Review

Indications, stains and techniques in chromoendoscopy

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Summary

Early detection of malignancies within the gastrointestinal tract is essential to improve the prognosis and outcome of affected patients. However, conventional white light endoscopy has a miss rate of up to 25% for gastrointestinal pathology, specifically in the context of small and flat lesions within the colon. Chromoendoscopy and other advanced imaging techniques aim at facilitating the visualization and detection of neoplastic lesions and have been applied throughout the gastrointestinal tract. Chromoendoscopy, particularly in combination with magnifying endoscopy has significantly improved means to detect neoplastic lesions in the gastrointestinal mucosa, particularly in ulcerative colitis and Crohn’s colitis. In addition, chromoendoscopy is beneficial in the upper gastrointestinal tract, especially when evaluating Barrett’s oesophagus (BO) for the presence of dysplasia. Furthermore, it also improves characterization, differentiation and diagnosis of endoscopically detected suspicious lesions, and helps to delineate the extent of neoplastic lesions that may be amenable to endoscopic resection. This review discusses the dyes, indications and advanced endoscopic imaging methods used in various chromoendoscopic techniques, and presents a critical overview of the existing evidence supporting their use in current practice with a particular emphasis on the role in inflammatory bowel disease and BO.

Introduction

Chromoendoscopy aims at a facilitated visualization and detection of dysplastic and malignant lesions in the gastrointestinal tract, which can be difficult to distinguish from normal mucosa. Chromoendoscopy is a diagnostic method in which, a chemical substance is sprayed onto the mucosal surface of the gastrointestinal tract to highlight specific areas or distinguish among different types of epithelia.

Screening colonoscopy for bowel cancer is an example of how chromoendoscopy can increase the diagnostic potential. Although standard definition ‘white light’ colonoscopy is efficient in reducing the incidence of colon cancer by 76–90%, it may miss significant numbers of smaller, flat lesions, particularly on the right side. As a result, anywhere between 10 and 24% of individuals may still develop interval colorectal cancer.1,2 Polypoid adenomas are usually easy to detect whereas only 20–50% of flat, intra-epithelial neoplasias may be picked up via conventional colonoscopy.1,4

Chromoendoscopic techniques improve the recognition of minute changes in the surface pattern by enhancing the contrast of raised and deepened areas. The stains used can be subdivided into ‘absorptive’ or ‘vital’ stains, which, as the name suggests,
are absorbed into tissue or into ‘non-absorptive’ contrast stains, which simply pool into mucosal tissue allowing better visual definition. Other agents are based on chemical reactions that indicate special functions of the underlying tissue and are eponymously named ‘reactive’ stains. Advanced imaging, such as magnification endoscopy, allows brilliant resolution of suspicious structures; although this latter technique is not widely available outside specialist units. Nonetheless, most chromoendoscopic methods can also be applied to standard endoscopes in an effort to improve the detection rate of pre-malignant lesions. Chromoendoscopy is particularly helpful in surveillance programmes aiming to detect dysplasia and pre-neoplastic lesions [e.g. in Barrett’s oesophagus (BO) or inflammatory bowel disease (IBD)] with the diagnostic yield of targeted ‘smarter’ biopsies being superior to random biopsies,5,6 thus reducing the histopathologic workload and potentially offsetting the costs for additional procedure time.

In this article, we summarize the indications, dyes and methodologies of various chromoendoscopic techniques, providing a critical review of the existing evidence pertaining to IBD and BO, as well as a comparison against digital, high-definition and virtual endoscopic techniques. Articles have been identified using PubMed, Medline and Ovid search engines, alongside pre-existing clinical management protocols and guidelines.

Equipments and general chromoendoscopic techniques

The staining substances are generally inexpensive and readily available, although not specifically marketed for chromoendoscopy. Spraying catheters allow the most controlled and precise application of the dye as a fine mist onto the gastrointestinal surface. They are disposable, flexible plastic sheaths with a luer lock and metal nozzle tip. A good bowel preparation is conditio-sine-qua-non for chromoendoscopy in the colon and up to 13% of patients may not meet this requirement.4 Some staining techniques also require pre-treatment with N-acetylcysteine as mycolyticum to clear excess mucus from the mucosal surface. The use of n-butylscopolamine is recommended to avoid bowel peristalsis and an uneven distribution of the dye.

The amount of staining solution required, depends on the surface area to be stained (e.g. pancolonic staining; or further characterization of a single lesion), but the smallest amount necessary should be applied to avoid dye pooling. For pancolonic staining, the endoscope is slowly withdrawn, while the endoscope tip with a 1–2 cm protruding spraying catheter is directed in spiral movements onto the mucosa and simultaneously, the dye is constantly sprayed. Step-by-step, segments of ~20 cm are stained and then carefully inspected.

