

Random Versus Targeted Biopsies for Colorectal Cancer Surveillance in Inflammatory Bowel Disease

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Abstract: For many years, cancer surveillance colonoscopy in ulcerative colitis patients has involved obtaining at least 30 biopsies of flat and abnormal-appearing mucosa. With the advent of better imaging techniques, biopsies can be better targeted to abnormal-appearing mucosa, thereby increasing the sensitivity of testing. Use of chromoendoscopy, narrow-band imaging, autofluorescence, or confocal endomicroscopy to target biopsies is likely to improve detection of dysplasia and identification of patients at high risk for developing cancer.

Individuals with long-standing ulcerative colitis (UC) and Crohn's colitis are at an increased risk for dysplasia and colorectal cancer (CRC). This increased risk is related to many factors but, most importantly, the duration and extent of disease.^{1,2} In a study by Rutter and colleagues,³ 600 patients with UC were followed for 30 years in a colonoscopic surveillance program in which 12.3% of the patients developed neoplasia. The authors also found the cumulative incidence of CRC, with respect to duration of colitis, to be 2.5% at 20 years, 7.6% at 30 years, and 10.8% at 40 years. These rates of incidence are significantly higher than those of the age-specific general population. Therefore, in order to decrease the risk of CRC, patients with UC or extensive Crohn's colitis (ie, at least one third of colonic involvement) are advised to undergo a surveillance colonoscopy every 1–2 years beginning 8–10 years after the onset of disease.² However, endoscopic detection of early neoplasia often proves to be difficult, as these lesions may be either invisible or very subtle. Thus, current recommendations include obtaining random, nontargeted 4-quadrant biopsies every 10 cm, starting from the cecum to the rectum, with a minimum of 32 biopsies, in addition to obtaining biopsies of suspicious lesions.² Despite this laborious and expensive examination, neoplastic lesions are still missed on endoscopy, as studies have demonstrated that only a very small fraction of the entire colonic mucosal surface is actually sam-

pled.⁴ Furthermore, there is growing evidence suggesting that finding flat, low-grade dysplasia during surveillance is a strong predictor of progression to advanced neoplasia; however, there is considerable disagreement regarding management of the dysplasia.⁵

Management of dysplastic lesions found on colonoscopy depends primarily upon the degree and focality of the dysplasia, with the mainstay of management involving either proctocolectomy or continued colonoscopic surveillance.^{4,5} Bernstein and associates⁶ reported a 19% frequency of CRC in 16 patients with low-grade dysplasia who underwent colectomy. A recent meta-analysis demonstrated that the positive predictive value of CRC for a patient with low-grade dysplasia was 22% and that patients with low-grade dysplasia carried a risk of CRC that was 9 times higher than that of patients who were dysplasia free.⁷

The ability to detect flat, low-grade dysplasia in patients with long-standing colitis proves to be a challenge, given the limitations in our existing technology and the relatively low number of biopsies obtained during colonoscopy. A study by Rubin and coworkers⁸ reported that a minimum of 33 biopsies must be obtained to rule out dysplasia with 90% certainty. Furthermore, 56–64 biopsies are needed to rule out dysplasia with 95% certainty. A survey of British gastroenterologists revealed that 57% obtained 10 or fewer biopsies per colonoscopy and only 2% obtained more than 20 biopsies.⁹ In the Netherlands, only 25% of gastroenterologists follow the national surveillance guidelines for biopsies in patients with UC, and in New Zealand, 50% of specialists obtain less than 17 biopsies per patient.^{10,11} German gastroenterologists report that 9% of clinicians follow practice guidelines in this area.¹² One may argue that perhaps the current recommendations of a minimum of 32 random biopsies with a meticulous examination for any sessile, flat, or minimally elevated lesions may be too laborious and expensive for the average endoscopist and that a more targeted examination may be needed in order to strengthen compliance with current guidelines. Given these findings, new technology is being developed in order to increase the detection rate of dysplasia without increasing total length of time during endoscopy. Four emerging techniques have been investigated to better detect low-grade dysplasia in patients with colitis. Here, we will discuss the potential benefits and limitations of these novel techniques.

