Differentiation of colonic polyps by confocal laser endomicroscopy

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Background and study aim: The real-time identification and removal of adenomas is a cost-effective strategy to improve the prognosis of colorectal cancer. Confocal laser endomicroscopy (CLE) could provide real-time histological-level observation. We aimed to evaluate the efficacy of CLE diagnosis using a simple classification system that differentiates adenomas from non-neoplastic polyps with intravenous fluorescein staining alone.

Patients and methods: An endoscope integrated confocal laser microscopy system was used in this study. CLE images of 35 colonic polyps, including 15 hyperplastic polyps and 20 adenomas confirmed by histology, were first evaluated to develop criteria for diagnosis of neoplastic and non-neoplastic polyps. The diagnostic criteria included goblet cell depletion, villous architecture, and microvascular alterations. We then performed a prospective study of colonic polyps found during CLE and classified them according to the established criteria. A total of 115 patients with 115 colonic polyps were included. The real-time CLE diagnosis was compared with that from histology. The stored CLE images were evaluated later by a blinded observer.

Results: The sensitivity, specificity, positive predictive value, and negative predictive value of real-time CLE in identifying colonic adenomas were 93.9% (95% confidence interval [CI] 85.4–97.6), 95.9% (95% CI 86.2–98.9), 96.9% (95% CI 89–99), and 92.2% (95% CI 81–97), respectively, compared with histological results. Interobserver agreement between real-time and post-CLE still-image evaluation was excellent (kappa = 0.929). Goblet cell depletion alone had a sensitivity of 84.9% (95% CI 73–92) and a specificity of 87.8% (95% CI 75–95), as well as excellent interobserver agreement (kappa = 0.824).

Conclusions: Endoscope integrated CLE with fluorescein staining may reliably assist in the real-time identification of colonic adenomas. Among three diagnostic categories, goblet cell depletion can be used to distinguish adenomas and hyperplastic polyps.

Introduction

To improve the prognosis of colorectal cancer (CRC), surveillance and treatment of early-stage CRC and precancerous lesions such as adenomas are cost-effective [1]. According to the adenoma–carcinoma sequence, the identification and removal of colonic adenomas is effective in reducing the morbidity and mortality of CRC [2]. Because the most common macroscopic type of colonic adenoma appears to be the polyp, the removal of all colonic polyps found during colonoscopy is recommended. However, given that a considerable proportion of colonic polyps are hyperplastic, with little or no potential for malignancy, the removal of all polyps could increase the risk of unnecessary complications and healthcare costs.

Real-time colonoscopy evaluation that predicts the histological diagnosis can assist in real-time diagnosis. In recent years, high resolution endoscopy or chromoendoscopy has been used in the differential diagnosis of adenomas and hyperplastic polyps. Real-time endoscopy diagnosis of colonic adenomas appears promising. Most studies used the Kudo classification of five pit patterns in colonic mucosa, with pit patterns > II associated with neoplasia [3].

Confocal laser endomicroscopy (CLE), a newly developed endoscopy technology, allows for detailed in vivo analysis of tissue and subcellular structures in 500- to 1000-fold images of the mucosa. Previous studies have shown a high sensitivity and specificity of the technique in identifying neoplasia in ulcerative colitis [4] and Barrett's esophagus [5]. Sanduleanu et al. reported on the

Study and criteria	Features of colonic neoplasia
Mainz [8]	Vessel architecture: Dilated and distorted vessels with elevated leakage; irregular architecture with little or no orientation to adjunct tissue
	Crypt architecture: Ridged/lined irregular epithelial layer with loss of crypts and goblet cells; irregular cell architecture with little or no mucin
Sanduleanu [7]	General architecture: Disturbed architecture: mild irregularity of the crypts, eventual villous transformation, simple to complex crowding, causing increased crypt/stroma ratio to completely altered morphology, crypt destruction
	Mild to moderate alterations of vascular pattern: dilated vessels, irregular aspect; neoangiogenesis, with capillary leakage
	Cytonuclear features: Incomplete to lack of epithelial surface maturation
	Slight cytonuclear atypia: basally localized, "pencillate" nuclei, loss of cell polarity with pseudostratification to severe cytonuclear atypia: more apically localized, enlarged, roundish nuclei, depletion of goblet cells
	Islands of malignant cells
Buchner [20]*	Same as the Mainz criteria
Current	Goblet cell depletion: Homogeneous darker epithelium of colonic crypts
	Villous architecture: Replacement of normal crypts by villi-like architecture in colon
	Microvacular alterations: Increased density and caliber of the microvessels with irregular fluorescein leakage in the lamina propria

* This study used the probe-based confocal laser microscopy system.

