ENDOSCOPIC OPTICAL ENHANCEMENT TECHNOLOGIES IN IBD

Gian Eugenio Tontini,[1,2] Maurizio Vecchi,[2,3] Markus F. Neurath,[1] Helmut Neumann[1]

1. Department of Medicine I, University of Erlangen-Nuremberg, Germany
2. Gastroenterology and Digestive Endoscopy Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy
3. Department of Biomedical Science for Health, University of Milan, Italy

ABSTRACT

Optical enhancement technologies are emerging as promising tools to improve diagnosis and clinical management of patients with inflammatory bowel diseases (IBD). The use of dye-based and dye-less chromoendoscopy may improve either characterisation of mucosal inflammation or detection of dysplastic and early neoplastic lesions. Confocal laser endomicroscopy and endocytoscopy both allow for *in vivo* and real-time microscopic analysis of the tissue. Moreover, the newly introduced molecular imaging has now also become feasible for *in vivo* diagnosis in IBD. This review focuses on the more recent progresses of advanced endoscopic imaging techniques in the setting of IBD and provides the reader with an updated overview on accepted clinical evidence and ongoing fields of research.

Keywords: Inflammatory bowel disease, Crohn's disease, ulcerative colitis, colonoscopy, advanced endoscopic imaging, dye-based chromoendoscopy, dye-less chromoendoscopy, confocal laser endomicroscopy, molecular imaging, endocytoscopy, surveillance, colorectal cancer.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), the two major entities of inflammatory bowel disease (IBD), are gastrointestinal chronic disorders that affect more than 1 million people in the United States and several million worldwide.[1,2] The intestinal mucosal layer is the main target of such disorders and represents the environment where exogenous and host related factors mould the immunological background that bears IBD pathogenesis. Accordingly, endoscopic imaging has a pivotal role for both diagnostic and therapeutic issues in patients with IBD. Indeed, the differential diagnosis among IBD entities and other gastrointestinal disorders is based on clinical evaluation and the combination of endoscopic, histological, radiologic, and biochemical results.[3-9] Moreover, patients with either CD or UC are at an increased risk of malignancies as severity, extent, and standing of chronic inflammation are recognised as the major risk factors of colitis-associated cancer (CAC).[10-12] Therefore, national and international guidelines strongly recommend colonoscopic surveillance protocols starting 8-10 years after the onset of symptoms, and every 1 to 2 years after that in extensive colitis.[5,13-16] This strategy is aimed at early detection of non-polypoid and early dysplastic lesions, which are the most reliable biomarker of concomitant or impending malignancy.[17,18] In 2005, an international consensus conference agreed that a minimum of 32 biopsies should be performed at each surveillance colonoscopy by obtaining four-quadrant biopsies every 10 cm separately retrieved, plus targeted sampling of macroscopically suspicious lesions.[16] However, this approach has raised several concerns as it failed to show concrete cost-effectiveness and to cut down the risk of overlooked neoplastic lesions.[11,18-27]

Consistently, growing efforts have been made to improve the efficacy of advanced endoscopic imaging techniques during endoscopic surveillance protocols.[26-33] In addition, emerging evidence has raised increasing attention to a new clinical topic, namely mucosal healing. This includes the precise staging of disease extent and activity, and the mucosal early response to biological therapy.[34,35] In this context, advanced endoscopic imaging techniques could refine our traditional approach of diagnosis in patients with IBD and may become the crucial diagnostic test for more disease-specific and patient-centred clinical strategies.[36,37] This review describes the concept of advanced endoscopic imaging for diagnosis and characterisation of patients with IBD, focusing on the newly introduced optical enhancement technologies.

CHROMOENDOSCOPY

Chromoendoscopy uses different staining techniques to enhance the mucosal detail and submucosal vascular pattern, thereby improving the detection of pathological lesions and enabling a more precise diagnosis.[28,36-38] Currently, chromoendoscopy is distinguished in dye-based and dye-less imaging techniques.