The additional time needed for chromoendoscopy, including preparation of the tissue surface, dye spraying, washing of excessive dye, inspection and interpretation, varies from 2 to 20 min. This is dependent upon staining objectives; a targeted lesion may need further, local characterization (e.g. colonic polyp), vs. an entire organ needing to be stained for detection of dysplastic areas (e.g. the colon in IBD).

Vital stains

Acetic acid
Acetic acid (vinegar) is a weak acid that breaks up the disulphide bridges of glycoproteins that build the mucus layer and results in a reversible denaturation of proteins. Acetic acid is not a colouring agent, but when sprayed onto the tissue surface, it can enhance the structural surface pattern similar to a contrast agent. It has been used in colposcopy for a number of years as it brings out dysplastic squamous lesions of the cervix.7

**Procedure.** Pre-treatment of the surface with mucolytic agents is not required. Concentrations of 1.5–3% (v/v) acetic acid are usually sprayed in 20 ml aliquots onto the oesophageal mucosa. Within a few seconds, a whitish discolouration of the epithelia is noted. This staining method has been introduced to predict the presence of specialized columnar-lined epithelium in the oesophagus using magnification endoscopy.

After acetic acid application onto suspected Barrett’s epithelium, different mucosal surface patterns can be observed (Table 1). When oesophageal biopsies are taken from type III/IV pit pattern areas (villous and cerebriform appearance), the diagnostic yield for specialized columnar-lined epithelium is >87%, whereas it is <11% when taken from type I or II areas (regular round pits or circular and oval pits).8

Methylene blue
Chromoendoscopy using methylene blue as a vital stain involves active mucosal absorption of the dye by small intestinal and colonic epithelium. The stain is not absorbed by non-absorptive mucosa such as squamous or gastric epithelium. Targeted biopsies should be aimed at heterogeneously stained or unstained areas, as high grade dysplasia (HGD)
and early cancers absorb the dye to a lower degree due to loss in goblet cells and decreased cytoplasm.

Procedure. Methylene blue chromoendoscopy requires prior mucus removal from the mucosal surface to ensure homogenous uptake of dye by epithelial cells in the upper gastrointestinal tract. This can be obtained by spraying 10% solution of N-acetylcysteine as a mucolytic onto the mucosal surface prior to the application of 0.5% methylene blue. Excess dye is carefully washed off with water until the staining pattern is stable.

With pan-colonic dye staining, segments of 20–30 cm of colon are sprayed and evaluated at a time. A slightly lower concentration (0.1%) of methylene blue is applied, using a spraying catheter onto the colonic mucosa. Excessive dye is removed by suction after a staining time of \( \frac{1}{2} \) min.

Side effects. Generally, chromoendoscopy with methylene blue is safe. However, methylene blue might induce oxidative damage to DNA in the epithelium in combination with photosensitization by white light endoscopy. Up till now, no increased risk of cancer development has been proven in patients undergoing methylene blue-based chromoendoscopy, although many centres prefer using indigo carmine (see below) for this reason, to avoid any potential DNA damage in patients who already have a pre-malignant condition. Chromoendoscopy using methylene blue may also cause a transient, harmless, blue discolouration of urine and faeces.

Cresyl violet—cytoendoscopy

Cresyl violet (gentian violet) solution is preferentially taken up in the crypts of Lieberkuhn, which appear as dots or pits, providing very clear definition of patterns having histological correlates. Cresyl violet staining can be combined with methylene blue and indigo carmine staining to detect small, early malignant changes in the colon. Cresyl violet has also been used as an intra-vital stain for the detection and characterization of early upper gastrointestinal cancers.

Procedure. Cresyl violet (0.05–0.2%) is usually applied in small amounts (1–2 ml) to avoid excessive darkening of stained surfaces. Combined with confocal laser endomicroscopy (CLE), cresyl violet may be applied topically to allow simultaneous chromoendoscopy and endomicroscopy, thereby providing accurate prediction of histology, as well as visualization of nuclear morphology.

Lugol

Lugol is an iodine-based solution, which is used to demarcate dysplasia and cancer in squamous epithelium (Figure 1). The iodine is incorporated in the glycogen, which is abundant within non-keratinized squamous epithelium. This results in a typical reptile skin-like endoscopic appearance after staining. Neoplastic tissue usually has low glycogen storage and therefore appears unstained. However, other conditions which result in depleted glycogen storage in the cells, such as inflammatory diseases (e.g. reflux oesophagitis) or BO might show a decreased or missing stain uptake. Lugol voiding lesions in patients with squamous cell cancer of the head and neck are a strong predictor for synchronous or metachronous oesophageal squamous cancer and identifies patients who should be under close endoscopic surveillance.

Procedure. Following initial inspection, 20–30 ml of 1–2% Lugol’s iodine solution (e.g. 12 g iodine + 24 g potassium iodide in 1000 ml water) is sprayed from the gastro-oesophageal junction to the upper oesophageal sphincter using a spray catheter.

