



Advanced gastrointestinal endoscopic imaging for inflammatory bowel diseases

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Abstract

Gastrointestinal luminal endoscopy is of paramount importance for diagnosis, monitoring and dysplasia surveillance in patients with both, Crohn's disease and ulcerative colitis. Moreover, with the recent recognition that mucosal healing is directly linked to the clinical outcome of patients with inflammatory bowel disorders, a growing demand exists for the precise, timely and detailed endoscopic assessment of superficial mucosal layer. Further, the novel field of molecular imaging has tremendously expanded the clinical utility and applications of modern endoscopy, now encompassing not only diagnosis, surveillance, and treatment but also the prediction of individual therapeutic responses. Within this review, we describe how novel endoscopic approaches and advanced endoscopic imaging methods such as high definition and high magnification endoscopy, dye-based and dye-less chromoendoscopy, confocal laser endomicroscopy, endocytoscopy and molecular imaging now allow for the precise and ultrastructural assessment of mucosal inflammation and describe the potential of these techniques for dysplasia detection.

Key words: Ulcerative colitis; Crohn's disease; Advanced endoscopic imaging; Chromoendoscopy; Mucosal healing; Colitis associated cancer; Confocal laser endomicroscopy; Endocytoscopy; Molecular imaging

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Core tip: Gastrointestinal luminal endoscopy is of paramount importance for diagnosis, monitoring and dysplasia surveillance in patients with Crohn's disease and ulcerative colitis. Within this review, we describe how novel endoscopic approaches and advanced endoscopic imaging methods such as high definition imaging, high magnification endoscopy, dye-based and dye-less chromoendoscopy, confocal

laser endomicroscopy, endocytoscopy and molecular imaging now allow for the accurate and highly resolved assessment of mucosal inflammation on the cellular level and describe the potential of these techniques for dysplasia detection.

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INTRODUCTION

Inflammatory bowel diseases (IBD) comprises the two major forms Crohn's disease (CD) and ulcerative colitis (UC) both of which are immunopathogenic complex diseases in which, on the basis of a genetic susceptibility host, an excessive mucosal immune response towards the complex enteric microbiota plays a role for the initiation and perpetuation of intestinal inflammation^[1-4]. Recent studies estimate that approximately 1.2 million people in the US and 3.7 million people in Europe suffer from IBD and in most parts of the world IBD incidence rates increase over time^[5,6]. With its profound involvement in diagnosis of UC and CD, in monitoring response to anti-inflammatory therapy as well as in dysplasia surveillance, gastrointestinal luminal endoscopy is of paramount importance for the management of IBD patients.

In the recent past, mucosal healing has increasingly been recognized as a key clinical treatment goal in IBD patients. Since it has been shown that complete mucosal healing is one of the most crucial aspects that predicts sustained clinical remission and resection-free survival of patients^[7], the precise and detailed assessment of the superficial mucosal layer in real-time becomes more important than ever for the medical management of IBD patients. In this context, advanced endoscopic imaging techniques including dye-based and dye-less chromoendoscopy, endocytoscopy and confocal laser endomicroscopy have been shown to allow precise, ultrastructural and even microscopic characterization of the inflammation within the luminal gastrointestinal tract in real time, thereby tremendously facilitating the diagnosis of IBD and the direct evaluation of response to medical therapy.

Apart from assessing the degree and extent of inflammation, regular surveillance endoscopies for dysplasia screening is another important aspect in the management of IBD patients. Several studies documented that the chronic inflammatory stimulus associated with IBD confers an increased risk for developing colitis associated cancer (CAC) in both, UC and CD patients^[8] and the individual colon cancer risk mainly depends on disease duration,

severity and anatomic extent of the disease^[9-13]. Due to the close association between disease duration and the development of CAC, current European and US guidelines recommending regular surveillance endoscopy starting 6 to 8 years after first manifestation of the disease^[14,15] and a large number of case series^[16-19] and case-control studies^[20-22] provided evidence of the clinical benefit of surveillance colonoscopy for IBD patients. However, at the same time routine white-light endoscopic examinations is limited for the accurate identification of dysplasia and intraepithelial neoplasia^[23] and at the same time, random biopsies have a low yield for dysplasia detection^[23,24].

While several reports have shown that traditional dye-based chromoendoscopy (DBC) with targeted mucosal biopsies is superior for dysplasia detection in IBD patients^[23,24], it is a time- and cost intensive procedure that requires a certain level of expertise and training. These confinements associated with DBC have led to the rapid evolvement of novel advanced endoscopic imaging techniques such as digital [*i.e.*, Fujinon Intelligent Color Enhancement (FICE), i-scan, Storz Professional Image Enhancement Systems (SPIES)] or optical [*i.e.*, Narrow band imaging (NBI), Compound band imaging (CBI)] dye-less chromoendoscopy which offer the advantage of enhancing mucosal vascular and mucosal surface pattern morphology by just pushing a button on the handle of the endoscope thereby potentially reducing time and costs associated with conventional dye-based chromoendoscopy^[25,26].

Within this article, we review the current literature on the role of advanced endoscopic imaging techniques such as high-definition and optical magnification endoscopy, dye-based and dye-less chromoendoscopy, confocal laser endomicroscopy and endocytoscopy for the assessment of the extent and degree of inflammation and the surveillance of colitis-associated cancer. Further, we provide an outlook on how first studies have utilized molecular imaging to visualize single molecular structures and also to stratify patients according to the expression of certain molecular targets, thereby allowing to make predictions of responses to medical therapies.