Dye-Based Chromoendoscopy

Dye-based chromoendoscopy (DBC) refers to topical application of dyes at the time of endoscopy in an effort to enhance tissue characterisation, differentiation, and diagnosis.[39] Dye spraying techniques were first described in the 1970s[40] and include absorptive agents (e.g. Lugol's solution, methylene blue, toluidine blue, and cresyl violet), contrast agents (e.g. indigo carmine and acetic acid), and reactive staining agents (e.g. congo red and phenol red).[28,41] DBC may allow for an improved diagnosis of disease severity and extent in subjects with IBD. Nevertheless, DBC has been implemented in clinical practice specially to improve detection of dysplastic lesions in long-standing IBD colitis.[21,42-47] In this context it has been estimated that methylene blue-aided chromoendoscopy yields a 2.2-fold increased dysplasia detection rate, particularly due to the enhanced detection of non-polypoid lesions.[21,47,48] Similar results have also been shown for indigo carmine-aided chromoendoscopy;[43,46] moreover, indigo carmine has no oxidative damage on DNA chains, thereby

theoretically avoiding the potential carcinogenic effect ascribed to the prolonged use of methylene-blue under white-light scanning.[49-52] A meta-analysis of six randomised controlled trials demonstrated a pooled sensitivity, specificity and diagnostic odds ratio of 83.3% (95%-CI=35.9-99.6%), 91.3% (95%-CI=43.8-100%), and 17.5% (95%-CI=1.2-247.1), respectively, for dysplasia detection in long-standing UC by using DBC compared with white-light endoscopy.[53] Accordingly, international guidelines have included the use of DBC in highly specialised centres to improve IBD surveillance protocols [4,13-16]. Potential limitations that could hamper the use of DBC in clinical practice include additional costs for the dye and the spraying catheter, operator training, and a non-uniform distribution on the mucosal surface.[28,33-37] Furthermore, there is no dye that provides a detailed evaluation of the mucosal vascular pattern (MVP), which is nowadays emerging as an important parameter for neoplasia detection and assessment of disease activity. [37]

Dye-Less Chromoendoscopy

Recently, dye-less chromoendoscopy (DLC) techniques have been implemented into daily routine practice to overcome the above-mentioned limitations of DBC. By pushing a button on the handle of the endoscope, these integrated endoscopic systems enable a detailed examination of both the mucosal surface and the MVP morphology, thereby providing high-contrast imaging in real-time and without the use of additional equipment. DLC encompasses optical chromoendoscopy and digital chromoendoscopy techniques.[37]

Optical chromoendoscopy techniques (Narrow Band Imaging or NBI, Olympus, Tokyo, Japan and Compound Band Imaging or CBI, Aohua, Shanghai, China) are based on optical lenses integrated within the light source of the endoscope, which narrow the bandwidth of spectral transmittance.[54] In contrast, digital chromoendoscopy (Fujinon Intelligent Color Enhancement or FICE, Fujifilm, Tokyo, Japan and i-scan, Pentax, Tokyo, Japan) rests on a digital post-processing of endoscopic images made in real-time by the video processor.[37]

As formerly discussed, detection of colorectal dysplasia in IBD is of paramount importance, being the most reliable biomarker of CAC.[17,18]

Consistently, several studies have recently addressed the potential of DLC, particularly NBI, in improving the accuracy and effectiveness of current surveillance programs in IBD. In a crossover randomised trial in which patients with UC underwent both NBI and high-definition (HD) white-light colonoscopy, van den Broek and co-workers[55] have found that NBI does not improve the detection of neoplastic lesions. In addition, NBI proved the suboptimal accuracy (73%) for differentiating neoplastic from non-neoplastic mucosa.[55] A further study by the same group confirmed that NBI has only a moderate accuracy for the prediction of histology (80%).[56]

Another prospective, randomised, crossover trial compared NBI to DBC with indigo carmine in 60 clinically inactive IBD patients 8 years after the onset of symptoms.[57] NBI detected significantly less false-positive biopsies, sparing time and yielding an equivalent true-positive rate. However, DBC scored slightly better than NBI identifying more neoplastic lesions and more neoplastic patients (p=0.2), thereby harbouring some concern about the use of this DLC technique as standard surveillance strategy in IBD.[56] Assessing the characterisation of early colorectal lesions in long-standing UC, Matsumoto et al.[58] combined NBI with magnification colonoscopy in a pilot study based on 46 patients. According to the modified classification for 'magnifying chromoscopic findings' the surface pattern of each lesion was defined as 'honeycomb-like', 'villous' or 'tortuous-like'. Dysplasia was positively correlated with the 'tortuous' pattern, therefore suggesting that NBI and magnified colonoscopy could improve dysplasia detection during surveillance in UC.[58] To the best of our knowledge, there are no studies on the use of digital chromoendoscopy techniques (i.e. i-scan and FICE) for detection and characterisation of intraepithelial neoplasia in IBD.