Table 1 Previously published and current confocal laser endomicroscopy diagnosis of colonic neoplasia.

endoscope integrated CLE diagnostic features of inflammatory cloacogenic polyps [6], and CLE was shown to have excellent sensitivity and specificity in the differential diagnosis of colonic polyps [7]. However, the previously used diagnostic criteria require knowledge of histopathology and require double staining with intravenous fluorescein and topical acriflavine.

In this study, we developed a classification system of categories of colonic adenomas to be used with CLE and intravenous fluorescein staining alone. We evaluated the sensitivity and specificity of the procedure and the criteria in a sample of patients by evaluating real-time CLE and still images immediately post-CLE and at 6 months and 1 year.

Patients and methods

Diagnostic criteria

The CLE used in this study was an endoscope integrated CLE system. CLE images of 35 polyps taken from 35 patients between 1 January and 30 May 2008 were reviewed by three experts (TY, XMG, and YQL). On histology, 15 of the 35 polyps were hyperplastic polyps and the other 20 were adenomas. The three experts reviewed the Mainz criteria [8] for CLE diagnosis of colonic neoplasia and extracted three CLE features that differentiate adenomas and hyperplastic polyps: goblet cell depletion, villous architecture, and microvascular alterations. On CLE, goblet cells are distinct in the human intestine [9] and in gastric intestinal metaplasia [10]. In addition, goblet cell depletion is an important feature identifying intraepithelial neoplasia [8]. Unlike the villi structure of the small intestine, normal colonic mucosa has no villi. From evaluation of the CLE images and histology of the 35 polyps, villous architecture in CLE images seems to be associated with tubulovillous or villous adenoma, which appears to be a replacement of normal round crypts by villi-like architecture. Because the blood volume of adenomas is higher than that of hyperplastic polyps [11], we evaluated the capability of CLE for in vivo assessment of microvascular alterations in inflammatory and neoplastic lesions [4,5,7,8]. Microvascular alterations in CLE images include increased density, dilation, and fluorescein leakage of microvessels.

After evaluating all combinations of the three categories, the most sensitive and specific strategy for diagnosis by CLE was as follows: a polyp with any of the three categories was considered an adenoma, and one with none of the three categories was nonadenomatous. The previously published criteria for colonic neoplasia and current diagnostic criteria for colonic adenomas are shown in **Table 1**.

Prospective study

Between 1 July 2008 and 30 April 2009, we included consecutive patients with polyps found during CLE. Patients were excluded if they were allergic to fluorescein sodium, had active gastrointestinal bleeding, were pregnant or breast-feeding, or had other contraindications for conventional colonoscopy. Patients with previously known histological diagnosis were also excluded. All patients had indications for colonoscopy. The study was approved by the Clinical Ethics Committee, Qilu Hospital, Shandong University. Patients provided written informed consent for CLE.

The endoscope integrated CLE device used was an EC3870K (Pentax, Tokyo, Japan). All patients underwent preparation for routine colonoscopy and scopolamine butylbromide, 20 mg (Yantai Luyin Pharmaceutical Co., China) was administered prior to the procedure. All patients first underwent colonoscopy by CLE in white-light mode. During withdrawal of the endoscope, once the first polyp had been located, 5 mL of 10 % fluorescein sodium (Baiyunshan Mingxing Pharmaceutical Co. Ltd., Guangzhou, China) was administered intravenously. For patients with multiple polyps, CLE examination was performed only on the polyp located in the most proximal segment of the colon. All patients had undergone allergy testing with 1 mL of 2 % fluorescein sodium 20 minutes before the injection. One endoscopist (TY), who had performed more than 50 CLE procedures previously, was responsible for all procedures. For observation of the polyps, the laser probe attached on the distal tip of the device was placed onto the polyp for tight and steady contact as soon as the endomicroscopy mode was turned on. CLE images of the polyps from the superficial to the deep layer were displayed on the endomicroscopy window. Digital images were also stored on computer. After real-time diagnosis based on our criteria, biopsy or polypectomy was performed. The biopsies or removed polyps were sent for routine histology. The histopathology diagnosis was based on the World

Health Organization classification of tumors of the digestive system, in which colonic adenomas mainly consist of tubular, tubulovillous, and villous adenomas [12]. In this study, hyperplastic polyps and inflammatory polyps were classified as non-neoplastic polyps and adenomas were classified as neoplastic polyps.