Chromoendoscopy

Chromoendoscopy, first described by Tada in 1976, is an examination involving endoscopic dye spraying of the colonic mucosa with the intent of highlighting subtle mucosal irregularities that would otherwise not be seen

with conventional white-light colonoscopy, thus improving the sensitivity of the endoscopic examination.¹³ The two main types of dye that have been used in colonoscopy are methylene blue and indigo carmine. During colonoscopy, a 0.1% methylene blue spray is applied to the colonic mucosa and absorbed by normal tissue, but less so in inflamed or dysplastic tissue.¹⁴ Indigo carmine, another contrast dye, highlights subtle mucosal irregularities or small/flat lesions between areas of normal colonic tissue.¹⁵ In a study by Kiesslich and colleagues,¹⁴ 165 patients who had UC for at least 8 years were randomized to either conventional colonoscopy or colonoscopy with chromoendoscopy using 0.1% methylene blue. In the chromoendoscopy group, 32 intraepithelial lesions were detected in 13 patients compared to 10 lesions found in 6 patients with conventional colonoscopy. Of the 32 lesions detected by chromoendoscopy, 24 were intraepithelial lesions detected in flat mucosa, whereas only 4 intraepithelial lesions were detected by conventional colonoscopy ($P=.0007$). In a later study by Rutter and colleagues,¹⁵ 100 patients with long-standing UC underwent 2 colonoscopic examinations back-to-back: a conventional colonoscopic examination followed by a second colonoscopy with chromoendoscopy using indigo carmine, with the goal of increasing the detection rate of subtle lesions. The investigators found a total of 114 additional abnormalities detected in 55 patients using chromoendoscopy compared to conventional colonoscopy. Of these 114 abnormalities, 7 were dysplastic. The authors concluded that a targeted biopsy examination with chromoendoscopy required fewer biopsies (157) but, more importantly, detected 9 dysplastic lesions, 7 of which were visible only after chromoendoscopy. Moreover, this study suggested that a careful examination of chromoendoscopy with targeted biopsies may be more of an effective surveillance strategy than obtaining multiple random, nontargeted biopsies.

In our experience at the Cleveland Clinic with a cohort of 39 colitis patients, 54 visible lesions were detected with conventional colonoscopy, 7 (13%) of which were found to be low-grade dysplasia; 28 additional lesions were detected with chromoendoscopy, 2 (7%) of which were found to be low-grade dysplasia; and 1 patient had flat, low-grade dysplasia with no visible lesions on random biopsies.¹⁶ Additionally, a later study by Marion and associates¹⁷ at Mount Sinai also demonstrated that chromoendoscopy with targeted biopsies revealed significantly more dysplasia than that of random biopsies.

Although these studies demonstrate that chromoendoscopy detects a greater number of dysplastic lesions as well as a greater number of patients with dysplasia, the increased number of patients has not always been statistically significant. This has proven to be a key finding,

as the detection of more dysplastic lesions in a patient who already has a dysplastic lesion found on conventional colonoscopy is not as clinically relevant as finding additional patients with dysplasia who had no dysplasia detected on conventional colonoscopy.⁴ Although the use of chromoendoscopy has not been formally implemented into the current guidelines for the screening of CRC in inflammatory bowel disease patients, several societies have recognized this technique to greatly enhance conventional white-light colonoscopy.^{18,19}

