PAPER

Magnification endoscopy, high resolution endoscopy, and chromoscopy; towards a better optical diagnosis

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In the past few years, optical magnification endoscopy and chromoscopy have gained renewed interest in the West as a means for the early detection of minute lesions in patients with Barrett's oesophagus and in patients referred for colonic cancer screening. In Barrett's oesophagus, the vast majority of data on the use of chromoscopy deals with the application of methylene blue. Conventional videoendoscopy in combination with methylene blue staining improves the detection of Barrett's mucosa. A correlation has been shown between variation and intensity of staining and histologically verified stages of dysplasia or cancer. Magnification endoscopy and chromoscopy improve the detection of colonic non-polypoid lesions associated with neoplasia and carcinoma. Pitt pattern analysis enables the distinction of non-neoplastic non-polypoid lesions (type I and II) from neoplastic type non-polypoid lesions (type III-V) with great accuracy. It is certain that "old fashioned" chromoscopy combined with advanced endoscopic technology carry a great diagnostic potential and should be further put to the test for use in daily clinical practice.

An optimal diagnostic endoscopic examination should be executed in a reasonable time frame, with a minimum amount of patient discomfort and risk, and have a maximal diagnostic yield. Preferably, it should be performed with equipment that is readily available using techniques that are easy to learn. Importantly, it must provide reproducible results. When searching for large pedunculated polyps using standard available videoendoscope equipment, failure to identify such a lesion because of poor image resolution is hardly an issue. This however, is very different in the case of searching for small areas of dysplasia or carcinoma in the oesophagus (Barrett's oesophagus), stomach (early gastric cancer), and colon (non-polypoid compared with polypoid lesions). In these cases the issue is the detection of minute lesions that often do not stand out from surrounding tissue with standard available techniques. Therefore, additional techniques must be used to improve their detection rate. Endoscopes equipped with high resolution charged coupled device (CCD) chips provide a superior image quality compared with endoscopes with standard CCD chips. In addition, magnification endoscopy provides an even more detailed image by optically enlarging the mucosal surface area. Endoscopic intravital staining techniques using absorptive and contrast stains can be used to enhance the visual characteristics of both normal and abnormal (that is, dysplasia and carcinoma) tissue creating a visual distinction for early detection and targeted biopsy. This review focuses on the principle, use, and yield of these techniques, single or in combination, in patients with Barrett's oesophagus and patients undergoing colorectal cancer (CRC) screening.

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TECHNIQUES

Magnifiying endoscopy and high resolution endoscopy

The commercial introduction of the first flexible fibre endoscope in 1961 marked the beginning of a revolution in the diagnosis and management of gastrointestinal disease. Since then, ongoing development has taken place in the area of endoscopy design and presently fibreoptic (video) endoscopes are largely replaced by electronic videoendoscopes. Conventional videoendoscopes are equipped with CCD chips of 100K to 300K pixels, meaning that each image is built up from 100 000 to 300 000 individual pixels. This technical feature, also referred to as pixel density, is important because it relates to the image resolution and hence to the ability to discriminate two closely approximated points. The higher the pixel density, the higher the image resolution, the more likely minute lesions will be discriminated and detected. The second generation of electronic videoendoscopes is equipped with CCD chips of 400K and recently endoscopes (both gastroscopes and colonoscopes) were introduced with 850K pixel density. Endoscopes with such a high resolution are referred to as high resolution endoscopes. This terminology can be confusing at times because the adjunct high resolution is sometimes also used to refer to magnifying endoscopes. In this article the adjunct high resolution relates to pixel density of the CCD chip.