HIGH-DEFINITION AND OPTICAL MAGNIFICATION ENDOSCOPY

The chips used in current *high-definition* (HD) endoscopes produce signal images with resolutions that range from 850000 pixels to more than 1 million pixels and the general definition of a HD image (or display) is one with more than 650 to 720 lines of resolution^[27,28]. In order to obtain true HD images, all components utilized (*i.e.*, endoscope video chip, processor, monitor, transmission cables) have to be HD capable. As compared to standard definition (SD) video chips, HD

chips have a considerably lower light sensitivity due to the smaller size of their pixels, thereby requiring a strong light source which is typically a 300 watt Xenon lamp or a laser source^[27].

As shown in a recent meta-analysis of over 4000 non-IBD patients comparing the diagnostic yield of colonic polyps between high-definition and standard white-light colonoscopy, HD exhibited only a marginally increased yield for the detection of colorectal polyps of 3.5% compared to SD and the number needed to treat was calculated to be 28^[29].

However, while HD offers only an incremental increase for the detection of sporadic adenomas, its relevance for the detection of dysplastic changes in IBD is remarkable. In patients with IBD, dysplastic lesions often develop as flat lesions within the mucosa rather than as protruding into the intestinal lumen. As shown in a retrospective cohort study based on 369 subjects with long-standing colonic IBD, HD colonoscopy could detect significantly more adenomas especially within flat or right-sided lesions, as compared to standard definition white light colonoscopy^[30]. Overall, the adjusted prevalence ratio of detecting dysplastic lesions on targeted biopsies was calculated as 2.99 for HD colonoscopy^[30].

Optical magnification endoscopy

Optical magnification utilizes a movable lens to vary the degree of magnification thereby allowing to magnify the mucosa of the gastrointestinal tract from 6-fold up to 150-fold^[31]. Since this magnification is based on an optical zoom, the image quality is maintained while zooming. In contrast to this optical magnification, electronic or digital magnification simply enlarges the image on the display, leading to decreased pixel density and decreased image quality^[27]. In one of the earliest studies, optical magnification endoscopy has been shown to be able to differentiate true neoplasms from non-neoplastic colonic lesions by analyzing the mucosal pit pattern. Hence, as shown in this landmark trial, magnification endoscopy can provide an accurate and instantaneous prediction of the histology of colorectal tumorous lesions^[31].

This observation in colorectal polyps has recently been extended to other neoplastic and non-neoplastic diseases in the upper and lower gastrointestinal tract, and was especially used in combination with dye-based chromoendoscopy^[32-38]. In one of the earliest prospective randomized trials, Kiesslich and co-workers were able to show that methylene blue-aided chromoendoscopy in combination with optical magnification not only allowed for a better assessment of the degree and extent of colonic inflammation compared to conventional colonoscopy, but also to detect significantly more intraepithelial neoplasia compared to standard white-light endoscopy^[24]. Consistent with these observations, in a prospective trial on 350 patients with long-standing UC, signi-

ficantly more intraepithelial neoplastic lesions were detected by magnification chromoendoscopy compared with 350 disease-matched controls that received conventional endoscopy. Importantly, of the 67 lesions with intraepithelial neoplasia that were found, 53 (79%) were detected using magnification chromoendoscopy alone. In addition, magnification endoscopy was able to predict neoplastic and non-neoplastic mucosal changes with high overall accuracy^[34]. Several lines of evidence now support the concept of enhanced dysplasia detection with magnification (chromo)-endoscopy and it appears that especially the high-resolution and high magnification visualization of the pit and vascular pattern is a key component and of paramount importance for the identification of dysplastic areas in IBD^[34,39-43].

Apart from dysplasia detection, high magnification chromoendoscopy has been utilized for the assessment of disease extent and disease severity in IBD. In one of the earliest pilot study, magnification endoscopy was utilized to grade the rectal network pattern and cryptal opening in 41 patients with mild to moderate UC. When both features were combined, patients with visible network pattern and cryptal opening had a lower clinical activity and lower grade of histologic inflammation compared to patients in which both findings could not be visualized. Therefore, these data provide evidence that magnification endoscopy is capable of predicting the histologic degree of inflammation^[44]. In a prospective trial on 113 patients with quiescent UC, Nishio and co-workers studied the role of magnification endoscopy for predicting the clinical course of disease^[45]. The authors classified the rectal pit pattern appearance into four groups according to its irregularity: grade 1, small, round, and regularly arranged pits; grade 2, rather large, oval pits, somewhat irregular in arrangement; grade 3, pits various shapes and sizes, and irregularly arranged; grade 4, dispersed pits varying in morphology, associated with small erosions. Using this classification, the authors found that magnification endoscopy grading correlated to histopathological findings and acts as a significant predictor of relapse with a relative risk of 2.0^[45]. Further, magnification endoscopy calculated a relapse risk of 60% in the following 12 mo in patients who had a grade 4 rectal pit pattern, but who had only mild or no endoscopic features of disease activity at the time of endoscopy^[45]. Consistent with these data, Hurlstone *et al.*^[35] found a good correlation between the Saitoh criteria for magnification imaging and Mats' histopathological criteria in a prospective trial including 325 UC patients. In a biphasic examination, all colonic segments were first inspected with conventional white-light endoscopy, followed by high magnification chromoendoscopy. It has been shown, that magnification imaging results in a significantly better prediction of disease extent than conventional endoscopy.