Side effects. The application of iodine can cause thyrotoxicosis in patients with underlying thyroid disease. Severe allergic reactions to iodine have been reported, and it should not be administered to patients with a history of iodine hypersensitivity. Retro-sternal discomfort induced by the mucosal irritation of iodine has been reported in up to 30% of patients. This side effect can be reduced by spraying 20 ml of 5% sodium thiosulphate solution after chromoendoscopy.
In 1998, 225 adults with balloon cytologic evidence of oesophageal dysplasia or carcinoma underwent conventional, standard definition, white-light endoscopy followed by staining with 1.2% Lugol’s iodine solution. Before staining, the sensitivity of visible lesions for identifying HGD or cancer was only 62% and the specificity, 79%; whereas after staining, the sensitivity of unstained lesions for identifying HGD or cancer, rose to 96%. Although no additional patients with invasive carcinoma were detected with staining alone, dysplastic lesions in 17 of 31 patients with moderate dysplasia (55%) and 8 of 35 patients with severe dysplasia (23%) were identified only after iodine staining. Chromoendoscopy with Lugol’s iodine may also prove useful for reducing misclassification in patients with reflux oesophagitis.

Non-absorptive contrast stains

Indigo carmine

Indigo carmine (E132; often used as food dye) is a contrast dye that neither reacts with nor is absorbed by the mucosa, but simply pools in the mucosal grooves and crevices, allowing better topographic definition (Figure 2).

Procedure. During continuous extubation, indigo carmine (0.4%) is gently applied to achieve diffuse coverage of the entire mucosal surface. Only a small volume of dye is applied to avoid excess dye accumulation. This continuous one-step low volume technique is much simpler than previously described multiple-step techniques involving segmental staining, followed by re-examination after excess dye has been aspirated. Indigo carmine is easily applied using a special dye-spray catheter.

Prior application of acetic acid has also been used in the upper gastrointestinal tract in some studies. In contrast to methylene blue, patients receiving indigo carmine dye spraying do not seem to have increased DNA damage induced to colonocytes. Indigo carmine appears to be photostable and poses little potential for damage to genetic material in vitro.

The different colonic staining patterns are categorized according to the Kudo pit pattern classification (type I: round pits; type II: reticular pattern, stellar or papillary pits; type III: tubular, large pits and IIIr: rounded, compact, smaller pits; type IV: elongated, branched and sulcus-like pits and type V: irregular, non-structural pits), which predicts histology with good accuracy. This classification is based on an early Japanese study where, 2050 colorectal tumour lesions were assessed by endomicroscopy, stereomicroscopy and histopathology. Based on stereomicroscopy, lesions with a regular type I or II pit pattern were non-tumours, whereas lesions with types IIIr, IIIl, IV and/or V pit patterns were neoplastic tumours. When the diagnosis by magnifying endoscopy was compared with the stereomicroscopic diagnosis, there was agreement in 1130 of 1387 lesions (81.5%).

Indigo carmine has also proven useful in delineating the extent of gastric lesions. In 2009, Lee et al. performed both conventional endoscopy and acetic acid–indigo carmine chromoendoscopy in 141 patients with early gastric cancer to clearly identify the extension of tumour. The latter technique clarified the border in a significantly higher percentage of differentiated adenocarcinomas (89.8% vs. 68.5%; P<0.001). However, the technique had no additional benefit in demarcating undifferentiated adenocarcinomas.
Reactive stains

Congo red

Congo red is a pH indicator that changes colour from red to dark blue or black when exposed to acidic environments (pH < 3). It has been used to map ectopic sites of excessive acid production and is useful in the evaluation of post-vagotomy patients. Congo red has been used for many years in screening early gastric cancers, and in combination with methylene blue, which stains gastric intestinal metaplasia.20

Procedure. This technique involves stimulation of acid production with 250 μg of pentagastrin given orally. During the endoscopy, 0.5% sodium bicarbonate solution is sprayed prior to a 0.3–0.5% Congo red solution. A positive reaction (black colour change) results within minutes that delineate acid secreting areas (blue/black) from non-acid-secreting areas (red).

A double staining technique using methylene blue and Congo red has been used to identify early gastric cancers as ‘bleached’ areas of mucosa that fail to stain with either methylene blue or Congo red. This is in contrast to the red or blue–red coloured mucosa of non-cancerous areas. A very early study by Ishi et al. demonstrated that the detection of synchronous early gastric cancers increased from 28% under standard imaging to 89% after methylene blue–Congo red combination staining. The technique also facilitates the detection of carcinomatous foci, 4–10 mm in size that are not visible with conventional endoscopy.21,22

Phenol red

Phenol red changes colour from yellow to red in the presence of an alkaline environment and has been used to detect and map the distribution of Helicobacter pylori infection within the stomach.

