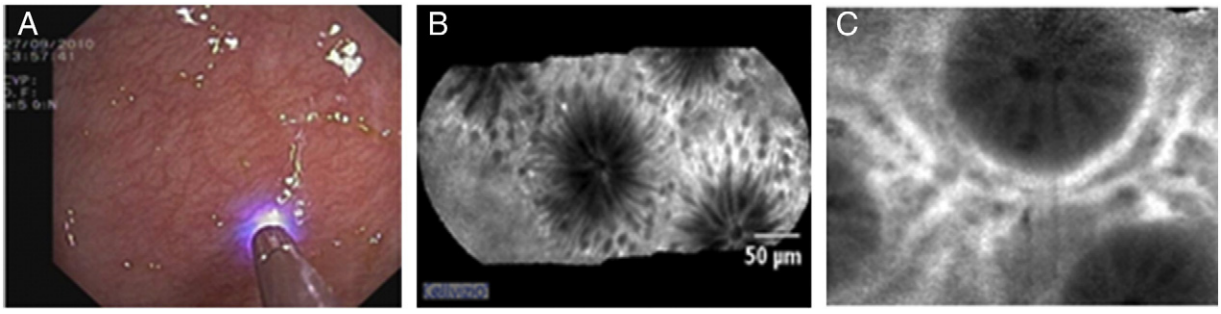

Figure 3 White light endoscopy image from a healthy subject showing the confocal probe and normal mucosa (A); corresponding confocal endoscopic imaging showing normal crypts (B) surrounded by an honey-comb network of capillaries (C).

3.1.2. Microvascular function

There was a significant difference in the fluorescence values between UC patients and controls (median fluorescence = 3451 pixel, IQR 2696–3912 vs. median fluorescence = 2404 pixel, IQR 2208–2793, $p < 0.01$). The 12 UC patients with inactive disease showed pericryptic fluorescent values higher than controls (median fluorescence = 3888 pixels, IQR 3560–4204 vs. median fluorescence = 2404 pixel, IQR 2208–2793, $p < 0.01$). Pericryptic fluorescence levels of UC inactive patients were also significantly increased compared to UC patients with quiescent disease (Fig. 5A–B) who showed similar values compared to controls (Table 2).

3.2. Endomicroscopic assessment of mucosal pattern

UC patients showed the so called "crypt fusion" which is consistent with an "en face" vision of the branched glands. In some areas crypt openings were star-shaped, a feature typical of hyperplastic glands. Crypt diameters were significantly longer in UC patients when compared with control patients (median diameter = 84 µm, IQR 76–94 vs. median diameter = 76 µm, IQR 71.2–81, $p < 0.05$); nevertheless UC patients showed some mucosal areas with a reduced crypt diameter. This was consistent with histology which demonstrated that the glands belonging to more atrophic areas showed shorter diameters than those in mildly inflamed areas. No difference was found between UC quiescent patients and controls (median diameter = 73 µm, IQR 70–77 vs. median diameter = 76 µm, IQR 71.2–81, $p = 0.25$). Interestingly, a significant difference between UC inactive patients and quiescent patients (median diameter = 92.5 µm, IQR 85.5–101 vs. median diameter = 73 µm, IQR 70–77, $p < 0.001$) was found. Although there was a trend towards increased intercryptic distance in UC patients compared to controls, this did not reach statistical significance (median distance = 73 µm, IQR 66–86 vs. median distance = 66 µm, IQR 62–69, $p = 0.07$). However, a significant difference in the intercryptic distance between UC quiescent patients and UC inactive patients (median distance = 66 µm, IQR 59.5–73.5 vs. median distance =82.5 µm, IQR 70.5–91.2, $p = 0.006$) was observed (Table 2).