Some endoscopes, including high resolution endoscopes, are equipped with an optical zooming facility comprising of a movable motor driven lens in the tip of the scope. By controlling the focal distance, the scope can move very close to the mucosal surface providing the magnified image. These scopes are referred to as magnifying endoscopes. Optical magnification is very closely related to the concept of high resolution. At the

Abbreviations: CRC, colonic cancer screening; CCD, charged coupled device

same level of magnification, a high resolution endoscope will provide a more detailed picture than an optical magnifying endoscope. The image resolution of the latter can be improved to the level of the high resolution scope by optically increasing the level of magnification at the expense of reducing the surface area that is visualised. Here lies probably one of the most promising aspects of use of high resolution endoscopes: the superior ability to discriminate detail in the non-magnified overview image. Such property is a key requirement of a screening tool: before a suspicious small lesion can be scrutinised and discriminated (by magnification endoscopy and chromoscopy) it must first be detected!

Image manipulation with an electronic zooming (magnification) facility is sometimes confused with optical magnification. There is, however, an important distinction. Electronic magnification can provide a more detailed image of a lesion, but only up to a certain level. Image quality is lost at some point because with every step of electronic magnification the image is composed of less pixels as compared with optical magnification.

Chromoscopy

Although around for many decades, chromoscopy is still widely underused in Western countries as compared with, for example, Japan. This seems unjustified given the fact that the equipment needed is readily available and cheap, the technique is not difficult to learn, and with some experience adds only a little extra time to the procedure.

Agents used for chromoscopy are categorised according to their working principle. Vital stains like Lugol's solution and methylene blue are absorbed into the cells. Contrast stains like indigo carmine are not absorbed but accumulate in pits and valleys between cells highlighting mucosal architecture. Reactive stains like Congo red and phenol red react to changing conditions of acid secretion and carry a potential with regard to the early detection of gastric cancer and *Helicobacter pylori* infection.

Lugol's solution contains potassium iodine and iodine that reacts with glycogen in non-keratinised squamous epithelium. Normal squamous epithelium stains deeply brown giving the oesophagus a snake skin-like appearance while areas with inflammation, dysplasia, or (early) cancer lack appropriate staining becasue of a depletion of glycogen. Lugol's solution has been used to delineate the extent of Barrett's oesophagus,[1] to screen for squamous cell cancer in the oesophagus in high risk populations,[2] and to detect (standard) endoscopy negative reflux disease in combination with high resolution endoscopy.[3] Usually a concentration of 2% to 3% Lugol's solution is used.

Methylene blue is taken up by absorbing tissues such as small intestinal and colonic cells. Areas of gastric metaplasia in the small intestine will highlight because they do not stain while the surrounding small intestinal mucosa does. Areas of intestinal metaplasia in the stomach or oesophagus will highlight because these areas stain positively while gastric mucosa and squamous oesophageal epithelium do not. Methylene blue has been used to screen for colonic neoplasia,[4] to diagnose villous atrophy,[5] and to diagnose Barrett's oesophagus[6] and screen for areas of dysplasia and carcinoma.[7] Usually a concentration of 0.5% of methylene blue is used.

Indigo carmine is a contrast stain that is not taken up by cells. Instead, it accumulates in pits and valleys between cells highlighting the mucosal architecture that becomes even more apparent with the use of magnification or high resolution endoscopy, or both. It has been used to diagnose Barrett's oesophagus,[8] evaluate villous atrophy,[9] and diagnose and discriminate polypoid and non-polypoid lesions in the colon.[10 11] In general, a concentration of 0.5% to 1% indigo carmine is used.

To perform chromoscopy little extra equipment is needed. To achieve appropriate delivery of the dye it is important that a special spraying catheter is used permitting optimal dispersion of the dye onto the mucosal surface. It is important to note that sensitivity and specificity of vital staining can be adversely affected by the presence of oesophagitis and ulcers.[12–14] In such cases it is best to obtain mucosal healing with proton pump inhibitors first, before vital staining is attempted. Another approach would be to carefully inspect the mucosa before staining and map ulcers and areas of oesophagitis.

To prepare the mucosa for optimal staining and clear it from mucus it is first washed with a mucolytic agent (N-acetylcysteine, 10 ml, 10%). Before applying the dye one must wait about two minutes to allow the mucolytic agent to work. Next, the dye is applied with a spraying catheter. In case of a vital stain like methylene blue a two minute waiting time is compulsory to let the dye be absorbed before the mucosa is washed again, this time by water, to remove excess dye. The last step should not be done in case of indigo carmine as this would remove the non-absorptive dye. After these steps, inspection of the stained mucosa may start.