Procedure. Prior to the endoscopy, the patient is given acid suppression therapy (either via a proton pump inhibitor orally the day before, or via intravenous therapy 30–60 min before the procedure), an oral anti-foaming mucolytic agent and an anticholinergic drug to suppress gastric motility. The entire surface of the stomach is sprayed over with 0.1% phenol red containing 5% urea. Positive staining of yellow to red usually occurs within 2–3 min.

The sensitivity of this method in detecting H. pylori approaches 100%, and specificity 84.6%.23,24 However, the clinical relevance of the phenol red technique is limited.

Chromoendoscopy in primary bowel cancer screening

Screening for colorectal cancer using faecal occult blood testing, sigmoidoscopy or colonoscopy is recommended in several countries, mostly in those patients aged >50 years with an average risk, and earlier in people with a strong family history or other risk factors. Adenomatous polyps are deemed to be precursors of colorectal cancer and removal of polyps and post-polypectomy surveillance decreases the incidence of colorectal cancer.25,26 Colonoscopy is considered to be the reference standard against which the accuracy and efficacy of other colorectal cancer screening tests is compared with; however, the miss rate for polyps is >20%.27

Several trials have evaluated the ability of chromoendoscopy to increase detection of adenomatous lesions during primary bowel cancer screening programmes in the general population. One of the first, large prospective trials used vital staining with
indigo carmine on all visible lesions in 100 consecutive patients without visible inflammatory changes. If findings on macroscopic examinations were unremarkable, the sigmoid colon and rectum were stained with indigo carmine over a defined segment (0–30 cm ab ano) and inspected for lesions visible only after staining. Using this technique, 178 additional lesions in the sigmoid colon were identified as being detectable only after dye spray. This study failed to control for withdrawal time, which may explain in part, the increased detection with indigo carmine.28 A second randomized trial of 260 patients comparing indigo carmine pan-chromoendoscopy vs. a control group of targeted biopsy colonoscopy (without dye spray) reported a higher number of adenomas in the pan-chromoendoscopy group (112 vs. 57; \(P<0.05\)). Flat lesions (82 vs. 54; \(P<0.05\)), right-sided lesions (79 vs. 31; \(P<0.05\)) and the number of patients with more than three adenomas (13 vs. 4; \(P<0.01\)) were also significantly higher in the pan-chromoendoscopy group.29

A recent study from Germany evaluated 1008 patients aged >45 years, who presented for primary bowel cancer screening over an 18-month period. Patients were randomized to receive standard colonoscopy or colonoscopy with pan-colonic dye spray using indigo carmine. There was a significant increase in the overall detection rate for adenomas in the pan-colonic dye-spray arm in comparison with the control group (46.2 vs. 36.3%; \(P=0.002\)). In the pan-colonic dye-spray group, the detection rate for both flat (0.56 vs. 0.28 per patient) and serrated (1.19 vs. 0.49 per patient) adenomas was nearly double that of the conventional colonoscopy arm (\(P<0.001\)). However, in the subgroup of advanced adenomas >1 cm, despite a trend towards greater detection in the dye-spray arm, this did not reach statistical significance, echoing the results of previous studies.3

Although the introduction of chromoendoscopy, both in isolation and in combination with magnifying endoscopy, has significantly improved the means to detect both pre-neoplastic and neoplastic changes, not all studies in primary bowel cancer screening have demonstrated clear benefits. One such randomized controlled trial of pan-colonic chromoendoscopy (indigo carmine) vs. standard colonoscopy included 259 patients, and although a statistically significant increase in detection rate was found for both <5 mm adenomas (89 vs. 36, \(P=0.026\)), as well as number of patients with \(\geq 3\) adenomas (15 vs. 3; \(P=0.002\)), no significant difference in the detection rate of adenomas overall was observed.30 The prospective trial by Le Rhun et al. included 203 patients and compared adenoma detection of standard resolution colonoscopy against high resolution pan-colonic chromoendoscopy (indigo carmine). Once again, although more polyps were detected by the latter, the total number of polyps detected per patient was not significantly different between groups.31

### Chromoendoscopy in IBD

The increased risk of colorectal cancer in ulcerative colitis (UC) has long been recognized.32,33 However, the previously estimated lifetime risk of \(\sim 30\%\) is probably greater than estimated, when applied to the present patient population. Recent population-based studies estimate the 30-year cumulative risk to be between 2.1 and 10.8%.34–36 The risk of cancer in Crohn’s disease with colonic involvement is less clear. Longstanding disease, extensive colonic involvement, a family history of colorectal cancer, the degree of inflammatory activity and the co-existence of primary sclerosing cholangitis37 are additional factors that increase the risk of colonic cancer in patients with pre-existing IBD. This led to the development of colonoscopy surveillance programs which centred upon multiple non-targeted random biopsies at pre-defined intervals based on the presence or absence of the aforementioned risk factors.38,39

