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Acetic acid-enhanced magnification endoscopy in the diagnosis of specialized intestinal metaplasia, dysplasia and early cancer in Barrett's oesophagus

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SUMMARY

Background
Barrett's surveillance is prone to sampling error.

Aim
To determine whether enhanced magnification endoscopy using acetic acid instillation improves diagnostic accuracy of specialized intestinal metaplasia/dysplasia in Barrett's oesophagus.

Methods
We examined the detection rate of the specialized intestinal metaplasia/dysplasia in 64 consecutive patients with Barrett's oesophagus using acetic acid to enhance mucosal pit patterns. Histology was compared with the previous findings at recent conventional surveillance in 62 patients. We also examined the inter-/intra-observer agreement in the assessment of the enhanced magnification endoscopy pit pattern findings.

Results
Histology revealed columnar-lined oesophagus in six (9%) patients, specialized intestinal metaplasia in 49 (77%), low-grade dysplasia in five (8%), high-grade dysplasia in one (2%), and adenocarcinoma in three (5%). There was discordance between the histologic findings from conventional surveillance with random biopsy. Fifteen patients (24%) had a histological upgrade with enhanced magnification endoscopy. There was a high detection rate of specialized intestinal metaplasia even in short segment Barrett's oesophagus (74%), and additionally, there were two cancers, one with 2-cm Barrett's oesophagus and one ultra-short (1 cm). The mean kappa values for inter- and intra-observer agreement in assessing the pit patterns were 0.571 (0.041) and 0.709 (0.038), respectively.

Conclusions
Enhanced magnification endoscopy allows clear visualization of the epithelial pit patterns within Barrett's oesophagus, and targeted biopsy results in a high yield of specialized intestinal metaplasia and dysplasia.

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INTRODUCTION

Oesophageal adenocarcinoma is increasing in incidence in the Western world, especially in the UK.[1] Barrett's oesophagus (BE) is the only identifiable premalignant condition responsible for this increased incidence. The evolution of adenocarcinoma in BE is believed to occur along a spectrum of specialized intestinal metaplasia (SIM) → low-grade dysplasia (LGD) → high-grade dysplasia (HGD) → adenocarcinoma.

Surveillance endoscopy and random biopsy are a common practice performed to detect SIM, dysplasia and early cancer. One of the main difficulties with BE surveillance is the reliability of diagnosis across this spectrum. There is a marked discordance between the endoscopic and histologic diagnosis of BE,[2] whilst at the cancer end of the spectrum, diagnosis is more alarmingly problematic. Areas of HGD and microscopic carcinoma in BE may only occupy a fraction of the total surface area of Barrett's mucosa, leading to false-negative sampling errors,[3] and biopsy by the Seattle protocol will only sample 4% of the Barrett's segment.[4]

In view of the above dilemmas, new endoscopic techniques for Barrett's evaluation have been developed, including the combined use of chromoendoscopy to stain the Barrett's segment,[5] and high resolution magnification endoscopy. Guelrud *et al.*[6] applied the technique called enhanced magnification endoscopy (EME) with instillation of acetic acid rather than the colouring agents, methylene blue or indigo carmine, and produced a classification of mucosal surface pit patterns that predicted the histologic findings. This technique offers the possibility of sensitive targeting of a hierarchy of pit patterns within the Barrett's segment at endoscopy to confirm SIM, dysplasia and early cancer. Similarly, in a recent study, Toyoda *et al.* showed that EME is useful for detection of intestinal metaplasia in distal oesophagus and oesophagogastric junction.[7]

We examined the detection of SIM, dysplasia and adenocarcinoma by EME in patients with BE, and subsequently compared the diagnostic yield of our findings with those found at previous routine endoscopy.

The descriptive criterion for pit pattern classification means that there is a great potential for subjective interpretation. We therefore evaluated the inter- and intra-observer agreement in the assessment of pit patterns, and recognition of areas more probable to harbour intestinal metaplasia or dysplasia in BE using the EME technique.

