Advances in endoscopic resection and radiofrequency ablation of early esophageal neoplasia
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Randomized trial on endoscopic resection-cap versus multiband mucosectomy for piecemeal endoscopic resection of early Barrett’s neoplasia


ABSTRACT

Background: Endoscopic resection (ER) is an important treatment for high-grade intraepithelial neoplasia and early cancer in Barrett’s esophagus. ER-cap requires submucosal lifting and positioning of a snare in the cap, making it technically demanding and laborious. Multiband mucosectomy (MBM) uses a modified variceal band ligator and requires no submucosal lifting or positioning of a snare. Objective: To compare ER-cap and MBM for piecemeal ER of early Barrett’s neoplasia.

Design: Randomized, controlled trial. Setting: Tertiary-care and community-care centers. Patients: This study involved 84 patients (64 men; median age 70 years) undergoing piecemeal ER of Barrett’s neoplasia. Intervention: Piecemeal ER was performed by using ER-cap (n = 42) or MBM (n = 42). Main Outcome Measurements: Safety, efficacy, procedure time, costs.

Results: Procedure time (34 vs 50 minutes; P = .02) and costs (€240 vs €322; P < .01) were significantly less with MBM compared with ER-cap. MBM resulted in smaller resection specimens than ER-cap (18 x 13 mm vs 20 x 15 mm; P < .01). Maximum thicknesses of specimens and resected submucosa were not significantly different. There were no clinically relevant bleeding episodes. Four perforations occurred, 3 with ER-cap, 1 with MBM (P = not significant). Limitations: Potential bias because of different levels of experience among participating endoscopists.

Conclusion: Piecemeal ER with MBM is faster and cheaper than with ER-cap. Despite the lack of submucosal lifting, MBM appears not to be associated with more perforations. Although MBM results in slightly smaller specimens, the clinical relevance of this may be limited because depth of resections does not differ between both techniques. MBM may thus be preferred for piecemeal ER of early Barrett’s neoplasia.
INTRODUCTION

Endoscopic resection (ER) is an important treatment modality for patients with Barrett’s esophagus (BE) containing early neoplasia (i.e., high-grade intraepithelial neoplasia [HGIN] or early cancer). Recent publications have demonstrated that ER is effective and safe in selected patients with early Barrett’s neoplasia, with 5-year survival rates up to 95%.\(^1\)\(^2\)\(^3\) Compared with surgical esophageal resection, ER of early neoplastic lesions is less invasive.\(^4\)\(^5\)

Different ER techniques have been developed, of which the ER-cap technique, first described by Inoue et al,\(^6\) is the most widely used in the esophagus. This technique involves the use of a specially designed transparent cap with a distal ridge, allowing placement of an electrosurgical snare. After submucosal fluid injection to lift the lesion from the deeper wall layers, a snare is positioned in the ridge of the cap. Subsequently, the lesion is sucked into the cap, and the snare is tightened, creating a pseudopolyp that can be resected by using electrocautery. However, submucosal lifting and snare placement make the ER-cap technique technically demanding and laborious, especially when used for piecemeal ER that requires multiple subsequent resections.\(^7\) For less-experienced endoscopists, the ER-cap technique might therefore be less attractive to apply. Recently, the multiband mucosectomy (MBM) device, a modification of the variceal band ligator, has been introduced for ER.\(^7\)\(^8\) For MBM, the mucosa is sucked into a cap, and by the release of a rubber band, a pseudopolyp is created that can then be resected with a hexagonal snare. It is hypothesized that the contraction force of the rubber band is not strong enough to hold in the muscularis propria and that submucosal lifting is therefore not necessary during use of MBM. Because the MBM cap holds 6 rubber bands, 6 subsequent resections can be performed without removing the endoscope and while using the same snare.

A previous study by our group, evaluating the feasibility of MBM for ER of early Barrett’s neoplasia and comparing results to matched historical ER-cap procedures, suggested that MBM allows safe and easy piecemeal resections, saves time and money, and appears to cause less bleeding.\(^7\) MBM, however, resulted in smaller resection specimens and therefore required more resections per procedure. The aim of this randomized study was to prospectively compare the ER-cap technique and the MBM technique for piecemeal resections in BE with early neoplasia.

PATIENTS AND METHODS

Patient selection

From January 2005 until June 2010, patients scheduled for piecemeal ER were included if they met all following criteria:

- BE with biopsy-proven HGIN and/or early cancer;
- No suspicion of submucosal invasion, based on the macroscopic appearance and/or endosonography;
No signs of lymph node and/or distant metastases on endosonography and CT-scanning of the thorax and abdomen;

Written informed consent.