CLINICAL DATA

Barrret's oesophagus

Barrett's mucosa is defined by the presence of specialised intestinal type columnar epithelium in a columnar lined oesophagus. Techniques providing improved endoscopic tissue diagnosis may aid in both confirmation of the presence of Barrett's mucosa as well as the identification of suspicious areas of dysplasia or carcinoma for targeted tissue biopsies and treatment (mucosal resection).

Canto and coworkers showed that methylene blue stains areas of specialised columnar epithelium with high accuracy.[6] In this study conventional videoendoscopes were used. Results were reproducible after four weeks. Of interest was the finding that in the control group methylene blue staining unexpectedly diagnosed areas of specialised columnar epithelium (confirmed by histology) in 42% of cases although previous investigations, including biopsies in some, were negative.

Sharma and coworkers studied a total of 75 patients with columnar appearing mucosa in the distal oesophagus shorter than 3 cm (short segment Barrett) with conventional videoendoscopy in whom the yield of methylene blue directed biopsies were compared with a historical control group of 83 patients with randomly obtained biopsies.[15] Specialised intestinal metaplasia was detected in 61% of cases in methylene blue directed biopsies and 42% of cases in random biopsies. Although statistically significant, the absolute difference in the number of biopsies between both methods was not convincing in terms of clinical relevance.

Canto and coworkers showed that in a group of 43 patients with biopsy verified Barrett's osophagus, methylene blue directed jumbo biopsies led to the identification of a much larger proportion of samples with specialised intestinal metaplasia compared with conventional standard four quadrant jumbo random biopsies despite fewer biopsies per patient.[7] Moreover, dysplasia and cancer were detected more frequently (44% of patients as compared with 28%) at half the costs of random biopsies. Additional cases that were identified included five patients with low grade dysplasia, one patient with high grade dysplasia, and one patient with cancer. Also in this study, conventional videoendoscopes were used.

In an attempt to use methylene blue staining not only to highlight areas of specialised intestinal metaplasia, Canto and coworkers performed both ex vivo and in vivo studies to test the predictive value of the degree of variation in the intensity of staining for identifying areas of dysplasia or cancer.[13] Ex vivo experiments were performed on five surgical specimens from patients who had an oesophagectomy for high grade dysplasia or early carcinoma. In vivo experiments were performed with conventional videoendoscopy in 47 patients

and entailed assessing the intensity of staining on a ordinal scale (absent, mild, moderate, or marked) and obtaining biopsy specimens from areas that were rated for histological confirmation and correlation. The authors found that the intensity of staining was significantly associated with the grade of dysplasia. Samples with no dysplasia were obtained from unstained or light blue staining areas in 38% of cases as compared with 92% and 82% of samples obtained from areas with high and low grade dysplasia, respectively. Furthermore, in almost all patients with severe dysplasia or cancer the staining pattern of the corresponding surface area showed moderate to marked heterogeneity (92%) as compared with only 21% in cases with low grade dysplasia and as low as 3% in cases without dysplasia. The authors conclude that increased heterogeneity and light to absent methylene blue staining are significant independent predictors of (severe) dysplasia and cancer.

Kiesslich and coworkers confirmed the ability of methylene blue staining to highlight areas of specialised intestinal metaplasia.[14] The study was performed in 51 patients with Barrett's osophagus and 21 control subjects (with a normal oesophagogastric junction) using conventional videoendoscopes. Targeted biopsies of stained areas provided histological proof of specialised columnar epithelium with a sensitivity of 98% and a specificity of 61%. Use of methylene blue staining increased the detection rate of areas with specialised columnar epithelium in patients already known with Barrett's oesophagus but, in agreement with observation by Canto also showed specialised columnar epithelium in control subjects. The authors observed a characteristic staining pattern of areas with high grade dysplasia or adenocarcinoma but noted that three of four cases had already been correctly identified by endoscopy alone before methylene blue staining was applied. In fact, some of the endoscopic features of a suspicious lesion such as cobblestone-like epithelial structure, niches, and pockets were more difficult to recognise after methylene blue staining.