PATIENTS AND METHODS

Patients

Consecutive unselected patients referred to a specialist BE clinic with an endoscopic diagnosis of BE were invited to undergo EME for confirmation of diagnosis and formation of a surveillance plan. Patients were referred from other gastroenterologists at our institution, or the endoscopy nurse practitioner. The previous endoscopies were performed with standard videoendoscopes, without chromendoscopy, by four-quadrant biopsy every 2 cm. After an informed consent from all patients, the study was conducted in accordance with the revised Declaration of Helsinki 2000.

Endoscopy procedure

Patients were offered conscious sedation with i.v. midazolam (2–5 mg), or local pharyngeal anaesthesia with xylocaine spray. All endoscopy procedures were carried out using the high resolution Olympus zoom gastroscope (GIF Q240Z; Olympus, Tokyo, Japan), Lucera CV-260 videoendoscopy processor and Olympus OEV181H high definition television monitor. The zoom gastroscope functions like a normal gastroscope, except that when magnification is required, a lever next to the up-and-down knob is depressed. When the lever is fully depressed, maximum magnification is achieved. A soft plastic cap (Olympus D-201-11802) is attached to the scope tip before staring the procedure. This cap helps keep the lens off the mucosa and fixes the focal length whilst obtaining maximal magnification. A routine upper gastrointestinal endoscopy was first performed and the endoscopist (PJF, GKA, KR) recorded the length of Barrett's segment, hiatal hernia and any other obvious (e.g. nodules, ulcer) abnormalities of note in the overview mode. Following this, 10–20 mL of 3% acetic acid was sprayed on the lower oesophagus and Barrett's epithelium with a spray catheter (Olympus PW-5L) introduced via the instrument channel. The oesophagus, Barrett's and gastric mucosa initially take on a white appearance (the aceto-white reaction), then the BE and gastric mucosa appear red. The Barrett's segment was viewed at 115× magnification at 2 cm intervals. The mucosa was scrutinized in magnified views at 2 cm intervals. The procedure was digitally recorded and still images recorded at each level. The various pit patterns were classified as follows, based on our personal observation in our unit and from the published literature:[4, 6]

Type I: Round or oval pit pattern.
Type II: Linear or tubular pit pattern.
Type III: Villous pit pattern.
Type IV: Cerebriform or gyri-like pit pattern.
Type V: Distorted pit pattern.
These are illustrated in Figure 1.

Biopsies were taken using 8-mm forceps (Olympus FB230K), targeted at the highest grade of pit patterns to maximize the detection of SIM and dysplasia. The Type I pit pattern was biopsied only if it was the sole pattern seen, as it usually represents cardiac or fundic type mucosa. Biopsy specimens were taken from distal to proximal areas, to allow blood from biopsy sites to trickle distally and avoid obscuring vision when biopsy is taken in the proximal areas. Any other abnormalities e.g. nodules were noted separately and biopsies placed in separate containers.

Inter- and intra-observer variability assessment

All endoscopic examinations were digitally recorded, analysed by the three endoscopists who performed the procedures, and still images were captured. The images were stored as JPEG files (file size about 100 Kb, pixel array 640×480, 24-bit colour). Exclusion criteria were duplicate pictures and images that were out of focus. The selected images were transferred to a software programme (Powerpoint; Microsoft, Redmond, WA, USA). The images were enlarged to fit a 35-mm slide format without distorting brightness, contrast or colour balance, and anonymized. The endoscopists (GKA, KR, PJF) who selected the pictures agreed on the predominant pit pattern in each selected image.

Six endoscopists were invited to assess these pictures for pit patterns, blinded to the histologic diagnosis. The six observer endoscopists are involved in a large endoscopic training programme and all had performed over 2000 upper endoscopies. Two hundred and fifty-five images were selected. Forty of them (eight of each pit pattern) were shown to the endoscopists as a reference guide of the five pit patterns seen at EME. Each endoscopist assessed the remaining 215 selected images independently. They were asked to state the predominant pit pattern in the pictures (the pattern occupying the majority of the picture). The assessment of pit patterns was repeated after 1 week, when the same images but in a different order were shown to the endoscopists. No time limit for reviewing the slides was imposed. The endoscopists recorded their results on preprinted forms.