More recently, DLC techniques have shown promising results for the characterisation of disease extent and activity in patients with mild or inactive IBD.[37,59] Kudo et al.[60] have focused their analysis on MVP comparing HD white-light endoscopy and NBI in UC patients by using histology as the reference standard. NBI was able to better characterise abnormal vessel structures, distinguishing between 'clear' and 'obscure' MVP where HD white-light endoscopy identified only a common 'distorted' MVP. Histopathology revealed that both acute and chronic signs of microscopic inflammation were remarkably correlated with the 'obscure' MVP (p<0.05), while only few signs of chronic inflammation correlated with the 'distorted and clear' MVP.[60] Additional research from the same group confirmed that MVP's analysis with NBI offers the concrete possibility to predict signs of acute microscopic inflammation in patients with quiescent UC.[60-63]

Very recently, our group evaluated the potential of i-scan to improve the characterisation of mucosal inflammation in IBD.[64] During pancolonoscopy, patients were examined using both HD white-light (Group A) and HD plus i-scan (Group B). Agreement between endoscopic prediction of disease severity and histological findings was 54% in group A and 90% in group B (p=0.066). The endoscopic prediction of the inflammatory activity's extent was 49% in group A and 92% in group B (p=0.001) using histology as reference standard, thereby suggesting that i-scan has the potential to improve both diagnosis of severity and extent of mucosal inflammation in patients with IBD. Therefore, this allows for a more precise diagnosis of mucosal inflammation compared to HD colonoscopy alone.[64] Taken together, even if DBC still represents the best choice to improve dysplasia detection in long-standing IBD, optical and digital DLC techniques have the potential to better quantify disease activity and mucosal healing, and currently appear as more practical tools to spread into daily routine clinical practice.

CONFOCAL LASER ENDOMICROSCOPY

Introduced in 2004, confocal laser endomicroscopy (CLE) has rapidly emerged as a promising approach to obtain real-time *in vivo* histology in luminal endoscopy as in several other clinical fields.[65] Briefly, this technique is based on tissue illumination with a blue laser light after topical or systemic application of fluorescence agents. In IBD, various studies have investigated the potential of CLE for disease classification and characterisation.[21,35,66-76]

In 2007, a study from Kiesslich et al.[21] clearly demonstrated that during surveillance of long-standing UC, the use of DBC-aided CLE

could detect 4.75-fold more neoplastic lesions compared with standard white-light endoscopy. In addition, the authors reported a remarkable biopsy sparing and an optimal accuracy (95%) in predicting the presence of neoplastic changes.[21] Consistent with this figure, Hurlstone and co-workers[76] described a high overall accuracy (97%) and excellent agreement with histological results (*kappa*=0.91) when using CLE for differentiation of dysplasia-associated lesion or mass (DALM) from sporadic adenoma (adenoma-like mass; ALM).

Beyond the characterisation of dysplastic changes, confocal imaging could also reveal signs of impaired intestinal barrier function, which is emerging as a crucial step in the pathogenesis of IBD.[68,73,74,77-82] The lining of the intestine undergoes a continuous renewal, resulting in epithelial gaps as a consequence of intestinal cell shedding.[68] A refined process, based on the redistribution of tight junction round the basolateral surface of the shed cell, preserves the barrier function at the gap site.[74] When this physiologic process is impaired, the intestinal barrier become permeable to the inward flow of antigens and microbes from the intestinal lumen into the bowel wall, paving the way to a prompt reaction of the immune system.[78] Accordingly, it has been hypothesised that the rate of epithelial cell shedding is increased in patients with IBD.[68] Recently, Liu et al.[69] have shown that CLE can be used to quantify *in vivo* the epithelial gap density of the terminal ileum during ongoing colonoscopy. They confirmed that epithelial gap density is significantly higher in IBD subjects than in negative controls. Nonetheless, ulcerative pancolitis and severe clinical disease were associated with lower gap densities compared with those observed in IBD with limited colitis and with mild-to-moderate clinical disease, thereby suggesting that gap density does not correlate with disease activity and neither with specific IBD entities.[69]