3.3. Pericrypt fluorescence, crypt diameter and subsequent disease flare

Receiver operating characteristic (ROC) curves were constructed to evaluate the cut-off values for pericryptic fluorescence and crypt diameter that best differentiated healthy subjects from those with inactive disease. These

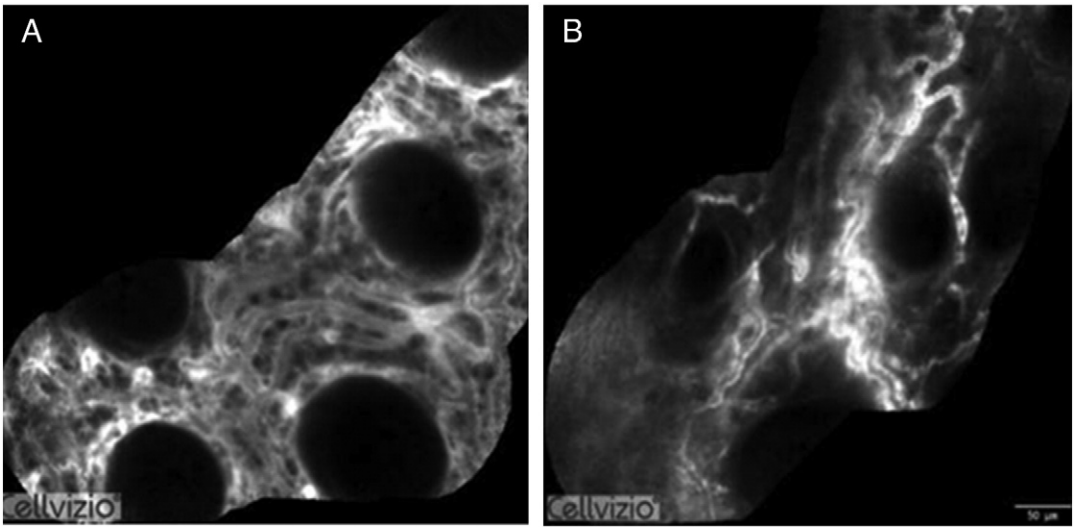

Figure 4 Confocal endoscopic imaging from UC patients with inactive disease showing increased vascular density (A) and tortuosity of capillaries (B).

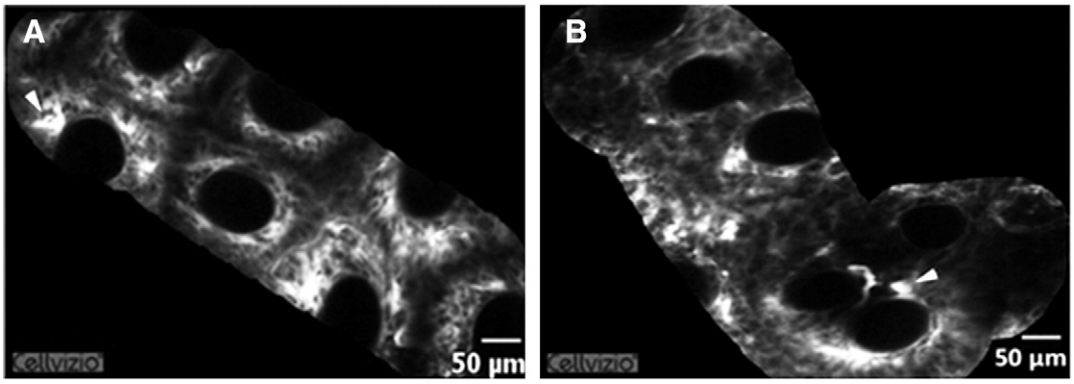

Figure 5 Confocal endoscopic imaging from UC patients with inactive disease. Mosaicing images showing fluorescein leakage in the extravascular area (arrow heads).

curves revealed that a cut-off of 3100 pixels was associated with a sensitivity of 100% and a specificity of 94% (area under the curve = 99.4%, 95% CI: 97.4–100) for the detection of inactive ulcerative colitis and a diameter <90 μm was associated with a sensitivity of 100% and a specificity of 95% (area under the curve = 98.1%, 95% CI: 93.5–100). During the 12 month follow up period, seven patients (37%, 6 on 5-ASA and 1 on azathioprine treatment) suffered a disease flare. When patients were stratified according to their pericrypt fluorescence and crypt diameter, those with pericrypt fluorescence >3100 pixel and a crypt diameter >90 μm (Group C, Fig. 6) had a significantly increased probability of presenting a disease relapse, compared to those patients with a pericrypt fluorescence and crypt diameter (Group A, Fig. 6) below the cut off values.