Histolopathologic assessment

Biopsy specimens for histological analysis were placed in 10% formalin solution and routinely processed. Routine H & E and Alcian blue stains were employed to identify the columnar glands and goblet cells within an intestinal metaplasia. The specimens were analysed by a single experienced gastrointestinal pathologist (PK), blinded to the endoscopic findings. The biopsy specimens were analysed individually for the presence of SIM and or dysplasia/cancer. Confirmation from a second pathologist was required for the diagnosis of dysplasia, using the five-tiered Vienna classification.[8]

Statistical analysis

The primary endpoint was to determine the diagnostic yield of SIM, dysplasia and adenocarcinoma by EME technique, using the descriptive statistics. As a secondary endpoint, we compared the findings with EME

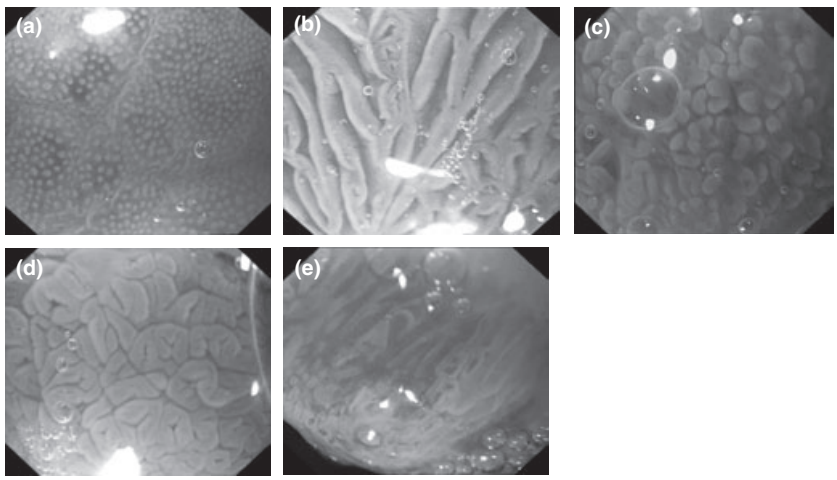

Figure 1. Pit pattern types as visualised by enhanced magnification endoscopy; a, round type; b, linear type; c, villous type; d, cerebriform type; and e, distorted type.

and previous random biopsy, using a weighted kappa, rather than kappa, as disagreement of one grade (e.g. CLE to SIM), is not as clinically important as bad as two grades (e.g. SIM to HGD).

To examine the chance adjusted agreement, kappa values were calculated for both inter- and intra-observer agreement. Each endoscopist's diagnosis was compared with each diagnosis of other endoscopists (interobserver, 15 pairs) and his/her own repeat diagnosis (intra-observer, six pairs). Inter-observer variation was calculated from the results of the first reading.

Given that pit patterns II–IV may harbour intestinal metaplasia, type I is cardiac or fundic type mucosa, and type V is usually seen in dyslastic areas, we also examined the accuracy, inter- and intra-observer variability between the endoscopists in terms of identifying areas most probable to harbour intestinal metaplasia or dysplasia. In all cases, mean values were expressed as mean (standard deviation). Statistics were analysed using STATSDIRECT statistics software package version 2.2.3, Cheshire, UK.

RESULTS

Patients

Sixty-four patients underwent EME as out-patients between January 2004 and January 2005. Males comprised 38 of 64 (59%). The mean age was 62 years, (range 26–83). Histology from previous surveillance endoscopy was available on 62 patients. Sixty-six per cent of the patients elected to have i.v. sedation; the rest had local pharyngeal anaesthetic spray. All patients were taking proton pump inhibitors.