A further study by Kiesslich and co-workers[73] confirmed that CLE can identify cell shedding and barrier loss at a microscopic level in real-time. Employing a murine model of cell shedding, they also demonstrated that an incomplete sealing at the site of cell shedding ('gap') can result in either outward flow, inward flow or bidirectional flow. This finding supports the hypothesis that outward flow of fluorescein into the intestinal lumen identified by CLE is a marker of loss of barrier function, as it implies the inward flow of antigens, toxins and microbes activating the mucosal immune system. Furthermore, the authors developed a grading system ('Watson grade') based on three CLE signs of barrier function impairment such as cell shedding, fluorescein flow into the intestinal lumen, and microerosions. The 'Watson grade' was shown to predict the relapse of IBD patients in remission within the subsequent 12 months (Watson grade II/III versus grade I: p<0.001), harbouring the use of CLE for on demand *in vivo* prediction of relapse during ongoing endoscopy.[73] Another pilot study based on both CD and UC patients with a median follow-up of 14 months has recently confirmed that gap density in endoscopically normal mucosa of the terminal ileum is a significant predictor for risk of major events such as hospitalisation or surgery.[74]

Moreover, several studies have recently established that CLE allows the characterisation of most microscopic architectural and inflammatory changes, which are conventionally regarded as histopathological hallmarks for the diagnosis of IBD.[9,66-72] In a study published in 2012, our group evaluated the feasibility of CLE for *in vivo* microscopic diagnosis of disease severity in patients with CD.[72] Consistent with histopathological results, CLE showed a sharp distinction between CD and controls based on different rates of the following findings: crypt morphology (number of colonic crypts, crypt tortuosity, crypt lumen), microerosions, vascularity, cellular infiltrate within the lamina propria and number of goblet cells. In addition, CLE was able to differentiate quiescent from active CD showing a high rate of crypt atrophy in the former group, as well as control subjects from quiescent CD, detecting a significant increase in crypt and goblet cell number as hallmarks of chronic inflammation.[72] Similar results were also shown in another study based on the use of CLE in patients suffering from UC.[71] Both assessment of crypt architecture (irregular arrangement, density, dilation, abscess) and fluorescein leakage into the crypt lumen with CLE showed good correlations with histological results (both p<0.001). Moreover, 57% of patients with normal mucosa seen on conventional white-light endoscopy (Baron score=0) showed acute inflammation on histology (Geboes index >3), whereas no patients with normal mucosa or

with chronic inflammation seen on CLE showed acute inflammation on histology.

In recent years, CLE has been integrated with the use of exogenous fluorescently labelled probes to specifically highlight neoplastic and inflammatory changes on the basis of their molecular signature; this novel and promising field in gastroenterology is called 'molecular imaging'.[8] In a pilot study, Atreya and co-workers[35] used CLE-based molecular imaging with monoclonal anti-tumour necrosis factor (TNF) antibodies to evaluate whether the therapeutic responses to Adalimumab correlate with the amount of mucosal membrane TNF receptor in patients with CD. The inflamed mucosa was coated with a newly developed fluorescent anti-TNF antibody (FITC-Adalimumab) during the colonoscopy prior to anti-TNF therapy. Fluorescein expression on a cellular level was quantified by CLE analysis focused on mucosal membrane-bound TNF+ (mTNF+) cells. During a follow-up period of 1 year, patients with a great density of mTNF+ cells showed significantly higher short-term response rates at week 12 (92%) upon subsequent anti-TNF therapy as compared to patients with low amounts of mTNF+ cells (15%). These data indicate for the first time that *in vivo* molecular imaging with fluorescent antibodies is feasible and safe, and could predict therapeutic responses to biological treatment, depicting promising and immediate potential for translational science and prompting effects on clinical practice.[35]

Multiphoton microscopy (MPM) is emerging as one of the most important *in vivo* imaging techniques for basic research. In comparison with the single photon excitation performed by CLE, MPM uses nonlinear optics, so that various molecular components can be discriminated without the need to apply fluorophores. The result is a superior effective resolution in thick tissue samples and an increased penetration depth, with images perfectly suited for the acquisition in 3D.[84] One recent article impressively demonstrated how MPM allows for a well-defined 3D visualisation of pathologic changes in tissue samples from patients with IBD without requiring exogenous fluorophores.[85]

Taken together, CLE appears as a versatile tool capable of enriching the power of endoscopy, predicting *in vivo* both several valuable cues of histology and the response rate to anti-TNF therapy. However, currently CLE is relatively expensive and time-consuming, therefore harbouring potential shortcomings that currently limit its implementation into daily routine clinical practice.