All CLE images of each polyp were stored in a specific folder on laser discs. Folders of polyp images were sorted randomly and renamed according to the sorting numbers. Another endoscopist (ZL), who did not participate in any of the CLE procedures, evaluated the images and made a still-image post-CLE diagnosis based on the same criteria as the real-time diagnosis. The endoscopist had performed 20 CLE procedures prior to this study and was familiar with the Mainz criteria. All images were then sent to the endoscopist (TY) who performed the original procedures for intraobserver variability analysis. This endoscopist made a diagnosis from the still images 6 months to 1 year after the original procedures.

Statistical analysis

Data are presented as mean ± SD and ranges, and median and interquartile ranges. The real-time CLE and post-CLE diagnoses were compared with histology results to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of CLE in diagnosing adenomas. Each category of the diagnostic criteria was also evaluated for sensitivity, specificity, and interobserver agreement. Real-time CLE and post-CLE diagnosis were evaluated in terms of interobserver agreement of the diagnosis of adenoma and each diagnostic category. Interobserver agreement for the real-time CLE and post-CLE diagnoses was determined by kappa value: values of 0.1 – 0.2 were considered slight agreement, 0.21 – 0.4 fair agreement, 0.41 – 0.6 moderate agreement, 0.61 – 0.8 substantial agreement, and 0.81 – 0.99 almost perfect agreement. For sensitivity and specificity, 95 % confidence intervals (CIs) were calculated. All data were analyzed by SPSS v13.0 (SPSS Inc., Chicago, Illinois, USA). This study was reported according to the STARD guidelines for reporting studies of diagnostic accuracy [13].

Results

Prospective study of patients with polyps

A total of 116 patients (116 polyps) were recruited and underwent CLE in white-light mode in the prospective study. One polyp from one patient was excluded because the endoscopist was informed about a previous histological diagnosis during the CLE procedure. Therefore, 115 patients (75 males) with 115 polyps were included in the final analysis. The mean age of patients was 51.6 ± 11.8 years. A total of 24 patients underwent colonoscopy for surveillance (19 postpolypectomy, three inflammatory bowel disease, and two with family history of CRC); the remaining 91 patients underwent colonoscopy for diagnosis.

The mean and median diameters of the 115 polyps were 7.5 mm and 6.0 mm, respectively. The diameters of 37 polyps (32.2 %) were ≥ 10 mm and 78 (67.8 %) were < 10 mm. Polyps were located in the cecum (n = 11, 9.6 %), ascending colon (n = 9, 7.8 %), transverse colon (n = 13, 11.3 %), descending colon (n = 20, 17.4 %), sigmoid colon (n = 24, 20.9 %), and rectum (n = 38, 33.0 %). Histology confirmed 45 hyperplastic polyps, four inflammatory polyps, 53 tubulous adenomas, and 13 tubulovillous adenomas. Neoplastic polyps had significantly larger mean diameter than non-neoplastic polyps: 9.2 mm (range 4 – 20 mm) vs. 5.1 mm (range 2 – 15 mm) (P = 0.002). Demographic, clinical, endoscopy, and histology data are shown in **Table 2**.

Table 2 Demographic, clinical, and histology data of the patients.

Age, mean ± SD, years	51.6 ± 11.8
Sex (male/female), n	75 / 40
Indications for colonoscopy, n	115
Lower abdominal pain	50
Changes of bowel habits	36
Hemafecia or occult blood in stool	5
Surveillance	24
Lesion size, mm	
Mean diameter	7.5
Median diameter	6.0
Lesion location, n (%)	
Cecum	11 (9.6)
Ascending colon	9 (7.8)
Transverse colon	13 (11.3)
Descending colon	20 (17.4)
Sigmoid colon	24 (20.9)
Rectum	38 (33.0)
Histology, n	
Non-neoplasia	49
Hyperplastic polyps	45
Inflammatory polyps	4
Neoplasia	66
Tubulous adenoma	53
Tubulovillous adenoma	13

Intubation into the cecum for CLE was successful for all patients. Except for transient yellow-stained skin and yellow urine, no severe side effects or complications were reported.