Older guidelines recommended 2–4 biopsies be taken every 10 cm in the colorectum, rendering 20–50 biopsies per examination. This approach was time-consuming, expensive and associated with significant miss rates of pre-neoplastic lesions. In 2003, Kiesslich et al. randomized 165 patients with longstanding ulcerative colitis to receive either conventional colonoscopy or colonoscopy with chromoendoscopy using methylene blue. In the chromoendoscopy group, there was significantly better correlation between the endoscopic assessment of degree and extent of colonic inflammation and the histopathologic findings, compared with the conventional colonoscopy group (89 vs. 52%; \(P<0.0001\)). More targeted biopsies were possible and significantly more intra-epithelial neoplasia was detected in the chromoendoscopy arm (32 vs. 10; \(P=0.003\)). Using the modified pit pattern classification, both the sensitivity and specificity for differentiation between non-neoplastic and neoplastic lesions were 93%.40 A Japanese study published in the same year followed up 57 patients with pancolitis between 5 and 7 years and found higher diagnostic accuracy for dysplasia in biopsies targeted by chromoendoscopy vs. standard definition colonoscopy (sensitivity 85.7 vs. 38.1%).41
In 2004, Rutter et al. conducted back-to-back colonoscopies on 100 patients with long standing, extensive ulcerative colitis. During the first examination, both visible abnormalities and quadratic, non-targeted areas (every 10 cm) were biopsied. Pan-colonic indigo carmine dye spraying was performed during the second examination and any additional visible abnormalities biopsied. The non-targeted biopsy protocol detected no dysplasia in 2904 biopsies. About 43 mucosal abnormalities (20 patients) were detected during the pre-dye-spray colonoscopy, of which, 2 (2 patients) were dysplastic. A total of 114 additional mucosal abnormalities (55 patients) were detected only following dye spraying, of which seven (5 patients) were dysplastic on histological analysis. Although there was a strong trend towards increased dysplasia detection following dye spraying, this did not quite reach statistical significance \((P=0.06)\). More recently, Ratiu et al. conducted a study \((n=55;\text{mean age 60 years})\) to see whether indigo carmine provides a greater adenoma detection rate in the distal colon during examination with flexible sigmoidoscopy. Chromoendoscopy with indigo carmine significantly improved the adenoma detection rate for lesions <5 mm (15 vs. 7 adenomas; \(P<0.01\)); however, no significant difference was detectable for lesions >5 mm between chromoendoscopy and conventional sigmoidoscopy. A larger study from Mt. Sinai Hospital, New York prospectively investigated 102 patients with either ulcerative colitis or Crohn’s colitis. Patients underwent standard surveillance colonoscopy with quadratic random biopsies every 10 cm (minimum 32 samples) or colonoscopy using a targeted biopsy protocol or finally, methylene blue (0.01%) dye-spray chromoendoscopy and targeted biopsy. Each patient had a single examination that included two passes of the colonoscope. Targeted biopsies with dye spray revealed significantly more dysplastic lesions than random biopsies (17 vs. 3; \(P=0.001\)) although the benefit over the targeted non-dye-spray approach did not reach statistical significance \((P=0.057)\).

A recent meta-analysis evaluating the efficacy of methylene blue or indigo carmine chromoendoscopy for detecting dysplasia in patients with ulcerative colitis demonstrated a pooled sensitivity of 83%, specificity of 91.3% and a diagnostic odds ratio of 17.5. A second meta-analysis found that the incremental yield of dysplasia detection between chromoendoscopy and white light endoscopy was 7% [95% confidence interval: 3.2–11.3] on a per-patient analysis, with a number needed to treat of 14.3 to detect one extra patient with dysplasia or cancer (Table 2). The difference in proportion of flat lesions detected by targeted biopsies was 44 vs. 27% in favour of chromoendoscopy. This evidence on the superiority of chromoendoscopy with targeted biopsies compared with standard colonoscopy has led to the recommendation of pan-colonic dye spray with targeted biopsies (where available), rather than random biopsies in the new guidelines for surveillance colonoscopy by the British Society of Gastroenterology and American Gastroenterological Association.

### Chromoendoscopy in BO

Longstanding gastro-oesophageal reflux can lead to the development of BO which affects ~2% of adults