Indications for piecemeal ER were: (1) monotherapy for removal of early neoplastic lesions (generally for patients with BE >5 cm), (2) as part of a stepwise radical endoscopic resection protocol of the whole BE in multiple sessions, or (3) removal of visible abnormalities before additional ablation therapy.

Endoscopic procedures

All ER procedures were performed at the Academic Medical Center Amsterdam, St. Antonius Hospital Nieuwegein, Catharina Hospital Eindhoven, or the University Hospitals Leuven. The first 34 patients were consecutively included and randomized at the Academic Medical Center and treated by an endoscopist with extensive experience in ER with the use of both techniques (J.B.). Fifty patients were included at the University Hospital Leuven, St. Antonius Hospital, or Academic Medical Center during hands-on training sessions for endoscopists training in ER. These patients were treated by endoscopists with limited experience in performing ER, who participated in a training program for endoscopic detection and treatment of early neoplasia in the upper GI tract (www.endosurgery.eu). Training consisted of a number of 2-day workshops with lectures, live demonstrations, and training on live pig models, followed by hands-on training in patients supervised by one of the trainers of the training program.

Endoscopic procedures were performed with patients under conscious sedation including midazolam with fentanyl or pethidine. During endoscopy, visible lesions were classified according to the Japanese classification for early gastric cancer: type 0-I being protruding, type 0-IIa elevated, type 0-IIb flat, type 0-IIc depressed, type 0-III excavated. In addition, the distance from the incisors, location, and estimated diameter were recorded for each lesion.

The area that needed to be resected was delineated by markings made with argon plasma coagulation (APC) (forced coagulation 20 W, gas flow 1.6 L/minute; ERBE Vio System; Erbe Elektromedizin GmbH, Tübingen, Germany). After the delineation, patients were randomized to the ER-cap or MBM technique, and timing of the procedure was started.

Multiband mucosectomy

MBM was performed by using the Duette MBM system (Cook Endoscopy, Limerick, Ireland), which consists of a transparent cap with 6 rubber bands (inner Ø 9 mm), releasing wires, a cranking device, and a 7 F hexagonal braided polypectomy snare that can be reused for multiple resections because of its shape stability. For MBM, the cranking device and transparent cap were assembled onto the endoscope before the endoscope was reintroduced. The target mucosa was sucked into the cap, and with the release of a rubber band, a pseudopolyp was created. The hexagonal snare was introduced, closed beneath the rubber band, and, by using pure coagulation current (ERBE Vio 40 W), the
pseudopolyp was resected. Immediately after the resection, the snare was retracted, the resected specimen was pushed into the stomach, and the resection wound was inspected. Subsequent resections were performed in the same way, allowing a small overlap (10% - 25%) between adjacent resections to prevent residual tissue bridges (Fig. 1). In the case of a residual tissue bridge, the bridge was lifted and removed by simple snare resection. If a bridge could not be captured in the snare, additional APC could be used to ablate the residual tissue.

**Figure 1.** Piecemeal resection of early Barrett’s neoplasia by using multiband mucosectomy (MBM). A: A 3-cm long, irregular tongue of Barrett’s mucosa at the 7 o’clock position. B: The tongue is delineated with argon plasma coagulation markings. C: View through the transparent MBM cap with the rubber bands. D: View of the pseudopolyp that is created by suctioning the mucosa into the cap and releasing a rubber band. E: A hexagonal snare is placed beneath the rubber band to resect the pseudo-polyp using electrocautery. F: After 2 MBM resections, the delineated area is completely removed.

**Endoscopic resection-cap technique**

For the ER-cap technique, an ER kit (Olympus GmbH; Hamburg, Germany) was used, which contains a spraying catheter, an injection needle, a hard, oblique cap (inner Ø 12 mm), and a crescent-shaped snare. The cap was attached to the tip of the endoscope, and the endoscope was reintroduced. First, the lesion was lifted by submucosal injection of diluted adrenaline (1:100,000 NaCl 0.9%). Then a snare was prelooped in the distal rim of the cap, the mucosa was sucked into the cap, and, by tightening of the snare, a pseudopolyp was created that was resected by using pure coagulation current (ERBE Vio 40 W). After the resection, the snare was disposed of, the specimen was pushed into the stomach, and the resection wound was inspected. For all subsequent resections, submucosal lifting was repeated, and a new snare was used (Fig. 2).