CLE diagnosis

Real-time CLE diagnosis accurately predicted adenoma or hyperplastic polyps for 109 of the 115 polyps. Four of the adenomas were misdiagnosed as hyperplastic polyps, and two hyperplastic polyps were misdiagnosed as adenomas. The sensitivity, specificity, PPV, and NPV of real-time CLE diagnosis of adenomas was 93.9 % (95 % CI 85.4 – 97.6), 95.9 % (95 % CI 86.2 – 98.9), 96.9 % (95 % CI 89 – 99), and 92.2 % (95 % CI 81 – 97), respectively, with total accuracy 94.8 % (95 % CI 89.1 – 97.6). The diagnostic yield of real-time CLE is shown in **Table 3**.

All polyps were then divided into two groups by diameter. For polyps < 10 mm, the CLE real-time diagnosis had a sensitivity of 90.3 %, a specificity of 95.7 %, and total accuracy of 93.9 %. For polyps ≥ 10 mm, the sensitivity was 97.1 %, specificity 100 %, and total accuracy 97.3 %. Real-time diagnoses were then divided into two phases by chronological order: the first half contained 58 polyps and the second half 57 polyps. Among the six polyps misdiagnosed with real-time CLE diagnosis, five occurred in the first half.

We acquired 9892 images of polyps from all CLE procedures for immediate post-CLE diagnosis. The mean number of images of adenomas and hyperplastic polyps was 101.3 and 67.5, respectively (P = 0.009). The endoscopist who performed the post-CLE diagnosis rated 7127 images as good quality (72.0 %), 1935 as medium quality (19.6 %), and 830 as poor quality (8.4 %) by the Mainz standards [8]. The interobserver agreement between real-time CLE and post-CLE diagnosis was almost perfect (kappa = 0.929). The stored CLE images were sent to the original endoscopist (TY) at 6 months to 1 year after the original real-time procedures for assessment of intraobserver agreement. Only two disagreements occurred between the original real-time CLE diag-

Table 3 Real-time confocal laser endomicroscopy and histology diagnosis of colonic polyps.

CLE diagnosis	Histology diagnosis				Total
	Tubulous adenoma	Tubulovillous adenoma	Hyperplastic polyps	Inflammatory polyps	
Adenoma	51	11	0	2	64
Hyperplastic polyp	2	2	45	2	51
Total	53	13	45	4	115

Data are number of cases.

Table 4 Diagnostic yield of combined confocal laser endomicroscopy (CLE) criteria categories and each diagnostic category in real-time diagnosis of colonic adenomas.

CLE diagnostic categories	Sensitivity	Specificity	Accuracy	PPV	NPV	Interobserver agreement (kappa)
Combined	0.94 (0.85 – 0.98)	0.96 (0.86 – 0.99)	0.95 (0.89 – 0.98)	0.97 (0.89 – 0.99)	0.92 (0.81 – 0.97)	0.929
Goblet cell depletion	0.85 (0.73 – 0.92)	0.88 (0.75 – 0.95)	0.86 (0.79 – 0.91)	0.90 (0.80 – 0.95)	0.81 (0.69 – 0.89)	0.824
Villous architecture	0.17 (0.10 – 0.27)	0.94 (0.83 – 0.98)	0.50 (0.41 – 0.59)	0.79 (0.52 – 0.92)	0.46 (0.36 – 0.55)	0.665
Microvascular alterations	0.67 (0.55 – 0.77)	0.76 (0.62 – 0.85)	0.70 (0.62 – 0.78)	0.79 (0.66 – 0.87)	0.63 (0.50 – 0.74)	0.689

Data are rate (95 % confidence intervals).
NPV, negative predictive value; PPV, positive predictive value.

nosis and post-CLE diagnosis, for almost perfect intraobserver agreement (kappa value = 0.964).

Goblet cell depletion gave the best diagnostic value compared with villous architecture or microvascular alterations. For use in diagnosing adenomas, goblet cell depletion had a sensitivity, specificity, and accuracy of 84.9 % (95 % CI 0.73 – 0.92), 87.8 % (0.75 – 0.95), and 86.1 % (0.79 – 0.91), respectively. Goblet cell depletion also had the best interobserver agreement between real-time and immediate post-CLE diagnosis (kappa = 0.824). Although in general, the sensitivity and specificity for villous architecture (16.7 % and 93.9 %, respectively) and microvascular alterations (66.7 % and 75.5 %, respectively) were not as high as for goblet cell depletion, the interobserver agreement between real-time and post-CLE diagnosis for the two categories was substantial (kappa = 0.665 and 0.689, respectively). The diagnostic yield for each category is shown in **Table 4**.