CHROMOENDOSCOPY

Chromoendoscopy enhances the mucosal architecture and/or submucosal microvasculature by the use of various dyes (Dye-based chromoendoscopy, DBC) or endoscopic optical and computer-based color programs (Dye-less chromoendoscopy, DLC). This contrast enhancement of the mucosal layer results in the improved detection of lesions that are often subtle or even nearly invisible at conventional white-light endoscopy.

DBC uses different dye agents which are divided into absorptive agents (Lugol solution, methylene blue, toluidine blue, and cresyl violet), contrast agents (indigo carmine, acetic acid) and reactive staining agents (congo red, phenol red), all of which are mostly applied *via* standard spraying or plain biliary ERCP catheters^[46]. As briefly mentioned above, DBC has been shown to improve detection of dysplasia in IBD, and chromoendoscopy is recommended as the preferred modality for surveillance in patients with colonic IBD by the British Society of Gastroenterology^[47] and the European Crohn's and Colitis organization^[48]. However, DBC also requires increased efforts, skills, time, and costs. These confinements associated with the use of traditional dye agents have finally led to the development of dye-less chromoendoscopy techniques (DLC).

DLC is further subdivided into optical chromoendoscopy (NBI, Olympus, Japan; CBI, Aohua Photoelectricity, Shanghai, China) and digital chromoendoscopy (i-scan, Pentax, Tokyo, Japan; FICE, Fujifilm, Tokyo, Japan; SPIES, Karl Storz, Tuttlingen, Germany). Optical DLC utilizes optical filters within the light source of the endoscope to narrow the bandwidth of spectral transmittance, thereby enhancing and facilitating the visualization of blood vessels. In contrast, digital DLC uses digital postprocessing algorithms that reconstruct the endoscopic image from the video processor in real time, thereby resulting in an improved contrast of the mucosal capillary pattern and an enhancement of the mucosal surface pattern morphology^[25,46].

Importantly, both optical and digital DLC are simple "push-of-a-button" techniques that are readily available during the endoscopic examination. Thus, compared to traditionally used dye-based chromoendoscopy, DLC theoretically offers the advantage of dye-enhanced mucosal imaging without the efforts in time and costs of applying contrast agents during the endoscopic examination. Further, data derived from the *in vivo* assessment of colorectal polyp histology impressively demonstrated that DLC can be readily learned even by "non-expert" endoscopists^[49-51]. Hence, endoscopists with varying levels of experience can accurately use digital chromoendoscopy after a single training session^[52,53] with comparable diagnostic accuracies between non-expert and expert endoscopists^[54].

In one of the earliest prospective randomized trials on the relevance of DBC for the assessment

of mucosal inflammation and dysplasia in ulcerative colitis, Kiesslich and co-workers directly compared DBC and conventional colonoscopy in a large cohort of UC patients. Importantly, DBC with methylene blue not only permitted a more accurate diagnosis of the extent and severity of the inflammatory activity in UC compared with conventional colonoscopy, but also significantly improved the early detection of intraepithelial neoplasia and CAC^[24]. Another "back-to-back" study evaluated pancolonial indigo carmine staining 0.1% for the detection of UC-associated dysplasia^[23]. As shown in this study, DBC with indigo carmine led to a higher dysplasia detection rate while at the same time reducing the total amount of biopsies^[23]. Consistent with these results, another prospective trial also included patients with Crohn's colitis (CC), and similarly, in both UC and CC, targeted biopsies after dye spraying (methylene blue) detected significantly more dysplastic lesions than random biopsies^[55]. Those results have been summarized and quantified in a recent meta-analysis of six randomized controlled trials, verifying that dye-based chromoendoscopy has a medium to high sensitivity and a high diagnostic accuracy for detection of dysplastic lesions in UC^[56]. In its totality, this profound evidence on the superiority of DBC for the detection of colitis-associated neoplasia, together with the knowledge of a cumulative colorectal cancer (CRC) risk in UC patients of 18% after 30 years of disease^[10], have led to the recommendation to perform chromoendoscopy with targeted biopsies as the surveillance procedure of choice in IBD patients in US and European guidelines^[14,15,47,48]. Furthermore, international multidisciplinary unifying consensus guidelines, the so-called SCENIC (Surveillance for Colorectal Endoscopic Neoplasia Detection and Management in Inflammatory Bowel Disease Patients: International Consensus Recommendations) recommendations, have been launched just recently. In these guidelines, performance of pancolonial chromoendoscopy with indigo carmine or methylene blue is the modality of choice for IBD surveillance, and also high-definition colonoscopy is preferred over standard white-light colonoscopy^[57,58].

While the superiority of DBC for dysplasia detection during IBD surveillance is undeniable, DBC is in general a time- and cost-consuming procedure, both factors working against its routine implementation into daily routine clinical practice. In terms of costs associated with DBC, a recent study evaluated whether DBC is cost-effective for colorectal cancer surveillance in UC patients and found that DBC with targeted biopsies is not only more effective but also less costly compared to conventional white-light endoscopy with four quadrant random biopsies taken every 10 cm^[59]. Thereby, from this study it appears that the cost-savings associated with targeted biopsies outcompetes the costs associated with increased time and materials required for DBC and targeted biopsies. In terms of additional time requirements, it has been shown that, even in expert hands, pancolonial

dye spraying requires 4 to 10 min additional time, resulting in a 30%-40% increase in total procedure duration^[60,61]. Finally, DBC has some other potential limitations other than increase in costs and efforts: the dye does not coat the mucosa evenly and dye pooling can lead to difficult observation and interpretation. In addition, DBC cannot provide a detailed evaluation of the mucosal vascular pattern morphology, which is a critical component in the diagnosis of disease activity and dysplasia detection in IBD.