Narrow-Band Imaging

Narrow-band imaging (NBI) is a novel imaging technique for endoscopy that employs a series of filters in a red-green-blue illumination system to project mostly blue light with shallow penetration into tissues.¹⁹ Vascular structures such as polyps and dysplastic lesions are darkly colored, whereas the surrounding mucosa and residual stool are lightly colored, thus allowing for better resolution of the gastrointestinal epithelium.¹⁹⁻²¹ Moreover, disorders of the vascular pattern in inflammation and the characteristic pit patterns (surface architecture of the epithelium) of early neoplastic lesions can be visualized more effectively with NBI compared to chromoendoscopy or conventional endoscopy. One potential advantage of NBI is the elimination of the need for dye spraying, which allows the operator to alternate from enhanced viewing techniques to white-light endoscopy by pressing a button. In a pilot study by Machida and colleagues,²² NBI colonoscopy was found to be superior to conventional colonoscopy and equal to chromoendoscopy in distinguishing neoplasia from nonneoplastic lesions. In a subsequent study by Matsumoto and coworkers,²³ the authors studied the surface patterns visualized with NBI to determine any correlation with dysplasia. Five dysplastic lesions were found in a total of 296 sites by NBI colonoscopy, and the rate of dysplasia was higher in the tortuous patterns (4/50; 8%) than in the honeycomb-like or villous patterns (1/246; 0.4%; $P=0.003$). In a prospective, randomized, crossover study by Dekker and colleagues,²¹ 42 patients with UC underwent both conventional colonoscopy with targeted biopsies, in addition to random biopsies and colonoscopy with NBI with only targeted biopsies 3 weeks apart. Neoplasia was identified in 11 patients: 4 patients by both techniques, 4 patients only by NBI, and 3 patients only by conventional colonoscopy. In addition to the targeted biopsies, 1,552 random biopsies were obtained, revealing an additional patient with dysplasia who was not previously detected. Although the studies suggested that a tortuous vascular pattern may help to identify dysplasia, the overall sensitivity of the NBI system is comparable to conventional colonoscopy.

Preliminary reports in Barrett esophagus have demonstrated that NBI improves the detection of dysplasia.²⁴ Likewise, the ease, convenience, and lack of additional stains make NBI an attractive imaging modality in the use of CRC surveillance in inflammatory bowel disease patients. However, with studies to date showing equal sensitivity to conventional colonoscopy in dysplasia detection, NBI has yet to become a standard of practice.

Autofluorescence

Improved dysplasia detection by autofluorescence imaging (AFI) relies upon endogenous fluorophores in tissue that undergo a change in excitation-emission spectra, depending upon whether the tissue is normal or has undergone malignant transformation.^{24,25} Differences in the proportion of mitochondria and lysosomes between normal, hyperplastic, and adenomatous tissue lead to differences in the composition of autofluorescent light and the emission spectra.²⁵⁻²⁷ For example, mitochondria, lysosomes, and submucosal collagen autofluorescence fall within the red spectrum when illuminated with blue light, whereas the spectrum of hemoglobin is purple, and that of non-neoplastic mucosa is green. Thus, dysplastic lesions will appear purple.²⁵⁻²⁷ Published studies in Barrett esophagus have reported increased detection rates of high-grade dysplasia when compared to conventional white-light endoscopy.^{24,26} A proof-of-concept study by Kara and associates²⁴ demonstrated an increased accuracy in detecting high-grade dysplasia in a cohort of 20 patients with Barrett esophagus. In this study, AFI detected 28 dysplastic lesions (100%), NBI detected 25 (89%), and conventional white-light endoscopy detected 17 (61%).

In the area of CRC surveillance, the use of AFI has been shown to help distinguish between adenomas and nonadenomatous polyps, without the need for additional chromoendoscopic dyes.²⁷⁻³¹ AFI has demonstrated its superiority over white-light endoscopy in differentiating between adenomas and hyperplastic polyps, and can detect more polyps in the right colon.²⁷⁻³¹ However, in a recent study by van den Broek and colleagues³² comparing AFI with high-resolution endoscopy, the authors found that AFI did not reduce the adenoma miss rate. Furthermore, they found that both AFI and NBI had a low diagnostic accuracy for differentiating between adenomas and non-neoplastic polyps (63% and 79%, respectively). In the only study to date looking at the role of AFI in neoplasia detection in UC patients, van den Broek and coworkers³³ found AFI to improve detection of neoplasia in UC patients when compared to conventional white-light colonoscopy. AFI proves to be a promising novel technology in the detection of neoplasia in often overlooked colonic mucosa.

Confocal Endomicroscopy

Confocal endomicroscopy is an endoscopic imaging technique that allows in vivo microscopic examination of the mucosal layer with cellular and subcellular visualization during endoscopy.³⁴ This is achieved by placing a miniaturized confocal microscope into the distal tip of a conventional colonoscope.³⁴ A fluorescent contrast agent is required to achieve high-contrast images. Agents can be applied either topically (as a spray) or systemically (intravenously) and include fluorescein, acriflavine, tetracycline, or cresyl violet.³⁴⁻³⁶ This new technology is a significant advancement, as it prevents the need for biopsy and formal histologic diagnosis of cellular and architectural abnormalities.