Wo and coworkers performed a prospective randomised crossover trial comparing the diagnostic yield of methylene blue directed biopsies with that of four quadrant 2 cm interval biopsies and found no additional benefit.[16] The study was performed using conventional videoendoscopes. A total of 47 patients were included. Sensitivity and specificity for specialised intestinal metaplasia were 53% and 51%, respectively. Relative frequencies for specialised intestinal metaplasia were 20% and 18% from methylene blue directed and conventional biopsies, respectively. There was no difference in results between patients with long segment compared with short segment Barrett's. Dysplasia was found in 10 patients with methylene blue directed biopsies compared with seven patients with conventional biopsies. Nearly all lesions showed indefinite or low grade dysplasia, which makes this study unsuitable for assessing the yield of methylene blue directed biopsies for the identification of high grade dysplasia or cancer.

Stevens and coworkers combined the use of chromoscopy and magnification endoscopy for diagnosing Barrett's oesophagus.[8] Firstly, they applied indigo carmine to highlight areas of possible Barrett's mucosa. Secondly, suspicious areas were visually scrutinised with means of magnification endoscopy (10 to 35 times). Barrett's mucosa was characterised by a slightly raised surface pattern with a villiform appearance. Results were confirmed histologically showing the presence of specialised columnar epithelium in these areas. No attempts were made to identify areas of dysplasia or cancer.

Guelrud and coworkers also tested the use of magnification endoscopy[17] to identify areas of specialised intestinal metaplasia. Their study included 49 patients with previously diagnosed Barrett's oesophagus without dysplasia who were being followed up in a surveillance programme. Before visual inspection, the distal oesophagus was irrigated with 10 ml to 15 ml of 1.5% acetic acid. No absorptive or contrast stains were used. Four different mucosal surface patterns were detected; type I or round pit pattern, type II or reticular pit pattern, type III no pits and fine villiform appearance of mucosa, and type IV no pits and ridged pattern with a thick villous convoluted shape with a cerebriform appearance of the mucosa. The yields with respect to accurate (histologically confirmed) prediction of the presence specialised intestinal metaplasia were 0%, 11%, 89%, and 100%, respectively. Areas of mucosal surface patterns type III and type IV (both highly predictive of the presence of specialised intestinal metaplasia), which were identified by magnification endoscopy and acetic acid spaying, were missed in almost all cases by standard endoscopy (61 of 63) or standard endoscopy combined with acetic acid (11 of 63). This study was performed in a group of Barrett's patients who had no dysplasia at previous surveillance endoscopies and no mention is made whether new areas of dysplasia were detected or magnification endoscopy was used to identify such areas.

In summary, most data regarding the use of chromoscopy in Barrett's oesophagus deal with methylene blue staining using conventional videoendoscopes. Comparatively few studies have looked at the use of indigo carmine. Methylene blue staining does improve the detection of Barrett's mucosa and areas of intestinal metaplasia are detected much more frequently than was previously recognised, even in people who were thought to have a normal oesophagogastric junction on regular endoscopy. The clinical consequence of the latter observation remains to be investigated. Although a correlation has been shown between variation and intensity of staining and stages of dysplasia or cancer based on histological examination, this needs to be confirmed by others (including inter-observer variation analysis) and tested against other surveillance techniques (for example fluorescence endoscopy). So far, most studies have focused on the use of conventional videoendoscopes, some with magnifying capabilities. The use of high resolution magnifying endoscopes in combination with chromoscopy may improve results even further. To date only a limited number of centres have such equipment. However, the appeal of a surveillance technique that is partly based on the use of equipment that is readily available, would be compromised. On the other hand, it does not seem unrealisticall to assume that in a not too distant future many conventional videoendoscopes will be replaced by high resolution endoscopes. It is not that long ago that videoendoscopy was regarded as just an other fancy tool that would not out perform or out live fibreoptic endoscopy.