4. Discussion

Endoscopic evaluation of mucosal inflammation in UC patients is important for the prognosis and the management of the disease. Clinical and endoscopic remission is considered the primary goal of treatment in IBD patients.[27] Assessment of symptom resolution and endoscopic mucosal healing is now recommended as a primary endpoint for clinical trials in patients with mild–moderate UC.[15] However, in some patients absence of endoscopic activity is associated with the persistence of histological markers of inflammation potentially predictive of disease relapse.[3–6] Early studies suggest that patients with no macroscopic signs of inflammation but with histologic inflammation suffer from an up to 3-fold risk for the development of an acute flare within the next 12 months.[28,29]

Although moderate to severe active inflammation can be reliably diagnosed by conventional white-light colonoscopy, the assessment of mucosal inflammation in patients with an endoscopically normal appearance has been shown to be inaccurate. In this study we have evaluated in vivo crypt architecture and microvasculature pattern in UC patients with clinical and endoscopic remission. Using CLE we were able to show that changes in microvasculature anatomy and function are present in up to one third of patients with clinical remission without apparent endoscopic stigmata of inflammation. In these subjects microvessels showed a tortuous shape, irregular distribution and loss of the honey-comb appearance; significantly increased fluorescein leakage was also noted compared to healthy subjects. Fluorescein sodium is predominantly protein-bound in the blood and is considered a good marker of mucosal blood flow distribution. Thus, the presence of fluorescein in the extravasal compartment, particularly in the pericryptal lamina propria, is related to an increased vascular permeability and consequent extravasation of blood components, representing an indirect sign of inflammation.

Crypt architectural abnormalities are an important feature in the histological assessment of inflammation in UC.[30] In a post-analysis of the confocal images, we found that crypt diameters were significantly increased in patients with structural and functional abnormalities of the microvasculature compared to controls. This is in keeping with the changes described by Watanabe et al. in UC patients in remission.[31]

Hypercellularity compatible with an inflammatory infiltrate of the lamina propria and goblet cell depletion in the crypt epithelium was also present and confirmed at the histological evaluation of the corresponding mucosal areas.

In our study fluorescence leakage and crypt diameter discriminated healthy subjects from patients with inactive

Table 2 Confocal endomicroscopy crypt architecture and pericryptic fluorescence assessment in relation to histology.

	Fluorescence pixels, median (IQR)	Crypt diameter (μm), median (IQR)	Intercryptic distance (μm), median (IQR)
Controls	2404 (2208–2793)	76 (71.2–81)	66 (62–69.7)
Quiescent disease	2696 (2502–3390)	73 (70–77)	66 (59.5–73.5)
Inactive disease	3888 (3560–4204) **	92.5 (85.5–101) *	82.5 (70.5–91.2) *

* $p < 0.05$, quiescent vs. control.
** $p < 0.01$, inactive vs. quiescent disease and vs. controls.

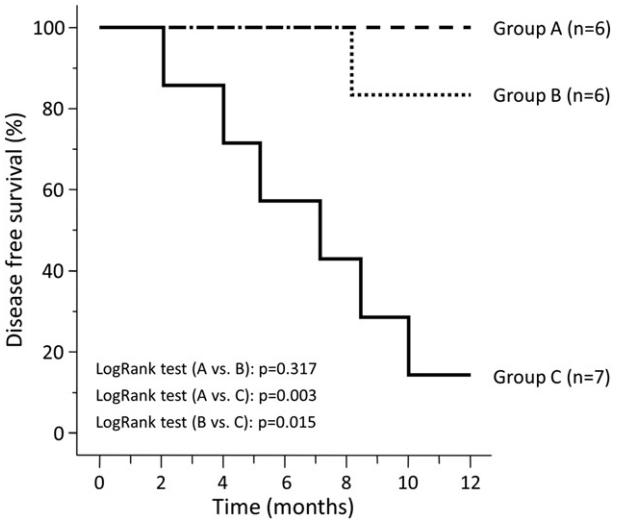

Figure 6 Kaplan–Meier plot of relapse of UC patients over 12 months after confocal laser endomicroscopy stratified according to their pericrypt fluorescence and crypt diameter. Group A: pericrypt fluorescence <3100 pixel and crypt diameter <90 μm. Group B: pericrypt fluorescence <3100 pixel and crypt diameter >90 μm. Group C: pericrypt fluorescence >3100 pixel and crypt diameter >90 μm.