In an attempt to overcome these potential limitations and hurdles associated with the use of DBC, dye-less chromoendoscopy techniques have rapidly evolved in the recent past.

The first description of optical dye-less chromoendoscopy (DLC; *i.e.*, NBI) for identification of colitis associated neoplasia was published in 2006. In this report on a 63-year-old man with longstanding ulcerative colitis and a previous history of dysplasia associated lesions or masses (DALM), it was shown for the first time that visualization of the pit pattern and the mucosal vascular pattern intensity by NBI might help in DALM detection and to discriminate dysplastic from non-dysplastic areas in ulcerative colitis^[62]. As shown in this report, especially the capillary vasculature exhibited a higher vascular pattern and appeared darker on NBI in dysplastic lesions compared to adjacent normal mucosa^[62]. Since then, various trials have studied the potential of NBI to assess the degree of mucosal inflammation and colitis associated preneoplastic and neoplastic changes. In one of the earliest prospective randomized trials, the accuracy of NBI for the detection of neoplasia in patients with longstanding ulcerative colitis was compared against standard colonoscopy^[63]. In general, more suspicious lesions were found with NBI, however the sensitivity of NBI for neoplasia detection was similar to conventional white-light endoscopy^[63]. Soon thereafter, the same group assessed the value of NBI for surveillance in UC in two other studies^[64,65]. In these studies, pit pattern analysis of neoplastic lesions exhibited only a moderate accuracy for the prediction of histology^[64]. Compared to high-definition endoscopy, NBI did not improve the detection of UC associated neoplastic lesions^[65]. Subsequently, trials comparing NBI against dye-based chromoendoscopy for the detection of colonic dysplasia in IBD found no significant differences between both techniques for dysplasia detection^[66,67].

Consistent with this, a recent meta-analysis of four studies comparing dye-based chromoendoscopy and NBI found a non-significant lower dysplasia detection rate with NBI^[57,58]. The result of this meta-analysis indicates that a meaningful benefit of NBI over chromoendoscopy is unlikely. However, at the same time the study also did not document a benefit of chromoendoscopy over NBI and clearly, higher powered studies are needed to address this question^[68,69]. Based on the above mentioned evidence, NBI is, at the moment, not recommended

to replace dye-based chromoendoscopy for cancer surveillance in IBD patients^[57,58].

Data on the relevance of digital chromoendoscopy for IBD surveillance are scarce to date. First evidence that digital chromoendoscopy with FICE might be superior to white-light colonoscopy with standard definition for the detection of dysplastic lesions in UC comes from a prospective, randomized trial that was just recently presented at the ECCO 2015 meeting^[70].

The role of dye-less chromoendoscopy to assess mucosal inflammation associated with IBD has also been studied. In one of the earliest reports, Kudo and colleagues analyzed the mucosal vascular pattern (MVP) in patients with asymptomatic or mildly active UC using NBI and HD white-light endoscopy^[71]. The authors found that areas with obscure MVP on NBI exhibit increased numbers of acute inflammatory cell infiltrates, goblet cell depletion and basal plasmacytosis and that evaluation of the MVP with NBI yielded a more precise determination of acute microscopic inflammation in patients with quiescent UC^[71]. The typical appearance of active UC and inactive, quiescent disease on NBI have been summarized by the same group of authors^[72]. In addition to that, another pilot study on 14 IBD patients was able to demonstrate that areas that appear normal on WLE, but positive on NBI (as defined by a stronger capillary vascular pattern), exhibit an increased leukocyte infiltrate and a significantly increased microvessel density on immunohistology, thus providing first evidence that NBI might allow *in vivo* imaging of intestinal angiogenesis in IBD patients^[73].

Data on the relevance of digital DLC for the assessment of mucosal inflammation in IBD patients are limited. To date, only one study evaluated FICE in IBD patients and showed that FICE is not helpful to improve the detection or delineation of ulcers and erosions in CD^[74]. Just recently, a study on 78 IBD patients that were randomized to receive either HD white-light endoscopy or HD endoscopy with i-scan, was able to demonstrate that i-scan allows a considerably improved prediction of disease extent and disease activity compared to white-light endoscopy (i-scan: 92% and 90% vs WLE: 49% and 54%)^[75]. Of note, examination time was not different between WLE and i-scan, consistent with the idea that dye-less chromoendoscopy is a push-of-a-button technology that can be readily incorporated into the existing examination^[75]. Although no studies have directly assessed the relevance of digital chromoendoscopy for the detection of colitis-associated neoplasia and cancer, it has been shown that HD endoscopy with i-scan can significantly detect more neoplastic lesions and more flat adenomas than standard resolution endoscopy^[76] and is as precise as dye-based chromoendoscopy for the characterization of small colorectal lesions in non-IBD patients^[77]. Based on these results, data on the assessment of colitis associated dysplasia by digital DLC are eagerly awaited.