Endoscopy findings

The mean length of BE was 4 cm (1–13). Twenty-seven (42%) of the patients had short-segment BE (SSBE), mean age 62 years, of whom 14 (51%) were male. Thirty-seven had long-segment BE (LSBE), of similar age (mean 62 years), with more males (24/37, 65%). Forty-three patients (67%) had a hiatus hernia associated with their BE.

Pit patterns observed with EME

All pit patterns were observed (Figure 1), and most patients exhibited more than one pit pattern. The highest grade of pit pattern observed for each individual was as follows: round or oval in one patient, linear/tubular 20, villous 32, cerebriform seven, distorted four. Both (HGD one and adenocarcinoma three) were inconspicuous on the overview mode, however, after EME, irregular/distorted pit patterns were noted, although the cancers also had cerebriform (in two) and villous patterns (one) associated with them.

Histology

The total number of biopsies from EME was 368 (mean 6 per patient), compared with 357, (mean 6) from the previous endoscopy. Histology from EME revealed columnar-lined oesophagus (CLE) in six (9%) patients, SIM without dysplasia in 49 (77%), LGD in five (8%), HGD in one (2%), and adenocarcinoma in three (5%). The relationship between pit pattern observed and histology is shown in Table 1.

Length of BE

A comparison of SSBE and LSBE is shown in Table 2.

Looking at yield of SIM by length of BE, of those with <3 cm (SSBE), 20 of 27 (74%) had SIM (with two cancers); for ≥3 cm 29 of 37 (97%) had SIM (with five LGD, one HGD).

If dysplasia and cancer are excluded, the yield of SIM was 20 of 25 (80%) for SSBE, and 29 of 30 (96%) for LSBE.

Histological change

There was a marked discordance between the histologic findings from conventional surveillance with random biopsy, and those from an EME (Table 3). There was significant disagreement using Maxwell's chi-squared test of overall disagreement (10, $P = 0.03$), whilst the level of agreement (Cohen's weighted kappa) was only 0.30 (95% CI 0.13–0.47; $P = 0.0002$).

Fifteen of the sixty-two patients (24%) who had prior endoscopy and random biopsy had a histological 'upgrade' after EME, two had a histology downgrade, and 46 (74%) stayed the same, as shown in Table 3. A greater proportion of patients with an SSBE <3 cm (8/27, 29%) had an upgrade than those ≥3 cm (7/37, 19%), although this was not statistically significant.

The mean interval between conventional random biopsy and EME was 6 months. The mean interval for the 15 patients who had a histological upgrade was

Table 1. Relationship between pit pattern and histology at EME

	Columnar-lined oesophagus (CLE)	Specialized intestinal metaplasia (SIM)	Low-grade dysplasia (LGD)	High-grade dysplasia (HGD)	Cancer	Total
Round	1	–	–	–	–	1
Linear/tubular	2	16	2	–	–	20
Villous	1	29	2	–	–	32
Cerebriform	2	4	1	–	–	7
Irregular/distorted				1	3	4
Total	6	49	5	1	3	64

Table 2. Characteristics of short-length Barrett's oesophagus (SSBE) and long-segment Barrett's oesophagus (LSBE)

	SSBE (<3 cm)	LSBE (≥3 cm)
n	27	37
Mean age (year)	62	62
Males	51%	65%
CLE	5	1
Intestinal metaplasia	20 (74%)	29 (78%)
LGD	0	5
HGD	0	1
Cancer	2	1

Table 3. Barrett's diagnosis before and after an enhanced magnification endoscopy (EME) $n = 62$ (previous histology not available for two patients)

	Enhanced magnification endoscopy + biopsy				
	CLE	SIM	LGD	HGD	Ca
Random biopsy					
CLE	4	7	1	–	–
SIM	2	40	3	1	2
LGD	–	–	1	–	–
HGD	–	–	–	–	1
Ca	–	–	–	–	–

CLE, columnar-lined epithelium; LGD, low-grade dysplasia; HGD, high-grade dysplasia; Ca, cancer.
Shaded boxes represent unchanged diagnosis. Boxes above the line represent a histologic upgrade, below a downgrade.