CLE images representing adenoma and hyperplastic polyp characteristics and corresponding histological features are shown in **Fig. 1**.

We also analyzed the real-time CLE diagnostic yields of combinations of two categories, as well as goblet cell depletion alone and with microvascular alterations or villous architecture. None of the combinations of categories was better than the original proposed diagnostic strategy, of any of the three categories alone indicating adenoma.

Discussion

In this study we aimed to evaluate the efficacy of CLE diagnosis using a simple classification system to differentiate adenomas from non-neoplastic polyps with intravenous fluorescein staining alone. We developed differential diagnostic criteria – goblet cell depletion, microvascular alterations, and villous architecture – for diagnosis of neoplastic and non-neoplastic polyps from CLE images of 35 colonic polyps, confirmed by histology as 15 hyperplastic polyps, and 20 adenomas. The prospective study of 115 colonic polyps revealed real-time CLE with excellent sensitivity and specificity in identifying colonic adenomas. Interobserver agreement between real-time and immediate post-CLE still-image evaluation was excellent. The sensitivity and specificity of goblet cell depletion alone was better than that of microvascular alterations and villous architecture. CLE with fluorescein staining alone may reliably assist in the real-time identification of colonic adenomas, especially by using the criterion of goblet cell depletion.

Polyp screening by colonoscopy is a meaningful approach for early detection and prevention of CRC [14]. Real-time differentiation of adenomas and hyperplastic polyps by chromoendoscopy, high-magnification colonoscopy, and narrow-band imaging are promising methods to strengthen the real-time decision. A multicenter study involving high magnification chromoendoscopy to classify colonic polyps revealed both sensitivity and specificity of 82 % [15]. The value of narrow-band imaging for differentiating neoplastic and non-neoplastic colon polyps was associated with high sensitivity and specificity in experienced hands [16].

Chromoendoscopy with indigo carmine was used to classify pit patterns into five types, with types III – V showing high rates of dysplasia. Kiesslich et al. reported a sensitivity of 92 % and a specificity of 93 % using chromoendoscopy with indigo carmine [17]. CLE could aid in diagnosis with cellular and subcellular analysis because of its high magnification imaging (500- to 1000-fold). Unlike previous studies involving both intravenous fluorescein and topical acriflavine [4, 7, 8], we could evaluate polyps by our diagnostic criteria using intravenous fluorescein alone.

Sanduleanu et al. reported that by using the integrated CLE system, the features of adenoma were lack of epithelial surface maturation, crypt budding, altered vascular pattern, and loss of cell polarity [7]. In our study, we summarized the CLE features of adenoma by three categories: goblet cell depletion, villous architecture, and microvascular alterations. The criteria applied in this study were extracted from but simpler than the Mainz criteria,