Non-polypoid colorectal neoplasia

Colorectal neoplasia can be divided into two groups: protruding polypoid lesions and superficial non-polypoid lesions. The second group can be subdivided into slightly elevated (small flat adenoma), lateral spreading tumours (large flat adenomas), and depressed type tumours.[18] The importance of making these distinctions is twofold. Firstly, it is being increasingly recognided that non-polypoid lesions are often missed, especially in the Western world, and that early detection and treatment of these lesions may be important in terms of disease management and outcome. Secondly, between the different types of non-polypoid lesions there are distinct differences in biological behaviour and likelihood of submucosal invasion in case of malignancy, which amounts to 2.1% for polypoid lesions, 0.05% for small flat adenomas, 8.2% for large flat adenomas, and 29.5% for depressed lesions.[18]

The key to identifying non-polypoid lesions is alertness to subtle mucosal changes such as small areas of colour changes, depression or elevation, and disruption of vascular architecture. The use 0.2% to 0.4% indigo carmine aids in identifying and differentiating such lesions. There is some debate whether chromoscopy should be used to identify suspicious lesions or

that it should only be used for classification of suspicious mucosal areas once they are identified by white light endoscopy.

With the ultimate goal in mind of developing an endoscopic technique providing an "optical biopsy", analysis of the colonic pit pattern has emerged as a promising technique. A pit is the mucosal opening to a crypt and its endoscopic (magnified) appearance has been used by many investigators, already dating back to the early seventies, to predict the histopathological correlate. The most commonly used classification was proposed by Kudo.[19 20] In this classification a total of five types are recognised with two categories divided into two subtypes: type I round pit, type II star shaped or asteroid pit, type III-S small tubular pit, type III-L large tubular pit, type IV dendritic or gyrus-like pit, type V-A irregular and non-uniform pit, type V-N amorphous or non-structural pit. The clinical relevance of this classification is based on the fact that in experienced hands it seems highly predictive of the final histopathological diagnosis with type I corresponding to a normal gland, type II to hyperplastic polyps, type III to neoplastic glands most often adenomatous lesions, type IV to neoplastic glands most often tubulovilleus adenomas with a small percentage of intramucosal and submucosal cancers (10% to 20%), and type V to cancerous glands with type V-N pointing towards to submucosal infiltration.[21]

Non-polyploid lesions comprise up to 32% to 45% of all early neoplastic colonic lesions in the Japanese population.[19 20 22] There has been some debate whether such lesions would actually exist in the Western population, but in recent years non-polypoid lesions including flat adenomas and depressed type lesions have been reported in patients from Sweden, the United Kingdom, and Germany. Jamarillo and coworkers detected 109 non-polypoid neoplastic lesions in 55 of 232 patients using magnifying endoscopy and indigo carmine staining.[23] Patients were randomly selected from a total of 2373 subjects who were referred for colonoscopy to the Karolinska hospital in Stockholm, Sweden, during a two year period. Patients with inflammatory bowel disease, hereditary non-polyposis coli, or familial adenomatous polyposis were excluded. Most patients with non-polypoid neoplastic lesions were over 60 years of age and no patient was younger than 40 years of age. Seventy one per cent of non-polypoid neoplastic lesions was smaller than 0.5 cm in size. Low grade dysplasia was found in 86%, high grade dysplasia in 12%, and adenocarcinoma in 3% of cases. Flat neoplastic lesions with a central depression showed a six times higher chance of high grade dysplasia compared with lesions without a central depression. Hart and coworkers performed screening sigmoidoscopies in 3000 patients who were randomly chosen from a cohort of 13 000 CRC screening cases in the Leicester area, United Kingdom, using standard endoscopy equipment without the use of chromoscopy.[24] This yielded a total of four flat lesions in three patients. Three lesions contained severely dysplastic tissue and one lesion a focus of adenocarcinoma. Importantly, three of these lesions were less than 5 mm in size.