7 months, and was only slightly higher for those who had a 'significant' upgrade to dysplasia or cancer (7 months).

Of note, 10 of the 15 upgrades were male, including all the significant upgrades (dysplasia and cancer), whereas the five women only upgraded from CLE to SIM. No women were diagnosed with dysplasia or cancer.

Inter- and intra-observer variability assessment

Two hundred and fifty-four sets of images were collected (rounded pit pattern 51, linear-type 51, cerebriform 77, villous-type 49 and distorted-type 26). The accuracy of predicting each pit pattern type by the six endoscopists is shown in Table 4. The mean accuracy for each type of pit pattern was type I 79% (67–98%), type II 74% (63%–81%), type III 65% (50–88%), type IV 64% (48–81%) and type V 86% (39–100%). The overall accuracy for predicting pit patterns more probable to harbour intestinal metaplasia was 79% (71–89%).

The mean kappa value for inter-observer agreement in assessing the various pit patterns was 0.571 (0.041) (range 0.491–0.636) (Figure 2). The mean kappa value for intra-observer agreement was 0.709 (0.037) (range 0.597–0.841) (Figure 3).

The mean kappa value for inter-observer agreement in predicting pit patterns more probable to harbour intestinal metaplasia or dysplasia was 0.548 (0.039)

Table 4. Accuracy of predicting the various pit patterns for each of the endoscopists on the first assessment

	PP I	PP II	PP III	PP IV	PP V
1st endoscopist	77	63	52	81	39
2nd endoscopist	74	67	55	61	83
3rd endoscopist	88	81	62	72	100
4th endoscopist	98	79	50	68	94
5th endoscopist	72	79	86	48	100
6th endoscopist	67	77	88	51	100

The values are given as percentage values.

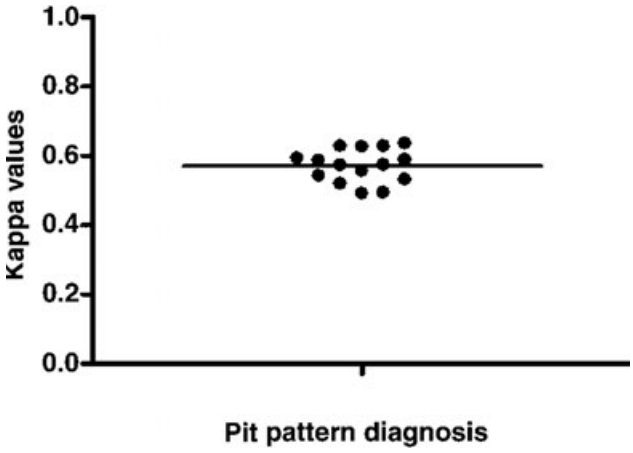

Figure 2. Inter-observer agreement in assessing the various types of pit patterns seen with EME.

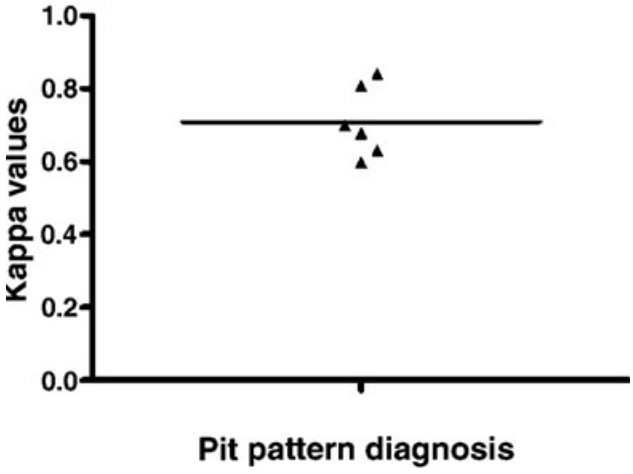

Figure 3. Intra-observer agreement in assessing the various types of pit patterns seen with EME.