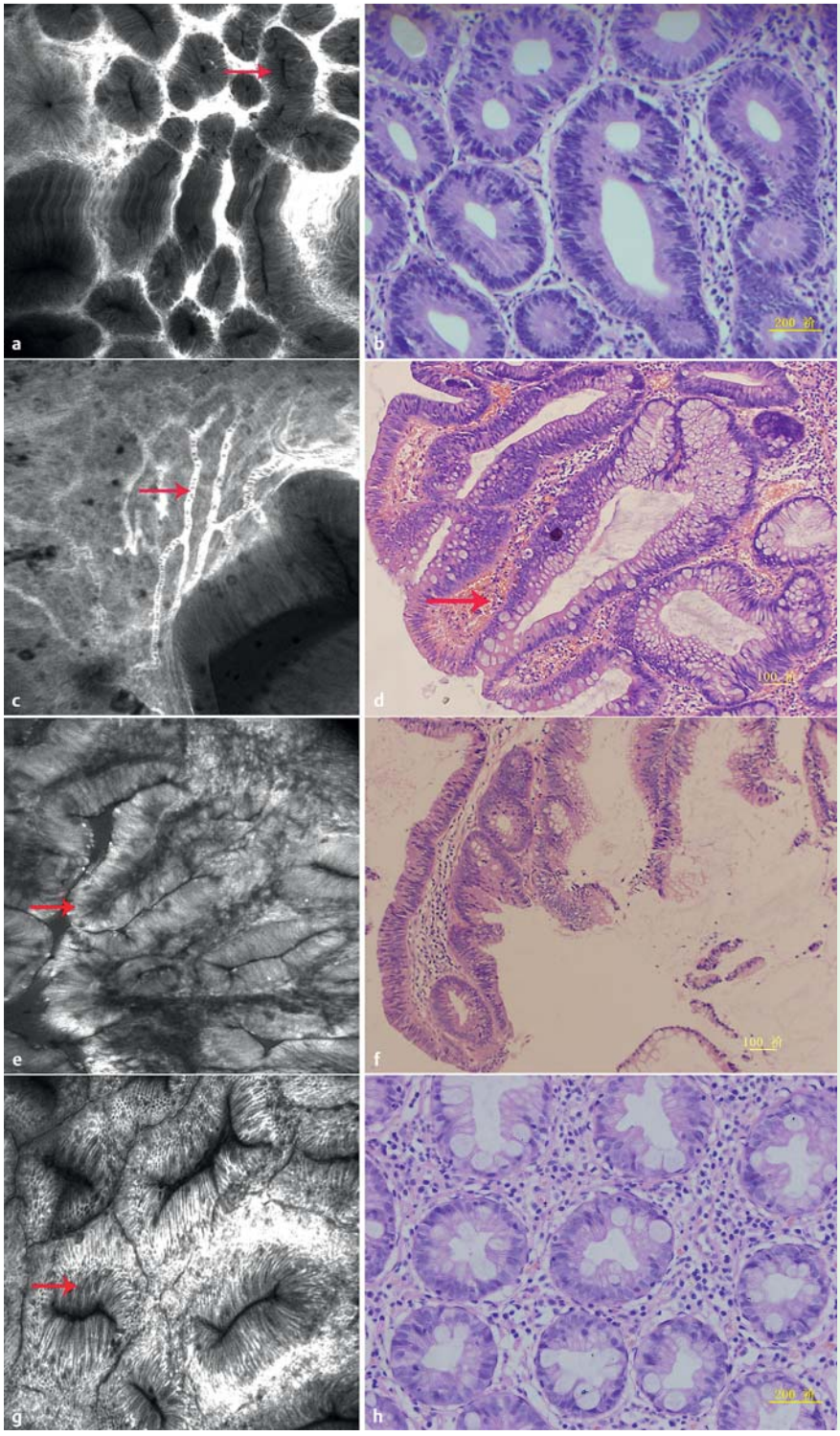

Fig. 1 Confocal laser endomicroscopy (CLE) images representing characteristics of adenoma (**a**, **c**, and **e**) and hyperplastic polyps (**g**) and corresponding histology results (**b**, **d**, **f**, and **h**). **a** CLE image of adenoma with darker epithelial cells indicating goblet cell depletion (arrow). Crypts are distorted and the density of microvessels is increased, with fluorescein leakage in the interstitium between crypts. **b** The corresponding histology results show goblet cell depletion in the epithelial lines and dilation of the crypts (hematoxylin and eosin [H&E], × 200) indicating tubulous adenoma. **c** CLE image of adenoma showing abnormal microvessels with increased number and dilated diameter near an adenomatous crypt (arrow). **d** Corresponding histology results showing abundant red blood cells in the axis of the villous (arrow) indicating dilated microvessel (H&E staining, × 100). **e** A villus is clearly visible on the CLE image (arrow). **f** Corresponding histology shows a finger-like villus. **g** CLE image of a hyperplastic polyp showing distorted and dilated crypt openings with normal goblet cells. **h** Corresponding histology results show dilated and distorted openings of crypts and normal goblet cells (H&E staining, × 200) indicating hyperplastic polyp.

with colonic pathology classified as normal, regeneration, and neoplasia based on both vessel and crypt architectures [8]. Whether the simplified criteria are easier for beginners of CLE in differentiating adenomas and hyperplastic polyps needs to be validated in further studies of the learning curve of CLE diagnosis.