Another study from the United Kingdom was performed by Rembacken and coworkers.[25] They screened a total of 1000 unselected patients who attended their unit for routine colonoscopy, for flat or depressed neoplasms. A total of 321 adenomas (36% flat, 0.6% depressed) were found in 225 patients. Ten per cent of all lesions contained areas of severe dysplasia. In addition, six Dukes's A adenocarcinomas together with 25 more advanced adenocarcinomas were identified. Dukes's A adenocarcinoma or severe dysplasia was found in 29% of large flat lesions and in 75% of depressed lesions illustrating the unique biological behaviour of these lesions and the clinical relevance for early detection.

In a recent German study from Kiesslich and coworkers using high resolution endoscopy or magnifying endoscopy (depending on the type of scope available at the time of investigation) and chromoscopy with indigo carmine, 52 of 100 patients had 105 visible lesions of which 89 were polypoid and 16 were non-polypoid (two depressed lesions).[26] Histopathological examination showed hyperplastic tissue in 45 lesions, adenomas in 54 lesions, and cancer in three. Of the 48 patients with no visible lesions, 27 patients showed 178 lesions after staining the distal 30 cm of the colon with indigo carmine (176 flat, two depressed). Histopathological examination showed hyperplastic polyps in 165 lesions, adenomas in 10 lesions, and neoplasia in three lesions (one high grade dysplasia and two low grade dysplasia). No cancers were found in these patients. The use of pit pattern analysis to distinguish between normal mucosa and hyperplastic polyps from neoplasia (type I and II compared with type III to V) showed a sensitivity of 92% and a specificity of 93%.

A special group of interest for CRC screening are patients with inflammatory bowel disease. These patients have an increased risk for developing colorectal cancer. For patients with ulcerative colitis the reported cumulative risk ranges between 9% to 14% at 25 years from the onset of symptoms.[27] Features such as long duration of disease, pancolitis, severe inflammation, and coexisting sclerosing cholangitis are risk factors.[28] Selected patients, therefore, enter an endoscopic surveillance programme. Magnifying or high resolution endoscopy, or both, combined with chromoscopy may prove a powerful tool for the early detection of areas of high dysplasia and cancerous lesions in these patients. The first studies on magnifying endoscopy in patients with ulcerative colitis dealt with the assessment of features of disease activity and remission.[29–32]

The only study on the use of magnifying endoscopy and chromoscopy (indigo carmine) as a screening tool for neoplastic lesions in patients with ulcerative colitis was performed by Jamarillo and coworkers.[33] They detected 104 lesions in 38 adenocarcinomas in 85 patients with longstanding (>10 years) and extensive disease. Most of these lesions were endoscopically flat (74%). A total of 23 lesions were neoplastic of which most were flat (65%). Low grade dysplasia was found in 21 lesions and high grade dysplasia in the remaining two. The authors conclude that magnifying endoscopy and chromoscopy are promising techniques for screening patients with inflammatory bowel disease enabling the early detection of flat neoplasms.

These data amount to the following conclusions. Non-polypoid lesions do exist in Western patient populations and are often present in patients with negative findings on standard endoscopy. Awareness of such lesions leads to recognition of some of these lesions even without the use of magnification endoscopy or chromoscopy, or both. Chromoscopy improves the detection of non-polypoid lesions. Pit pattern analysis by means of chromoscopy and magnification endoscopy enables the distinction of non-neoplastic non-polypoid lesions (type I and II) from neoplastic type non-polypoid lesions (type III–V) with great accuracy. Whether the search for these lesions using high resolution or magnification endoscopy, or both, in combination with chromoscopy is clinically relevant and worthwhile in terms of patient management and cost effectiveness is difficult to assess and depends on many variables. On the one hand it may save costs by obviating the need for biopsing type I and II lesions, which comprise the majority of all non-polyploid lesions. On the other hand better inspection yields more suspicious lesions. Even more important, whether early detection of non-polypoid lesions actually decreases mortality and saves lives remains to be confirmed.

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