Table 1 Technical characteristics of probe based and integrated integrated confocal laser endomicroscopy devices

	Endoscope based CLE		Probe based CLE		
	iCLE	GastroFlex	GastroFlex ^{UHD}	ColoFlex	ColoFlex ^{UHD}
Image-plane depth (μm)	0-250	70-130	55-65	70-130	55-65
Lateral resolution (μm)	0.7	3.5	1	3.5	1
Field-of-view (μm)	475 × 475	600 × 600	240 × 240	600 × 600	240 × 240
Frames per second	0.8-1.6	12	12	12	12
Magnification	1000-fold	1000-fold	1000-fold	1000-fold	1000-fold
Required operating channel (mm)		≥ 2.8	≥ 2.8	≥ 2.8	≥ 2.8
Length (cm)	120 and 180	300	300	400	400

iCLE: Integrated confocal laser endomicroscopy; UHD: Ultra-high definition.

CONFOCAL LASER ENDOMICROSCOPY

Confocal laser endomicroscopy (CLE) can visualize structures at the (sub)cellular level^[78], and since its introduction in 2003 CLE has emerged as a technique that can be utilized for precise histologic real time *in vivo* imaging of various diseases^[79-83]. Technically, after topical (acriflavine hydrochloride, cresyl violet) or systemic (fluorescein sodium) administration of contrast agents, CLE emits a low power blue laser light onto the tissue, which is then reflected from the tissue and refocused on the detection system by the same lens, leading to microscopic imaging at 1000-fold magnification in real time^[81]. Currently, two different FDA-approved and CE-certified CLE devices are available^[84]: (1) a probe based CLE system that can be advanced through the accessory channel of a standard endoscope (pCLE, Cellvizio, Mauna Kea Technologies, Paris, France); and (2) an integrated device where the CLE probe is integrated into the distal end of a high-resolution endoscope ("integrated", iCLE; Pentax, Tokyo, Japan). Both pCLE and iCLE emit blue laser light with wavelength of 488 nm, and light reflection from the tissue is detected at wavelengths between 205 and 585 nm. The iCLE-system acquires images at a manually adjustable scan rate of 1.6 frames/s with a resolution of 1024 × 512 pixels, or at 0.8 frames per second with a resolution of 1024 × 1024 pixels. The depth of scanning and the laser power can be adjusted from 0 to 250 μm and from 0 and 1000 μW, respectively. The optical slice thickness is 7 μm, with lateral and axial resolution of 0.7 μm and a confocal image field of view of 475 μm × 475 μm.

The pCLE system utilizes separately available confocal probes, and specific probes for different indications throughout the entire gastrointestinal tract are available. Probe-based CLE utilizes a fixed laser power and a fixed imaging plane depth for image acquisition. Lateral resolution ranges between 3.5 μmol/L and 1 μmol/L, resulting in a field of view of 600 μmol/L-240 μmol/L, depending on the confocal probe used. Images are acquired at 12 frames/s, leading to real-time videos. Single video frames either in real time or post processed with an increased field of view (4 mm × 2 mm) can be reconstructed using

a special computer algorithm (Mosaicing, Mauna Kea Technologies, Paris, France). Probe based CLE in IBD is mostly being performed by using the ColoFlex UHD probe, which requires a 2.8 mm working channel. Hence, these probes can be advanced through the working channel of most endoscopes used in clinical practice.

Both CLE-systems offer unique advantages and specifications and their utilization depends on the clinical scenario. Advantages of iCLE are its higher resolution, the possibility to alter the laser power and imaging plane depth and it also allows to simultaneously take biopsies and thus to directly compare confocal imaging with histopathological results. The major advantages of the pCLE system are its *ad hoc* usage in existing endoscopes and the possibility to perform real time video recording.

Technical characteristics of the iCLE systems and different CLE probes are summarized in Table 1.

The utilization of confocal endomicroscopy and the interpretation of images in IBD patients require only a short learning curve. In this regard, data from a prospective evaluation demonstrated that after the initial three examinations, performance parameters of CLE imaging improved with a significant decrease in the overall CLE procedure time and an increase in images in focus acquired. Further, agreement between CLE and histopathology improved over time with kappa values of 0.81 after twenty-six cases. Thus, CLE is a procedure that can be readily learned in a short time frame and that can be successfully applied in IBD patients^[85].

In one of the first *in vivo* randomized trials on the relevance of CLE for UC surveillance, it was shown that by using chromoendoscopy (with methylene blue) together with endomicroscopy, 4.75-fold more neoplasias could be detected than with conventional colonoscopy, although 50% fewer biopsies were required and CLE exhibited an excellent diagnostic accuracy for the prediction of neoplastic changes^[86]. In addition, using the modified Mainz confocal criteria for the *in vivo* diagnosis of adenoma-like mass (ALM) and DALM in a pilot study on 16 UC patients, it has also been shown that CLE can accurately differentiate between DALM and ALM with an almost perfect

kappa coefficient of agreement between CLE and histopathologic evaluation^[87]. This is important since such an approach can significantly facilitate the clinical decision whether patients should receive endoluminal endoscopic resection or be rather referred for proctocolectomy^[87]. Importantly, these studies utilized the integrated CLE system (iCLE) and subsequently, another pilot study on 22 UC patients demonstrated that surveillance in UC is also feasible with the stand-alone CLE probe (pCLE) with reasonable diagnostic accuracy of the pCLE system for dysplasia detection^[88]. These results were also confirmed by a recent meta-analysis that included a total of fifteen studies involving 719 patients with either sporadic polyps or IBD. In this work, it has been calculated that CLE can distinguish neoplasms from non-neoplastic tissue in IBD patients with a sensitivity of 83% and specificity of 90%, thereby confirming that CLE can indeed differentiate between neoplastic and non-neoplastic tissue^[89]. Morphologically, dysplasia on CLE is in general characterized by dark cells with crypt density attenuation, a ridged-lined irregular epithelial layer with loss of crypts and dilated and distorted vessels with elevated leakage and irregular vascular architecture. In contrast, inflamed mucosa is in general characterized by dilation of crypt openings, enlarged spaces between crypt, and microvascular alterations with fluorescein leaks into the crypt lumen therefore making the lumen brighter than the surrounding epithelium^[78,90].