ENDOCYTOSCOPY

Endocytoscopy (EC) is another advanced imaging technique implemented for *in vivo* microscopic imaging at a magnification up to 1390-fold.[86] Based on the principle of contact light microscopy, this technique enables the visualisation of the very superficial mucosal layer at a cellular and subcellular level.[87] EC has recently been evaluated in gastrointestinal endoscopy, particularly to detect neoplastic changes in aberrant crypt foci and to differentiate neoplastic from non-neoplasic colorectal lesions.[88-90] Our group has recently published the results of a pilot study designed to assess the feasibility of EC in distinguishing single inflammatory cells in patients with IBD.[91] It has been observed that EC enables a sharp characterisation of several cellular (cell size, arrangement and density) and subcellular details (size and shape of nuclei and nucleus-to-cytoplasm ratio). Consistently, EC could reliably distinguish different inflammatory cells with the following respective sensitivities and specificities: neutrophilic (60% and 95%), basophilic (74% and 94%), eosinophilic granulocytes (75% and 91%), and lymphocytes (89% and 93%). Furthermore, intestinal disease activity assessed by EC was perfectly in agreement with histopathological results (100%).[91] Taken together, these data seem to nominate EC as a new promising method to characterise and assess the severity of mucosal inflammation in IBD.

CONCLUSION

Recent technological advances in optical imaging and luminal endoscopy are greatly improving the quality of gastrointestinal imaging, enabling microscopic and molecular analysis in real-time during ongoing endoscopy. Converging lines of evidence suggest many promising applications of these optical advanced imaging techniques in several IBD clinical settings. DLC is emerging as a practical method to enhance in real-time mucosal subtle details, thereby potentially improving the detection and characterisation of dysplastic lesions, as well as the accuracy in assessing disease activity and extent. CLE and EC allow

for real-time *in vivo* histology during ongoing endoscopy with predictable benefits for diagnosis and surveillance of IBD subjects. The newly introduced molecular imaging can even overcome the limits of traditional morphological analysis, driving the endoscopic imaging towards the quantification of specific biochemical processes, thereby promoting the examination of functional data. However, further clinical studies are still required to assess the cost-effectiveness and the best strategies for the correct use of these optical enhanced techniques in clinical practice.

Acknowledgements

REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(121):2066-78.

2. Cominelli F. Inhibition of leukocyte trafficking in inflammatory bowel disease. N Engl J Med. 2013;369(8):775-6.

3. Dignass A et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. J Crohns Colitis. 2012;6(10):965-90.

4. Annese V et al. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis. 2013;7(12):982-1018.

5. Van Assche G et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 3: special situations. J Crohns Colitis. 2013;7(1):1-33.

6. Mowat C et al. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011;60(5):571-607.

7. Lichtenstein GR et al. Practice Parameters Committee of American College of Gastroenterology. Management of Crohn's disease in adults. Am J Gastroenterol. 2009;104(2):465-83.

8. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol. 2010;105(3):501-23. Erratum in Am J Gastroenterol. 2010;105(3):500.

9. Magro F et al. European consensus on the histopathology of inflammatory bowel disease. J Crohns Colitis. 2013;7(10):827-51.

10. Eaden JA et al. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48:526-35.

11. Collins PD et al. Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. Cochrane Database Syst Rev. 2006;2:CD000279.

12. Ullman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011;140(6):1807-16.

13. Farraye FA et al. AGA technical review on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. Gastroenterology. 2010;138(2):746-74.

14. Cairns SR et al. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups. Gut. 2010;59:666-89.

15. Rex DK et al. American College of Gastroenterology guidelines for colorectal cancer screening 2009. Am J Gastroenterol. 2009;104(3):739-50.