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Preferred staining technique</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia detection in BO</td>
<td>Acetic acid 1.5–3%</td>
<td>95.5–100</td>
<td>~80</td>
<td>Neoplasia detection rate increased by 2.5-fold in one study.(^5)</td>
</tr>
<tr>
<td></td>
<td>Indigo carmine 0.4–0.8%</td>
<td>83</td>
<td>88</td>
<td>High sensitivity and specificity restricted to long (≥3 cm) Barrett’s intestinal metaplasia segments having an irregular, distorted pattern (pit-pattern III/IV). Unable to distinguish low-grade dysplasia from non-dysplastic intestinal metaplasia.</td>
</tr>
<tr>
<td>Dysplasia detection in IBD</td>
<td>Indigo carmine 0.4%</td>
<td>93</td>
<td>Overall: 91</td>
<td>Data extracted from recent meta-analysis with an AUROC of 89%.(^4)</td>
</tr>
<tr>
<td></td>
<td>Methylene blue 0.1%</td>
<td>72</td>
<td>Overall: 91</td>
<td></td>
</tr>
</tbody>
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**Table 2** Indications and preferred dyes in surveillance chromoendoscopy
in the West, and is more common in men aged >50 years. The lifetime oesophageal adenocarcinoma risk in this pre-malignant condition is in the order of 3–5%.\(^{47}\) This is 30–100 times higher than in the general population. In view of this, endoscopic surveillance programs have been instituted with an aim of detecting dysplasia or neoplasia at an early stage.\(^{30,49}\) Most guidelines recommend surveillance endoscopy every 2–5 years in patients with BO to detect early signs of HGD, which poses a risk of progressing to adenocarcinoma of 6–19% per year,\(^{49}\) as well as early, treatable neoplastic lesions.

There has been a longstanding practice for surveillance endoscopies to take random four quadrant biopsies every 1–2 cm unless obvious nodules or mucosal irregularities are present (‘Seattle protocol’). However, high grade dysplasia and early cancer often present as flat lesions that are difficult to identify. The Seattle protocol is time consuming, expensive and may still miss 41–66% of high grade dysplasia.\(^{5}\) Therefore, great effort has been undertaken to improve the visualization of subtle mucosal changes, which indicate the histological presence of early neoplasia. Although methylene blue has proven useful at diagnosing gastric lesions,\(^{50}\) its use in Barrett’s surveillance has produced discrepant results. A meta-analysis by Ngamruengphong et al. concluded that the diagnostic yield for the detection of specialized intra-epithelial metaplasia and dysplasia is only comparable, but not superior to taking random biopsies.\(^{51}\) As methylene blue staining is cumbersome, time consuming and bears at least a potential risk of DNA damage, it cannot widely be recommended for routine Barrett’s surveillance. However, it might be helpful to delineate dysplastic or malignant lesions for further endoscopic therapy (endoscopic mucosal resection or endoscopic submucosal dissection).

Whereas methylene blue chromoendoscopy does not seem to increase the diagnostic yield in the context of BO, highlighting irregularities in the surface pattern by acetic acid spraying (Figure 3) and taking targeted biopsies from said areas does result in a higher detection rate of dysplasia and cancer.\(^{52,53}\) Although many studies apply acetic acid staining in combination with magnification endoscopy, it is also helpful when applied to conventional, standard definition ‘white light’ endoscopes.\(^{46}\) Staining with the naturally brownish-coloured balsamic vinegar combines the advantage of chromoendoscopy and surface structure enhancement by the acetic acid.\(^{54}\) Pech et al. prospectively evaluated the diagnostic value of staining with balsamic vinegar, and in combination with high resolution video-endoscopes without magnification, balsamic vinegar staining obtained an accuracy of 90%, sensitivity of 100% and specificity of 82% in predicting the presence of Barrett’s epithelium compared with histology.\(^{54}\) A recent study from Portsmouth (UK) collected data on 190 patients undergoing upper gastrointestinal endoscopy and found acetic acid chromoendoscopy to have a sensitivity of 95.5% and specificity of 80% for the detection of neoplasia. In this study, visible neoplasia was observed during 43 procedures with white-light endoscopy and in 102 following dye spray with acetic acid (\(P=0.001\)), yielding an improved neoplastic-lesion detection rate of \(~2.5\)-fold when compared with conventional white light endoscopy. Moreover, a high degree of correlation between lesions predicted to be neoplastic by acetic acid chromoendoscopy and those diagnosed by histological analysis was found (\(r\)-value = 0.99).\(^{46}\) However, the results of a recent randomized study (\(n=137\)) were more sobering, with targeted biopsies using enhanced magnification acetic acid chromoendoscopy, yielding specialized intestinal metaplasia at the same frequency as standard endoscopy, calling into question, the diagnostic utility of this technique.\(^{55}\) Nonetheless, as advanced endoscopic imaging techniques using acetic acid identify the vast majority of early neoplasia, targeted biopsies using acetic acid chromoendoscopy and high resolution endoscopes may yet replace random quadrant biopsies as part of Barrett’s surveillance programmes. Moreover, acetic acid localizes neoplastic lesions in the majority of patients and could potentially represent significant cost savings in high-risk patients with BO and suspected neoplasia.\(^{56}\)