Apart from dysplasia detection, several reports have assessed the value of CLE for the real-time *in vivo* assessment of the histologic degree and the extent of mucosal inflammation associated with IBD. One of the earliest pilot studies evaluated the morphologic differences of the colonic mucosa between active and inactive UC. The crypts in UC patients with non-active disease were small, round and slightly irregular in arrangement with a small and round crypt lumen. In contrast, colonic crypts in active UC were large, variously shaped and irregular in arrangement whereas colonic crypts in active UC appeared large, variously shaped, irregularly arranged with numerous inflammatory cells and capillaries in the lamina propria^[91].

These results were also confirmed by several other studies. A study by Li and colleagues on 73 UC patients assessed the inflammatory activity by CLE with a 4-grade CLE classification system of crypt architecture, as well as by analysis of microvascular alterations and fluorescein leakage, demonstrating that all three parameters (crypt architecture, fluorescein leakage, microvasculature) showed good correlations with histopathology^[92]. Of note, 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 seen on CLE showed acute inflammation on histology^[92]. Another recently published study by the same group showed that CLE can be used

to predict disease relapse in UC. In this report, UC patients with macroscopic normal mucosa on WLE, but endomicroscopic signs of active inflammation (as assessed by analysis of the crypt architecture with CLE), exhibited a significantly higher relapse rate than patients with non-active disease on CLE^[93]. Finally, Buda *et al*^[94] have further confirmed that mucosal changes detected *in vivo* by CLE in remittent UC patients can predict disease relapse.

Results from our own group demonstrate that CLE can also reliably assess Crohn's disease activity and also be utilized to differentiate Crohn's disease from ulcerative colitis^[95,96]. In a study on 54 patients with Crohn's disease, we were able to show that a significantly higher proportion of patients with active CD had increased colonic crypt tortuosity, enlarged crypt lumen, microerosions, augmented vascularization, and increased cellular infiltrates within the lamina propria while, in quiescent CD, a significant increase in crypt and goblet cell number was detected compared with controls^[95] and based on these findings, the Crohn's Disease Endomicroscopic Activity Score (CDEAS) for the assessment of CD activity *in vivo* was proposed. The CDEAS does not only allow differentiating between quiescent CD and controls but also between quiescent and active disease and showed strong correlation to serum levels of the C-reactive protein^[95]. In a recently published prospective trial on 79 IBD patients we were able to show that patients with CD showed significantly more discontinuous inflammation, more focal cryptitis and more discontinuous crypt architectural abnormality on CLE than patients with ulcerative colitis. Conversely, ulcerative colitis was associated with severe, widespread crypt distortion, decreased crypt density and irregular surface and based on these findings, the so-called IDEA (IBD Differentiation based on Endomicroscopic Assessment) scoring system was developed exhibiting an excellent diagnostic accuracy when compared with the historical clinical diagnosis and histopathology^[96].

Hence, the CLE based scoring systems such as the CDEAS or the IDEA appear as precise tools for the accurate prediction of disease severity in CD patients and for the differential diagnosis between CD and UC^[95,96]. However, validation trials are highly warranted to proof the early results.

Another important feature for the endomicroscopic evaluation of inflammatory activity by CLE are the so-called epithelial gaps^[97]. Epithelial gaps, originally discovered in the terminal ileum and rectum of patients undergoing surveillance colonoscopy, have a diameter of an individual epithelial cell (10 μ m) and pathomorphologically result from shedding of epithelial cells. In this context it has been shown that in IL-10 deficient mice, as a murine model of inflammatory bowel diseases, as well as patients with CD exhibited a significantly higher epithelial gap density compared to controls^[98]. A study including 21 CD and 20 UC

patients with a median follow-up of 14 mo provided first evidence that assessment of epithelial gaps by CLE has important clinical relevance. In this report, patients with elevated gap density were at significantly higher risk for hospitalization or surgery^[99]. Further, patients with elevated gap density were at increased risk for hospitalization and surgery and gap density was a significant predictor for risk of major events, with a hazard ratio of 1.10 associated with each increase of 1% in gap density. Additionally, gap density also correlated with IBD disease duration^[99]. These results are corroborated by another trial on 47 UC and 11 CD patients in clinical and mucosal remission in which increased cell shedding with fluorescein leakage was associated with subsequent relapse within 12 mo after endomicroscopic examination. Further, endomicroscopic grading of the local barrier dysfunction by the so called Watson grade exhibited a very high specificity and good overall accuracy for predicting disease flares^[100].