disease confirmed at histology, with a high sensitivity and specificity. Indeed, when these parameters were combined, they were able to predict disease relapse in our group of patients with clinical and endoscopic remission during a 12 month follow up period.

These data are in line with a previous pilot study by Kiesslich et al. [32] By analyzing cell shedding with CLE, the authors were able to predict disease relapse in patients with IBDs. CLE not only can identify in vivo barrier loss but also can offer visualization of dynamic changes in vascular structure during intestinal inflammation and mucosal repair, features not evident at standard histology. Microscopic characterization of the intestinal mucosa can not only increase our knowledge on the pathogenesis of gastrointestinal diseases, but also guide subsequent clinical management.

Potential limitations of our study have to be addressed. First, although we used a standardized prospective setting, no sample size calculation was performed thereby potentially affecting statistical analysis. Therefore, we recommend the validation of our results by a prospective multicenter study. Second, only one experienced investigator performed all endomicroscopic and post hoc analysis which may have result in a potential bias. However, the investigator was blinded to all clinical and histologic information. Additionally, we have used standardized, image processing algorithms which were investigator independent. Third, although the investigator had good experience in performing and interpreting endomicroscopy, this may not have been sufficient to reliably obtain and interpret endomicroscopy images. Fourth, the post hoc interpretation of the images represents a potential limitation for a widespread clinical application of this technique in clinical practice. However, using this method we were able to differentiate different architectural and functional patterns among subjects with a similar normal endoscopic mucosal appearance and obtain objective measures of the parameters considered. Lastly, due the limited number of patients considered and the relatively small number of flares, the subgroup analysis is best viewed as exploratory and the interpretation warrants caution. However, based on the results of this pilot study, future studies with larger population can be proposed in order to validate our findings and perform more detailed subgroup analysis to unravel the potential clinical importance. Although data from community hospitals have been recently reported,[33] the use of confocal endomicroscopy is currently mainly under research protocols in academic centers. Moreover, the cost of the equipment, medico-legal liability and the substantial amount of time to add to the endoscopic procedure represent major hurdles before this technology can be incorporated into routine clinical practice.

Mucosal healing has gathered major interest in the last decade as an emerging parameter of treatment effectiveness in patients with IBDs.[6] However, its importance as a surrogate prognostic marker is still under debate. Healing of the mucosa in some patients will only prolong the symptom free interval. In both our, and others' experience,[34] disease relapse following biologically induced remission usually occurs at the same colonic site affected prior to the occurrence of mucosal healing. Subcellular morphologic and functional imaging of the mucosal microvasculature and architecture obtained by new technologies such as CLE could contribute to a better definition and application of these parameters as a clinical endpoint of mucosal healing.

In conclusion, our data confirm that in UC patients CLE can accurately detect and quantify in vivo intramucosal changes during the clinical and endoscopic remission phase and that these changes are potentially predictive of disease relapse. However, additional data should be available before this technology can be proposed into clinical practice, outside the research setting.

Statement of authorship

AB conceived and carried out the study, interpreted data, and wrote the manuscript. GH conducted the study, interpreted data, and assisted with the writing of the manuscript. HN participated in the design of the study, interpreted data and helped to draft the manuscript. RD participated in the design of the study and helped to draft the manuscript. PP performed the statistical analysis. CM carried out pathological examination. JJ contributed to data analysis and helped to draft the manuscript. MB contributed to the interpretation of data. GCS participated in the design of the study, data interpretation and analysis. All authors read and approved the final manuscript.

Conflict of interest

Author declares that there are no conflict of interest.

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