Several studies have demonstrated that confocal endomicroscopy is an excellent tool for investigating suspicious lesions initially located by other means. In a study by Kiesslich and colleagues,³⁷ 161 patients with long-standing UC were randomized to conventional colonoscopy with random and targeted biopsies or endomicroscopy with targeted biopsies only. The authors demonstrated that with endomicroscopy, 4.75-fold more neoplastic lesions were detected when compared to the conventional colonoscopy group, and nearly 50% fewer biopsy specimens were required. This study signifies the increased diagnostic yield with the combination of chromoendoscopy and confocal microscopy, in which chromoendoscopy helps identify the flat lesions while the targeted endomicroscopy examination differentiates between neoplastic and non-neoplastic tissue.

Confocal endomicroscopy has been shown to be most appropriate for use in conjunction with other techniques because of the minute scanning area. Confocal endomicroscopy is only appropriate for scanning tissues at a site already detected by standard or optically enhanced colonoscopy techniques such as NBI or AFI. One potential drawback of confocal endomicroscopy is the need for a contrast agent. Of the agents mentioned above, the most commonly used are fluorescein sodium (10%) and topically applied acriflavine (0.2%). Fluorescein is a well-known agent with long-standing US Food and Drug Administration approval for angiography or angiography of the retina and iris vasculature.³⁸ The colonic mucosal structures that can be identified with fluorescein include enterocytes, cellular infiltrate, surface epithelial cells, blood vessels, and red blood cells. Fluorescein is a highly safe agent whose major side effects in the short term include yellowish discoloration of the skin and 1–2 days of bright yellow urine. A survey of 16 international academic centers with active research protocols in confocal endomicroscopy did not show any serious adverse events.³⁹ Mild adverse events occurred in

1.4% of individuals and included nausea/vomiting, transient hypotension without shock, injection site erythema, diffuse rash, and mild epigastric pain.³⁹

Topical acriflavine is a commonly used alternative agent that stains the acidic constituents of cells and the nuclei of superficial layers of the mucosa.³⁶ Although there is a hypothetical concern regarding the risk of mutagenesis upon exposure to this agent, no adverse events have occurred. However, these concerns have reduced the use of acriflavine in humans. One important point to note is that fluorescein does not typically stain nuclei; thus, differentiations cannot be made between low-grade dysplasia, high-grade dysplasia, and mucosal cancer.³⁴⁻³⁶ However, by taking into consideration confocal pattern classifications, the shape and size of single cells, and the structure of goblet cells, fluorescein was used for in vivo diagnosis of intraepithelial neoplasias in UC with high sensitivity (94.7%) and specificity (98.3%).³⁷

Confocal endomicroscopy represents an exciting new frontier for gastroenterologists to begin interpreting histopathology in addition to gross morphology of suspicious lesions in inflammatory bowel disease patients. Gastroenterologists will need additional education in this area as well as close collaboration with pathologists in order to adequately interpret endoscopic images.

Conclusion

There is a growing body of literature suggesting that current conventional colonoscopy results in a significant number of missed lesions and that perhaps adjunctive or alternative techniques are needed to decrease the risk of CRC. The 4 novel techniques discussed in this paper allow for targeted or “smarter” biopsies and increase the yield of the examination. Moreover, these techniques afford better resolution and detection of subtle lesions that are not otherwise visible with conventional colonoscopy. Chromoendoscopy has once again become an area of interest after decades of its application. Although studies have shown that chromoendoscopy increases the number of detected dysplastic lesions, the increase in the number of patients detected with dysplasia has not been significant. Thus, further studies are needed prior to incorporation into clinical practice. Studies using NBI and AFI have demonstrated that these techniques are useful and convenient adjuncts to conventional colonoscopy. Likewise, studies with confocal endomicroscopy have also suggested a role in CRC screening in inflammatory bowel disease patients. However, further studies are needed to prove its efficacy and cost-effectiveness. One may conclude that the future of CRC screening in inflammatory bowel disease patients centers on increased attention to histologic and biochemical characteristics of normal and

dysplastic colonic tissue. The era of obtaining random biopsies throughout the colon may soon be in the past, as these advances move us closer to a surveillance model that is targeted, has high sensitivity and specificity, and allows for real-time diagnosis and intervention.

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