Chromoendoscopy using indigo carmine in BO may also be helpful. In 2003, Sharma et al. performed indigo carmine dye spray and magnification chromoendoscopy in 80 patients with suspected >3 cm BO. The yield of intestinal metaplasia was 97% when a ridged, villous pattern was observed and 100% for high grade dysplasia when an irregular, distorted pattern could be seen. Although all patients with long segment BO were identified using this technique, the value dropped to 82% when evaluating for short segment diseases.\(^{57}\) A more recent, multi-centre study prospectively evaluated 56 patients with BO using indigo carmine magnification chromoendoscopy. This study also found that the presence of an irregular, distorted pattern throughout the Barrett’s segment was highly sensitive (83%) and specific (88%) for high grade dysplasia.\(^{58}\) However, indigo carmine is not able to accurately distinguish low-grade dysplasia from non-dysplastic intestinal metaplasia.\(^{57}\)
Chromoendoscopy in combination with high definition and magnifying endoscopy

Good resolution and appropriate magnification are essential for achieving high quality visualization during any endoscopic examination. Video resolution is defined as the ability to optically distinguish two closely approximated points or objects. High-resolution imaging improves the ability to discriminate detail, whereas magnification enlarges the image. Several studies have argued that high-resolution imaging endoscopy may be enough to detect BO, with no additional benefits being obtained with acetic acid, or in detecting HGD or early cancer with indigo carmine chromoendoscopy. However, even with high-resolution endoscopes, four-quadrant biopsies are still necessary, and acetic acid spraying may yet improve visualization of suspicious lesions.

Recent advancements in endoscopic technology have produced high magnification endoscopes that allow real time visualization of mucosal morphology in greater detail. The clinical utility of this modality had been limited by the size of the endoscope in the past. However, improvement in the design of the charged-couple device, an electronic light-sensing apparatus located at the tip of the endoscope, has given rise to less bulky and more manageable apparatus. Magnification endoscopy has been used in combination with indigo carmine to diagnose BO with a sensitivity of 97%, and HGD with a sensitivity of 100%. As already discussed, enhanced magnification endoscopy with acetic acid allows clear visualization of the epithelial pit patterns within BO; however, despite the increasing availability of high resolution magnification endoscopes, there is a lack of diagnostic criteria for magnified endoscopic images.

There are also large cases’ series that report the utility of using magnification colonoscopy and pit-pattern analysis to differentiate neoplastic and non-neoplastic lesions. One prospective trial randomized patients (n = 660) to either magnification chromocolonoscopy with indigo carmine or conventional (non-magnified) chromocolonoscopy and found that the accuracy of the former in distinguishing neoplastic from non-neoplastic lesions <10 mm in size (92%) was significantly higher compared with
the latter (68%). The higher accuracy of magnification chromocolonoscopy has been validated in other reports. In the colon at least, it may be that the mucosal magnification in combination with tissue staining is more important than high-resolution imaging, as the number of lesions detected between high-resolution colonoscopy without tissue staining and standard colonoscopy does not appear to be different between modalities.

Magnification chromoendoscopy in combination with methylene blue dye spray has also been studied in ulcerative colitis, and is significantly better than magnification endoscopy alone at identifying intra-epithelial neoplasia. Prospective trials with targeted chromocolonoscopy (indigo carmine) confirmed these findings and demonstrated improved detection of intra-epithelial neoplasia compared with random quadrantic biopsies. In small studies, magnification chromocolonoscopy was also used to assess colitis disease severity and may even predict disease relapse.

**How does chromoendoscopy compare with other endoscopic advancements?**

All chromoendoscopic methods have to compete with newer, quicker and neater imaging techniques, which offer on-demand imaging of the gastrointestinal mucosa during the endoscopic examination whenever further characterization of a suspicious area is required. For many of these novel techniques the clinical value over conventional white light and high-definition endoscopy in the detection of adenomatous, dysplastic and neoplastic lesions of the gastrointestinal tract has yet to be rigorously evaluated. Moreover, few studies provide a ‘head-to-head’ comparison of these newer endoscopic enhancements vs. chromoendoscopy.

Narrow-band imaging (NBI; Olympus, Tokyo, Japan) offers better visualization of the superficial mucosal detail and vasculature by pressing a button on the hand control of the endoscope without the need for any additional dyes. The NBI system has special red–green–blue filters in which the band-pass ranges have been narrowed and the relative contribution of blue light has been increased. NBI has become increasingly popular in the upper gastrointestinal tract, as it assists in unmasking pre-malignant and neoplastic lesions in patients with BO. However, despite initial enthusiasm and promise, well-designed prospective randomized controlled trials and meta-analysis have failed to prove superiority of narrow-band imaging in screening or surveillance colonoscopy, compared with standard definition white light endoscopy. Nonetheless, improved further characterization of already detected colonic polyps by NBI may allow prediction of the histology with acceptable accuracy, thereby allowing a ‘resect and discard’ strategy, which might be cost-effective and safe for diminutive colonic polyps.