(range 0.440–0.703). The mean kappa value for intra-observer agreement in predicting pit patterns more probable to harbour intestinal metaplasia or dysplasia was 0.701 (0.040) (range 0.546–0.815).

DISCUSSION

EME allowed clear demarcation of the Barrett's segment with high definition of mucosal pit patterns, which were selectively targeted according to a hierarchy of pit patterns (I–V). This produced a remarkably high prevalence of dysplasia (10%) and cancer (5%); pooled data from large surveillance programmes in the US estimate the combined incidence and dysplasia to be 1%/year.[9]

Confirmation of SIM is a prerequisite to the confirmation of BE. Excluding dysplasia and cancer, SIM rather than only CLE was found in 40 of 47 (85%) of patients, compared with 48 of 60 (80%) with their previous conventional surveillance endoscopy (Table 2). However EME found an additional four LGD, one HGD and three cancers over a short interval.

A plethora of new techniques have been applied to overcome the limitations of surveillance by random biopsy, including spectroscopy, optical coherence tomography, confocal microscopy and narrow band imaging.[10] Most of these techniques require expensive instruments and are technically demanding. Narrow band imaging combined with magnification endoscopy seems to be a promising new endoscopic imaging technique, aiming to enhance the mucosal surface contrast without the use of dyes. Yet, future studies are awaited to demonstrate its efficacy in the surveillance of patients with BE.[10]

A more accessible technical advancement has been the combination of chromoendoscopy and high resolution magnification endoscopy. Various dyes, including Lugol's iodine, methylene blue, and indigo carmine, have been used.[11–15] We have employed acetic acid to enhance the appearance of the Barrett's segment, because of its simplicity to use, safety, and long track record in the field of colposcopy. Acetic acid results in reversible alteration of the proteins in the cell, and more specifically of the bundling of cytokeratins, modifying their optical properties. A reversible alteration of the nucleoprotein wrapping after the application of acetic acid modifies the refraction index of the nucleus (the aceto-white reaction). The columnar epithelium becomes swollen and the villi and pit pattern becoming more apparent.[16]

Guelrud produced a classification of different mucosal surface patterns observed [6]: I, round pits (representing gastric fundic columnar epithelium); II, reticular; III, villous; and IV, ridged or cerebriform. The yields for detecting SIM according to endoscopic patterns were 0%, 11%, 87%, and 100%, respectively. This technique offers the possibility of sensitive targeting of a hierarchy of pit patterns within the Barrett's segment at endoscopy to confirm intestinal metaplasia, and we have achieved comparable high yields of at least SIM.

Short segments of BE bring their own-specific problems in diagnosis and subsequent management decisions. There is a poor correlation between the endoscopists suspicion of BE and histologic confirmation.[17] SSBE did not appear to be particularly benign

when EME was employed in our study: 74% of patients with SSBE had SIM, and in addition there were two cancers, one with 2-cm BE and one ultra-short (<1 cm). This contrasts with a prevalent attitude that SSBE is less worthy of surveillance, indeed a recent survey of the UK gastroenterologists found that 62% considered that BE <3 cm does not warrant surveillance.[18] Other groups have found a much lower incidence of SIM, with only a quarter of <3 cm (SSBE) yielding SIM in Rudolph *et al*'s study.[19] In another study[17] of 146 patients with endoscopic BE, the yield of SIM fell steadily with the declining length of columnar-lined segment, from >65% (>5 cm) to 50% (3–5 cm) to 25% (<3 cm).