16. Itzkowitz SH, Present DH. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. Inflamm Bowel Dis. 2005;11(3):314-21.

17. Goldmann H. Significance and detection of dysplasia in chronic colitis. Cancer. 1996;78(11):2261–3.

18. Rubin CE et al. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. Gastroenterology. 1992;103(5):1611-20.

19. Rutter MD et al. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. Gastroenterology. 2006;130(4):1030-8.

20. Leung K et al. Ongoing colorectal cancer risk despite surveillance colonoscopy: The Polyp Prevention Trial Continued Follow-up Study. Gastrointest Endosc. 2010;71(1):111–7.

21. Kiesslich R et al. Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. Gastroenterology. 2007;132(3):874-82.

22. Robertson DJ et al. Colorectal cancer in patients under close colonoscopic surveillance. Gastroenterology. 2005;129(1):34-41.

23. Rutter M et al. Endoscopic appearance of dysplasia in ulcerative colitis and the role of staining. Endoscopy. 2004;36(12):1109-14.

24. Ransohoff DF et al. Ulcerative colitis and colonic cancer. Problems in assessing diagnostic usefulness of mucosal dysplasia. Dis Colon Rectum. 1985;28(6):383-8.

25. Eaden JA et al. How gastroenterologists screen for colonic cancer in ulcerative colitis: an analysis of performance. Gastrointest Endosc. 2000;51(2):123-28.

26. Vienne A et al. Low prevalence of colonoscopic surveillance of inflammatory bowel disease patients with longstanding extensive colitis: a clinical practice survey nested in the CESAME cohort. Aliment Pharmacol Ther. 2011;34(2):188-95.

27. Ullman TA et al. Biopsy specimen numbers in the routine practice of surveillance colonoscopy in ulcerative colitis (UC).Gastroenterology. 2004;126:471.

28. Kiesslich R, Neurath MF. Chromoendoscopy in inflammatory bowel disease. Gastroenterol Clin North Am. 2012;41(2):291-302.

29. Bernstein CN. The color of the most effective tool to use in dysplasia surveillance in inflammatory bowel disease. Gastrointest Endosc. 2011;74(4):849-51.

30. Efthymiou M et al. Cancer surveillance strategies in ulcerative colitis: the need for modernization. Inflamm Bowel Dis. 2011;17(8):1800-13.

31. Bessissow T, Bisschops R. Advanced endoscopic imaging for dysplasia

surveillance in ulcerative colitis. Expert Rev Gastroenterol Hepatol. 2013;7(1):57-67.

32. Ullman TA. Chromoendoscopy should be the standard method and more widely used for cancer surveillance colonoscopy in ulcerative colitis. Inflamm Bowel Dis. 2007;13(10):1273-4.

33. Kiesslich R, Neurath MF. Chromoendoscopy: an evolving standard in surveillance for ulcerative colitis. Inflamm Bowel Dis. 2004;10(5):695-6.

34. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. Gut. 2012;61(11):1619-35.

35. Atreya R et al. In vivo molecular imaging using fluorescent anti-TNF antibodies and confocal laser endomicroscopy predicts response to anti-TNF therapy in Crohn's disease patients. Nature Medicine 2013. [In press].

36. Neumann H et al. New endoscopic approaches in IBD. World J Gastroenterol. 2011;17(1):63-8.

37. Tontini GE et al. Review article: newer optical and digital chromoendoscopy techniques vs. dye-based chromoendoscopy for diagnosis and surveillance in inflammatory bowel disease. Aliment Pharmacol Ther. 2013; 38(10):1198-208.

38. Neumann H et al. Advanced endoscopic imaging for diagnosis of Crohn's disease. Gastroenterol Res Pract. 2012;2012:301541.

39. Wong Kee Song LM et al. ASGE Technology Committee. Chromoendoscopy. Gastrointest Endosc. 2007;66(4):639-49.

40. Tada M et al. On the dye spraying method in colonofibreoscopy. Endoscopy. 1977;8(2):70-4.

41. Canto MI. Staining in gastrointestinal endoscopy: the basics. Endoscopy. 1999;31(6):479-86.

42. Subramanian V et al. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. Aliment Pharmacol Ther. 2011;33(3):304-12.