Newer generation endoscopy systems include Fuji intelligent chromoendoscopy (Fujinon) or i-Scan (Pentax); both also from Japan. These modalities utilize the light reflected from the intestinal mucosa, which is then modified by ‘post-processor computer algorithms’ that allow different forms of enhancements leading to accentuation of the vasculature, surface architecture or tissue pattern visualization. Although these techniques provide accurate classification and differentiation of colorectal polyps and neoplastic lesions, a clear advantage in adenoma detection rate over chromocolonoscopy or high resolution white light endoscopy has not yet been shown.

Autofluorescence imaging (AFI) endoscopy uses short wavelengths of light to stimulate endogenous substances, so called ‘fluorophores’ such as nicotinamide adenine dinucleotide (NADH), collagen, aromatic aminoacids and porphyrines in the tissue to emit fluorescent light of a longer wavelength. Due to different content of such fluorophores, normal and neoplastic tissues differ in their autofluorescence spectra. Whereas normal mucosa appears green, neoplastic areas are ‘flagged up’ in violet. Modern endoscopic techniques combine white-light endoscopy, AFI and NBI (trimodal endoscopic imaging). However, studies have not consistently demonstrated the superiority of this method compared with high resolution endoscopy alone, for detection of colonic or oesophageal dysplasia. Thus, AFI-guided biopsies are not currently able to replace random biopsies during surveillance endoscopies.

CLE is a technique that offers in vivo imaging of the mucosal layer at cellular and subcellular resolutions without the fixation artefacts one experiences with histological specimens. In this system, fluorescent dyes are applied either locally or systemically, and subsequently excited by a low-power laser. The intensity of the fluorescent energy is then captured as an image. Commonly used agents are fluorescein sodium and acriflavine. Studies have shown the high accuracy (96.7%) with which CLE allows differentiation from low-grade to high-grade intra-epithelial colorectal neoplasia. Moreover, chromoendoscopy together with targeted CLE and endomicroscopy is able to increase the diagnostic yield of intra-epithelial neoplasia by 4.75-fold (P = 0.005) in patients with chronic ulcerative colitis.
Other emerging in vivo endoscopic techniques include light-scattered spectroscopy (LSS), a type of reflectance spectroscopy which determines tissue structure by determining elastic light scattering,86,87 and optical coherence tomography, a probe-based technique using infrared light which allows deeper tissue penetration than CLE.88,89 However, the clinical benefit of these novel modalities has not yet been fully appreciated and further technological improvements for gastrointestinal endoscopy are warranted before they enter widespread usage.

Tumour markers and chromoendoscopy

Biomarkers of malignancy or pre-malignancy in stool or blood are a potentially attractive means of detecting colorectal dysplasia and neoplasia, particularly for high-risk populations. Unfortunately, many conventional tumour markers such as CEA, CA19-9 and EpCAM reflect inflammation, rather than pre-malignant change; therefore do not have an established place in cancer, or pre-cancer detection. However, specific molecular alterations (p53 mutations, DNA aneuploidy, chromosomal instability, K-ras mutations, p14 and p16 hypermethylation, microsatellite instability, age-related methylation, telomere length shortening) have been identified at a higher frequency in the non-neoplastic epithelium of UC patients with neoplasia, compared with non-neoplastic epithelium from UC patients without neoplasia.90 These emerging tools may assist in the risk-stratification of future endoscopic surveillance strategies and therapeutic intervention to patients at highest risk of developing cancer. Although these novel biomarkers do not improve absolute lesion detection rates during endoscopy, emerging molecular imaging methods could revolutionize the detection of dysplasia in conjunction with chromoendoscopy by providing a wide field of view while simultaneously highlighting molecular abnormalities in ‘real time.’ One such example is the use of specific lectin probes that recognize coordinated changes in glycan expression that accompany the transition from BO through dysplasia to adenocarcinoma.91,92 Such advancements may not only help delineate the extent of disease involvement, but also inform the future choice of treatment if they can be validated as an endoscopic tool in vivo.

Conclusions

Chromoendoscopy enables targeted biopsies and thus improves the diagnostic yield of dysplastic alterations. It also improves further characterization, differentiation and diagnosis of endoscopically detected suspicious lesions. Chromoendoscopy is considered a safe, relatively inexpensive procedure aside from the additional endoscopy time required. The chromoendoscopic staining methods are not technically demanding and easy to learn, but require experience in the interpretation of the observed staining pattern. The accessories needed to perform chromoendoscopy, the dye agents and spraying catheters, are readily available. Standardization of staining protocols, a consensus in terminology and classification of staining patterns are still awaited. Virtual chromoendoscopy allows further characterization of detected lesions and can predict their histology with accuracy. Therefore, an optical triage approach to ‘diagnose, reject, discard’ or ‘diagnose and leave behind’ might reduce the costs of polypectomy. Newer evolving technologies are likely to definitively change the clinical algorithm of gastrointestinal endoscopy in which hopefully the proportion of missed adenomas, dysplastic lesions and carcinomas will substantially decrease.

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References