Evidence for cancer risk in SSBE is also equivocal, with findings that increasing length of BE has a consistent but non-significant trend for an increased cancer risk and the presence of aneuploidy.[19] The proposal that only long (e.g. 8 cm) segment BE is followed up would seem to be risky,[20] and the American Gastroenterology Association (AGA) workshop on BE concluded that no discrimination between SSBE and LSBE should be made in determining the surveillance strategy.[21]

Fifteen patients (24%) had a histological upgrade, including eight of particular clinical relevance (Table 2). There are two notable features about these 'significant' upgrades. The first is that they occur within a short mean surveillance interval (7 months). The screening interval for the three cancers was just 1, 6 and 3 months, with previous histology of HGD, SIM and SIM, respectively.

Our study raises the possibility that significant findings which may be amenable to life-saving intervention, such as HGD or carcinoma *in situ*, are missed by sampling error when conventional surveillance endoscopy is employed.[3] In patients who undergo oesophagectomy for HGD, cancer may be present in over a third of the resected specimens subsequently reviewed by the pathologist.[22] However, the commonly held risk for malignant transformation is 1% per year,[23] so this may be because of the finding of three cancers by chance skewing the results.

The second significant finding with histological upgrades is that they all occurred in men. BE is more common in white men, among whom the incidence of oesophageal adenocarcinoma has more than quadrupled over the past few decades.[24] This confirms previous findings that BE is more common in males, and they are at greater risk of subsequent adenocarcinoma[24, 25] Males were more likely than females to have LSBE (65% vs. 35%), but this did not account for the increased risk.

A classification system is useful, when it can be reproducible in clinical practice, so that its application has a reliable outcome. In our study, the accuracy of predicting the pit patterns ranged from 63% to 86% between observers. The mean kappa values for inter- and intra-observer agreement in the diagnosis of pit patterns were 0.571 (0.041) (range 0.491–0.636) and 0.709 (0.038) (range 0.597–0.841), respectively, among endoscopists experienced in endoscopy. The mean kappa values for inter- and intra-observer agreement in predicting histology were 0.548 (range 0.440–0.703), and 0.701 (range 0.546–0.815), respectively, which can be regarded as moderate to substantial.

The technique of EME has several limitations. It is more time-consuming than conventional endoscopy, which may have led us to offer sedation prior to gastroscopy to more patients than we would normally. The acetic acid staining wears off after 4–5 min, so familiarity with the technique and co-ordination with the assisting endoscopy nurse is required. After the first few biopsies, a combination of bleeding and reflux obscures the view and obliterates the pit pattern. This requires frequent washing with water flush via the instrument channel and sometimes the use of antimotility agents like hyoscine. Although the biopsies are targeted, the mean number of biopsies taken was no different to conventional surveillance.

Although our study appears to show dramatic results, it has several limitations and caveats. The comparison between endoscopies is not like-for-like. The previous endoscopy was at a mean interval of 6 months. This seems to be too short for the natural history of BE to account for the change, but a synchronous controlled or cross-over study would control for confounding factors, such as any particular enthusiasm on the part of the endoscopist using EME. The high yield of dysplasia may reflect the total contribution of a specialist Barrett's clinic taking referrals and performing confirmatory endoscopies rather than a true advantage of the technique over the current surveillance techniques.

Endoscopy in BE is notoriously unreliable, from measurement of the length of Barrett's, to sampling error, and the pathologist's interpretation. The changes could have occurred by chance, as seen by the Munich group,[2] although the direction of change is towards a histological upgrade rather than downgrade.

In conclusion, EME allows clear resolution of epithelial pit patterns within BE, and allows targeted biopsy with a high yield of SIM and dysplasia, even in

SSBE. This may lead to a significant upgrade in histology. Additionally, the recognition of pit patterns and histology prediction in BE using the EME technique is reproducible. This indicates that a uniform description of the diverse manifestations of Barrett's mucosa after acetic acid instillation is feasible and practical in clinical practice. Therefore, EME with acetic acid has the appeal of offering a pragmatic, reliable surveillance tool that could be taken up by non-specialist or research-orientated units, the so-called 'real world'. This technique warrants further validation in a large-controlled study.

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