43. Hurlstone DP et al. Indigo carmine-assisted high-magnification chromoscopic colonoscopy for the detection and characterisation of intraepithelial neoplasia in ulcerative colitis: a prospective evaluation. Endoscopy. 2005;37(12):1186-92.

44. Hurlstone DP et al. Further validation of high-magnification chromoscopic colonoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. Gastroenterology. 2004;126(1):376-8.

45. Marion JF et al. Chromoendoscopy-targeted biopsies are superior to standard colonoscopic surveillance for detecting dysplasia in inflammatory bowel disease patients: a prospective endoscopic trial. Am J Gastroenterol. 2008;103(9):2342-9.

46. Rutter MD et al. Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. Gut. 2004;53(2):256-60.

47. Kiesslich R et al. Methylene blue aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. Gastroenterology. 2003;124(4):880-8.

48. East JE et al. Magnification pan-chromoendoscopy remains the mainstay for colitis dysplasia detection and differentiation. Gastroenterology. 2007;133(1):366-7.

49. Olliver JR et al. Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus. Lancet. 2003; 362(9381):373-4.

50. Hardie LJ et al. Chromoendoscopy with methylene blue and the risk of DNA damage. Gastroenterology. 2004;126(2):623; author reply 623-4.

51. Davies J et al. Methylene blue but not indigo carmine causes DNA damage to colonocytes in vitro and in vivo at concentrations used in clinical chromoendoscopy. Gut. 2007;56(1):155-6.

52. Dinis-Ribeiro M, Moreira-Dias L. There is no clinical evidence of consequences after methylene blue chromoendoscopy. Gastrointest Endosc. 2008;67(7):1209.

53. Wu L et al. The diagnostic accuracy of chromoendoscopy for dysplasia in ulcerative colitis: meta-analysis of six randomized controlled trials. Colorectal Dis. 2012;14(4):416-20.

54. Kuznetsov K et al. Narrow–band imaging: potential and limitations. Endoscopy. 2006;38(1):76-81.

55. van den Broek FJ et al. Narrow-band imaging versus high-definition endoscopy for the diagnosis of neoplasia in ulcerative colitis. Endoscopy. 2011;43(2):108-15.

56. van den Broek FJ et al. Endoscopic tri-modal imaging for surveillance in ulcerative colitis: randomised comparison of high-resolution endoscopy and autofluorescence imaging for neoplasia detection; and evaluation of narrow-band imaging for classification of lesions. Gut. 2008;57(8):1083-9.

57. Pellisé M et al. Narrow-band imaging as an alternative to chromoendoscopy for the detection of dysplasia in long-standing inflammatory bowel disease: a prospective, randomized, crossover study. Gastrointest Endosc. 2011;74(4):840-8.

58. Matsumoto T et al. Magnifying colonoscopy with narrow band imaging system for the diagnosis of dysplasia in ulcerative colitis: a pilot study. Gastrointest Endosc. 2007;66(5):957-65.

59. Tontini GE et al. Advanced endoscopic imaging techniques in Crohn's disease. J Crohns Colitis 2013;doi:10.1016/j.crohns.2013.09.004. [Epub ahead of print].

60. Kudo T et al. Mucosal vascular pattern in ulcerative colitis: observations using narrow band imaging colonoscopy with special reference to histologic inflammation. Int J Colorectal Dis. 2009;24(5):495-501.

61. Matsumoto T et al. Magnifying NBI colonoscopic findings in ulcerative colitis. Stomach Intestine. 2007;42:877-81.

62. Matsumoto T et al. Autofluorescence imaging colonoscopy in ulcerative colitis: comparison with conventional and narrow-band imaging colonoscopy. Dig Endosc. 2007;19:139-44.

63. Esaki M et al. Endoscopic findings under narrow band imaging colonoscopy in ulcerative colitis. Dig Endosc. 2011;1:140-2.

64. Neumann H et al. Virtual chromoendoscopy for prediction of severity and disease extent in patients with inflammatory bowel disease: a randomized controlled study. Inflamm Bowel Dis. 2013;19(9):1935-42.

65. Neumann H et al. Confocal laser endomicroscopy: technical advances and clinical applications. Gastroenterology. 2010;139(2):388-92.