Of the three categories used to diagnose adenomas, goblet cell depletion was found to be excellent, not only for its good sensitivity and specificity but also because of excellent interobserver agreement between real-time CLE and immediate post-CLE assessments. On CLE images, the depletion of goblet cells appeared to be uniform, with absence of flourescein in epithelial cells, which is easy to discriminate from the heterogeneous fluorescein density caused by the radial arrangement of goblet cells in normal colonic mucosa. Goblet cell depletion was also found to be important in identifying colonic neoplasia by CLE in other studies [4, 8, 18]. In addition, fluorescein depletion can reveal mucin depletion of the epithelium covering adenomas [19]. Although we found goblet cell depletion alone both sensitive and specific for diagnosis of adenomas, the sensitivity and specificity were still lower than that in the diagnosis strategy recommended by our preliminary study of 35 polyps: any of the three categories indicating adenomas.

Although microvascular alterations are specific to adenomas, the sensitivity of this category in diagnosis of adenomas was poor. As the scanning depth of CLE is only 250 µm, microvessels in adenomatous interstitium might be difficult to observe. Villous architecture showed good specificity but poor sensitivity, which could be explained by the low proportion of tubulovillous or villous adenomas (only 13 of 66 adenomas were tubulous and none was villous).

Of note, the sensitivity, specificity, and total accuracy of the post-CLE diagnosis were all similar to the real-time CLE diagnosis, and the interobserver and intraobserver agreements were almost perfect. Although the immediate post-CLE diagnosis was performed blinded to the CLE procedures and white-light endoscopy information, we could not rule out the effects of white-light video and imaging on the real-time CLE diagnosis, even though the mean size of adenomas was larger than that of non-adenomatous ones under the white-light endoscopy mode.

We performed a preliminary evaluation of the learning effects in CLE diagnosis in this study and found that the second half of real-time diagnoses showed higher accuracy than the first half; thus the learning effects by cumulative practice should draw more attention. The slightly higher accuracy of real-time diagnosis than the immediate post-CLE blinded diagnosis may be attributed to the learning effects.

The advantage of CLE over conventional colonoscopy should be the real-time differential diagnosis of colonic polyps, but not the surveillance of colonic polyps. In fixing the laser probe onto the distal tip of the system, aiming at a small polyp is not easy. Probe-based CLE might be easier for aiming at small lesions. Buchner et al. reported that probe-based CLE had similar sensitivity and specificity to virtual chromoendoscopy in the diagnosis of colonic polyps [20]. In addition, the CLE image quality can be affected by poor bowel preparation, artifacts caused by abdominal pulse or an unsteady touch on pedicled polyps.

Our study contains some limitations. First, in contrast to other studies, we included polyps > 10 mm [15]. Although immediate post-CLE blinded assessment of still images and real-time assessment showed similar accuracy and excellent interobserver agreement, real-time judgement based on lesion size and gross morphology could have been affected. Second, only one endoscopist performed all of the procedures and only one endoscopist performed all of the immediate post-CLE diagnoses, so the reliability of real-time and still-image diagnosis could not be assessed. Third, adenomas were not classified according to dysplastic grades because fluorescein sodium alone could not provide sufficient visualization to allow for clear analysis of nuclear structure, which is a key to grading neoplasia. Although not studied in humans, acriflavine has been shown to carry a risk for mutagenicity in experimental data [7]. Goetz et al. used cresyl violet staining in diagnosing colonic lesions by CLE and suggested its promise for white-light endoscopy and CLE nuclei assessment [21]. Fourth, for patients with multiple polyps, CLE examination was performed only on the most proximal polyps, which might suggest bias in polyp selection. Fifth, sessile serrated adenomas (SSAs) are difficult to differentiate from hyperplastic polyps by the current criteria. However, SSAs are difficult to differentiate even by conventional histopathology. The differentiation depends highly on specific markers with immunohistochemistry [22]. With the development of in vivo molecular diagnosis [23], CLE may be able to be used for diagnosis of SSAs by use of appropriate molecular markers. Sixth, the diagnostic accuracy was poor for inflammatory polyps in this study, and therefore the current criteria need further amendments. The small proportion of inflammatory polyps in our study (4 of 115) may also contribute to the poor accuracy.

In conclusion, our study suggests that endoscope integrated CLE with intravenous fluorescein alone may reliably assist in the real-time diagnosis of colonic adenomas. Of three diagnostic categories, only goblet cell depletion showed good sensitivity and specificity. Further well-designed studies are needed to validate the reliability of our proposed criteria.

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Competing interests: None

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