The role of CLE for the assessment of mucosal inflammation, for the prediction of therapeutic response and for cancer surveillance in IBD has recently been summarized in a systemic review^[101]. In their totality, the above discussed studies demonstrate that CLE can be used to reliably assess the macro- and microscopic inflammatory activity in IBD patients and to obtain optical biopsies in real-time. Since the precise determination of mucosal inflammation is one of the most critical components for defining and achieving mucosal healing, which itself has been identified as a key prognostic parameter and important treatment goal in IBD patients^[7], it is likely that CLE will become more important in the foreseeable future not only to facilitate and optimize the management and surveillance of IBD patients but also to prospectively identify patients that are under risk of experiencing a disease flare.

ENDOCYTOSCOPY

Endocytoscopy (EC; Olympus, Tokyo, Japan) is an advanced imaging technology that uses contact light microscopy with a fixed-focus, high-power objective lens to allow *in vivo* microscopic imaging of the GI tract with up to 1390-fold magnification^[102-104]. The depth of field of EC ranges from 0 to 50 μm , thereby only the very superficial mucosal layer can be visualized. As a prerequisite before image acquisition, EC requires thorough mucolysis (*e.g.*, with *N-acetyl-cysteine*) and staining of the mucosa with an absorptive agent (*e.g.*, methylene blue, toluidine blue, cresyl violet). Data from *ex vivo* studies suggest that optimum conditions for endocytoscopic observation can be obtained after staining with 0.25% toluidine blue in the colon, after 60 s of exposure to the dye^[105], but a combination of different dye agents might be required for optimal tissue contrast^[106].

Similar to CLE, a probe based endocytoscopy system (pEC) as well as an integrated endocytoscopy

(iEC) device are available^[103]. The integrated devices use two different lenses and are integrated into endoscopes for the upper (103 cm in length) and lower (133 cm in length) GI tract. iEC provides a 580x-fold image magnification and also allows conventional optical magnification with narrow band imaging capabilities. Recently, another integrated endocytoscopy system (GIF-Y0002) was introduced which contains only one lens that allows to continuously increase the zoom from the conventional endoscopy level up to 380-fold (tissue field of view, 700 mm \times 600 mm) using a hand lever. Using digital magnification (\times 1.6) in addition, the magnifying power can be increased to 600-fold, providing a tissue field of view measuring 440 mm \times 380 mm^[104,107]. With these characteristics, this new endoscope-generation allows continuous magnification from standard overview to the (sub)cellular level.

For the pEC device, two different probes are available, providing either 450-fold (XEC 300F) or 1390-fold (XEC 120 U) magnification^[103,104]. The horizontal observation field is 300 μm \times 300 μm (0.09 mm²) for the 450-fold magnification probe and 120 μm \times 120 μm for the 1390-fold magnification probe and both probes require an accessory channel of 3.7 mm.

Compared with CLE, less data on the assessment of mucosal inflammation in IBD are available for EC. In an initial report on 55 UC patients, a newly introduced endocytoscopy scoring system (ECSS) was compared with Matt's histopathological grading to evaluate the degree of inflammation. As shown in this report, assessing the shape and distance between crypts as well as the visibility of superficial microvessels with a 450-fold magnification showed a strong correlation with Matts' histopathological grading^[108] and further, the ECSS showed a high reproducibility between different investigators^[108]. Recently, our group tackled the issue whether EC can be used not only for the determination of mucosal inflammation, but also for the identification and visualization of single inflammatory cells. For this purpose, we used the probe-based EC system with 1390-fold magnification^[109]. In this report, we were able to demonstrate that EC is able to visualize not only different cellular structures within the intestinal mucosa such as size, arrangement, and density of cells but also ultrastructural patterns such as size and shape of nuclei and the nucleus-to-cytoplasm ratio^[109]. With these features, EC allowed to reliably distinguish single inflammatory cells, namely neutrophilic, basophilic and eosinophilic granulocytes, and lymphocytes^[109]. Further, concordance between endocytoscopy and standard histopathologic grading of disease activity was 100% and EC exhibited a substantial interobserver and almost perfect intraobserver agreement^[109].

The detection of colitis-associated neoplasia or cancer with EC has not been studied to date. However, first evidence suggests that EC can identify dysplasia in aberrant crypt foci as the earliest precursor lesions of colorectal cancer in the dysplasia-

carcinoma sequence^[110] and in colonic polyps, EC is capable to even detect and distinguish focal high-grade intraepithelial neoplasia^[111]. Potential clinical applications of endocytoscopy in the upper and lower GI tract as well as at other mucosal surfaces have been recently reviewed^[104].

MOLECULAR IMAGING

One limitation of the above discussed techniques is that, although they allow precise visualization of cellular structures, they are dependent on the presence of morphologic changes to detect pathology. In the recent past, techniques such as CLE have been combined with the utilization of fluorophores with specificity towards a defined molecular target, thereby enabling the visualization and quantification of biochemical structures or processes on the molecular level in real-time during ongoing endoscopy. This novel field of gastrointestinal endoscopy is referred to as "molecular imaging" or "*in vivo* immunohistochemistry" and is now a rapidly evolving field in gastroenterology. Since molecular imaging is based on the utilization of exogenous probes, criteria for the ideal probes utilized for molecular imaging in the gastrointestinal tract have been defined. As such, ideal probes for labeling molecular structures should exhibit a high diversity, high affinity binding, rapid binding kinetics within minutes, adequate tissue penetration, low immunogenicity, ability for large scale synthesis and fluorescent labelling^[112]. These criteria can be fulfilled by different agents and so far the following substance classes have been successfully utilized for molecular imaging: antibodies, lectins, affinity peptides, activatable probes, nanoparticles and physiological substances^[113-115].