66. Watanabe O et al. Confocal endomicroscopy in patients with ulcerative colitis. J Gastroenterol Hepatol. 2008;2:S286-90.

67. Krauss E et al. Characterization of lymphoid follicles with red ring signs as first manifestation of early Crohn's disease by conventional histopathology and confocal laser endomicroscopy. Int J Clin Exp Pathol. 2012;5(5):411-21.

68. Liu JJ et al. Mind the gaps: confocal endomicroscopy showed increased density of small bowel epithelial gaps in inflammatory bowel disease. J Clin Gastroenterol. 2011;45(3):240-5.

69. Liu JJ et al. Increased epithelial gaps in the small intestines of patients with inflammatory bowel disease: density matters. Gastrointest Endosc. 2011;73(6):1174-80.

70. Trovato C et al. Confocal laser endomicroscopy for the detection of mucosal changes in ileal pouch after restorative proctocolectomy. Dig Liver Dis. 2009;41(8):578-85.

71. Li CQ et al. Classification of inflammation activity in ulcerative colitis by confocal laser endomicroscopy. Am J Gastroenterol. 2010;105(6):1391-6.

72. Neumann H et al. Assessment of Crohn's disease activity by confocal laser endomicroscopy. Inflamm Bowel Dis.

2012;18(12):2261-9.

73. Kiesslich R et al. Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse in inflammatory bowel disease. Gut. 2012;61(8):1146-53.

74. Turcotte JF et al. Increased epithelial gaps in the small intestine are predictive of hospitalization and surgery in patients with inflammatory bowel disease. Clin Transl Gastroenterol. 2012;3:e19.

75. van den Broek FJ et al. Pilot study of probe-based confocal laser endomicroscopy during colonoscopic surveillance of patients with longstanding ulcerative colitis. Endoscopy. 2011;43(2):116-22.

76. Hurlstone DP et al. Confocal endomicroscopy in ulcerative colitis: differentiating dysplasia-associated lesional mass and adenoma-like mass. Clin Gastroenterol Hepatol. 2007;5(10):1235-41.

77. Su L et al. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. Gastroenterology. 2013;145(2):407-15.

78. Salim SY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. Inflamm Bowel Dis. 2011;17(1):362-81.

79. Meddings J. The significance of the gut barrier in disease. Gut. 2008;57(4):438e40.

80. Wyatt J et al. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet. 1993;341:1437e9.

81. Marchiando AM et al. The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. Gastroenterology. 2011;140(4):1208e18.

82. Su L et al. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. Gastroenterology. 2009;136(2):551-63.

83. Atreya R, Goetz M. Molecular imaging in gastroenterology. Nat Rev Gastroenterol Hepatol. 2013;doi: 10.1038/nrgastro.2013.125. [Epub ahead of print].

84. Zipfel WR et al. Nonlinear magic: multiphoton microscopy in the biosciences. Nat Biotechnol. 2003;21(11):1369-77.

85. Schürmann S et al. Label-free imaging of inflammatory bowel disease using multiphoton microscopy. Gastroenterology. 2013;145(3):514-6.

86. Neumann H et al. Review article: in vivo imaging by endocytoscopy. Aliment Pharmacol Ther. 2011;33(11):1183-93.

87. Neumann H, Kiesslich R. Endomicroscopy and endocytoscopy in IBD. Gastrointest Endosc Clin N Am. 2013;23(3):695-705.

88. Pohl H et al. Endocytoscopy for the detection of microstructural features in adult patients with celiac sprue: a prospective, blinded endocytoscopy-conventional histology correlation study. Gastrointest Endosc. 2009;70(5):933-41.

89. Cipolletta L et al. Endocytoscopy can identify dysplasia in aberrant crypt foci of the colorectum: a prospective in vivo study. Endoscopy. 2009;41(2):129-32.

90. Neumann H et al. Image of the month. Endocytoscopy-based detection of focal high-grade intraepithelial neoplasia in colonic polyps. Clin Gastroenterol Hepatol. 2011;9(2):e13.

91. Neumann H et al. Endocytoscopy allows accurate in vivo differentiation of mucosal inflammatory cells in IBD: a pilot study. Inflamm Bowel Dis. 2013;19(2):356-62.