To date, mucosal inflammation as well as cancer development within the GI tract has been successfully studied with molecular imaging, in both, mice and humans. Just recently, Mitsunaga and co-workers utilized a topically applied enzymatically activatable probe (*gGlu-HMRG*), which exhibits a fluorescent signal in the presence of the enzyme γ -glutamyltranspeptidase (GGT) to visualize cancer development by fluorescence colonoscopy in the AOM/DSS mouse model of colitis-associated cancer^[116]. Fluorescent lesions were detected 5 min after topical administration of *gGlu-HMRG*, even in small lesions, and fluorescence signal persisted for at least 30 min. Fluorescence guided biopsy revealed that all fluorescent lesions contained cancer or dysplasia and although microscopic inflammatory infiltration also had variable fluorescence, these signals were general approximately 10-fold lower than the cancerous tissue^[116]. Consistent with these observations, other studies utilized protease-sensing probes, such as a cathepsin reporter probes^[117,118], MMP-activatable probes^[119], GGT substrates^[116,120], or certain peptides^[121,122] to detect and visualize intestinal dysplasia and polyps in murine and xenograft models.

Furthermore, molecular targets that are upregulated from colorectal cancer cells and which are already established therapeutic targets, such as epidermal growth factor receptor (EGFR) or vascular endothelial growth factor receptor (VEGFR), have been utilized as fluorescent probes for the visualization and precise discrimination of cancer tissue^[123-125].

Just recently, a first prospective human study *in vivo* on the molecular labelling of EGFR was conducted in 37 patients with CRC^[126]. In this study, an Alexa 488 conjugate-labeled mouse anti-hEGFR antibody was topically applied *via* a spraying catheter and subsequently, confocal imaging was performed. An EGFR-specific fluorescence signal was present in 18 out of 19 patients with CRC and 12 out of 18 patients with intestinal adenomas while normal mucosa exhibited no or only weak fluorescence^[126]. Importantly, no side effects were observed during *in vivo* CLE imaging and at the 4-6 wk follow-up. Further, no human-anti mouse antibodies were detected serologically, although this aspect was assessed in serum samples of only 4 patients^[126].

A potential clinical relevance of the visualization of EGFR during ongoing endoscopy has been portrayed out in a recent study in which colon cancer xenografts were induced by transplanting either cetuximab-sensitive (HT29) or cetuximab-resistant (SW620) human CRC cell lines into nude mice. CLE was performed 48 h after injection of a fluorescently labelled cetuximab test dose and the initial fluorescence intensity was examined in relation to clinical readouts such as tumor growth, thriving and mortality. The initial fluorescence signal was significantly stronger in cetuximab-treated HT29 tumors than in HT29 controls or cetuximab-treated SW620 tumors and accorded with significantly slower tumor progression, better overall survival and better physical condition. Further, cetuximab sensitivity could be predicted from fluorescence intensity at day 0 with a high positive predictive value. Importantly, molecular imaging was for the first time linked to an early prediction of response to targeted therapy in models of human CRC. Therefore, these results may indicate a promising principle for early patient stratification.

These findings were directly translated into clinical applications and one first clinical trial has already proven that the visualization of molecular targets can be used to make a risk stratification of IBD patients prior to the initiation of treatment into responders and non-responders, thereby allowing a prediction on the therapeutic success. In this landmark phase 1 clinical trial, a FITC (fluorescein isothiocyanate) labeled anti-TNF antibody was manufactured under GMP conform conditions and topically applied during endoscopy to the inflamed mucosa of IBD patients that were naïve to anti-TNF antibody treatment. Subsequently, the amount of intestinal mTNF+ cells was quantified *via* CLE^[127]. Importantly, patients with high numbers of mTNF+ cells showed significantly

higher short-term response rates (92%) at week 12 upon subsequent anti-TNF therapy as compared to patients with low amounts of mTNF(+) cells (15%), despite comparable severity of mucosal inflammation in both patient groups. This clinical response in patients with high amounts of intestinal mTNF+ cells was sustained over a follow-up period of 1 year and was associated with mucosal healing observed at follow-up endoscopy^[127].

These data were the first to provide direct evidence that molecular imaging with fluorescent antibodies and CLE can be utilized to predict therapeutic responses to biological treatment in Crohn's disease prior to the initiation of therapy and thus might be used for an *a priori* risk stratification according to their treatment response. The establishment of such a personalized medicine approach and its integration into clinical routine and patient care would have a tremendous impact since it would allow avoiding unnecessary risk exposure associated with biological therapies and lead to a considerable economization of the treatment regimens at the same time.

CONCLUSION

In recent years, emerging strategies for the management of patients with IBD have radically changed the role of advanced endoscopic imaging techniques in both, research trials and clinical practice. Image-enhanced technologies including high-definition, optical magnification, and chromoendoscopy can remarkably refine the characterization of mucosal inflammation and clearly have the potential to improve the detection of non-polypoid dysplastic lesions in daily clinical practice. In addition, recent developments in optical biopsy techniques such as endocytoscopy and confocal laser endomicroscopy have made available the microscopic assessment of mucosal changes in real time during ongoing endoscopy, thereby predicting several histologic features with a high level of accuracy. The path traced by rapidly evolving molecular imaging technologies promises to transcend the known spectrum of plain morphological assessment of conventional luminal gastrointestinal tract to visualization of molecular structures and pathways in IBD patients that allow individual predictions about therapeutic responses.

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