After the last resection, the wound edges were inspected to assess whether all markings used to delineate the target area had been removed. The resection specimens
were retrieved from the stomach by using a foreign-body retrieval basket (disposable 2.5 mm foreign body Roth net; US endoscopy, Mentor, Ohio). Specimens were pinned down on paraffin (mucosal side up) and preserved in formalin for histological evaluation. Timing of the procedure was stopped when the endoscope was removed, after retrieval of all resection specimens and treatment of any complications.

**Histological evaluation**

All specimens were dehydrated in a series of alcohol solutions, sectioned in 2-mm slices, and embedded in paraffin. At a minimum of 4 levels, 200-µm thick slices were cut, mounted on glass slides, and routinely stained with hematoxylin and eosin. All slides were evaluated by a senior pathologist and subsequently independently reviewed by an expert GI pathologist (F.t.K., M.V.) solely for the purpose of the study; both were blinded for the allocated ER technique. Grading of intraepithelial neoplasia was in concordance with the World Health Organization classification. In addition, the maximum diameter of resected specimens was measured macroscopically, and the thickness of the specimens and the thickness of the submucosal stroma in the specimens were measured microscopically by the same pathologist.
Outcome parameters

The following parameters were assessed and compared between both techniques:
- Number of resections per procedure;
- Procedure time (from randomization until the end of the procedure, including treatment of any complications);
- Number and severity of acute (during procedure) and early (0-48 hours) complications. Complications were recorded only if they were clinically significant and graded as “mild” (unplanned hospital admission, hospitalization <3 days, hemoglobin drop <3 g, no transfusion), “moderate” (4-10 days hospitalization, <4 units blood transfusion, need for repeat endoscopic intervention), “severe” (hospitalization >10 days, intensive care unit admission, need for surgery, ≥4 units blood transfusion), or “fatal” (death attributable to procedure <30 days or longer with continuous hospitalization);
- Maximum and minimum diameter of resection specimens (mm);
- Maximum thickness of resected specimens and resected submucosal stroma (mm);
- Radicality of the resections at the deep resection margins;
- Costs of disposables.

Sample size calculation and randomization

It was anticipated, based on a prior study by the Amsterdam group, that ER-cap and MBM would be equally effective and safe for piecemeal ER in BE. However, piecemeal ER by using the ER-cap technique is technically more difficult and requires a greater number of disposables. It was therefore hypothesized that MBM for piecemeal ER might significantly reduce procedure time and costs compared with ER-cap. From a clinical perspective, MBM would be a valid alternative to ER-cap if such a significant reduction of procedure time and costs for piecemeal ER were found, while maintaining a success rate comparable to that of ER-cap. A total number of 84 patients would be required for this study when using a significance level of 0.05 and a power of 80%. This patient number would allow the detection of any significant reduction in costs (>20%) and duration (>30%) of the procedure.

Patients were randomly assigned following a simple randomization procedure to one of the two ER techniques. For the randomization procedure, a set of serially numbered, opaque, sealed envelopes containing an even amount of ER-cap and MBM lots was distributed over the participating centers. Envelopes were opened after patients were considered eligible for inclusion and when the target area was delineated with APC markings.

Statistical and ethical issues

The ethics committees at the Academic Medical Center Amsterdam, St. Antonius Hospital Nieuwegein, Catharina Hospital Eindhoven, and the University Hospitals Leuven reviewed and approved the study protocol and the patient informed consent form. The trial was registered at www.trialregister.nl (NTR1435).
Statistical analysis was performed with SPSS 16.0.2 Software for Windows. For descriptive statistics, mean (± standard deviation) was used in case of a normal distribution of variables, and median (interquartile range [IQR]) was used for variables with a skewed distribution. Where appropriate, the t test and the Mann-Whitney test were used. Median difference between groups and confidence intervals for median differences between groups were calculated with the Confidence Interval Analysis package.16

RESULTS

Patients

During 365 ER procedures for HGIN/early cancer in BE, patients were screened for inclusion (Fig. 3). Eighty-four patients were included: 64 men, 20 women, median age 70 years (IQR 63.3-76.0). Patients were randomized to ER-cap (n = 42) or MBM (n = 42). Baseline characteristics did not differ significantly between groups (Fig. 2).

Endoscopic procedures

Number of resections and procedure time

A total of 84 ER procedures were performed (MBM, n = 42; ER-cap, n = 42). Results are shown in Table 1. In the MBM group, a median number of 5 resections (IQR 3-7) were required to remove the delineated area; in the ER-cap group, a median number of 3 pieces (IQR 2-7) were resected (P = not significant [ns]). Time per resected specimen was significantly shorter with MBM compared with ER-cap, with a median of 7 minutes (IQR 5-10) per piece for MBM and a median of 11 minutes (IQR 8-15) per piece for ER-cap (P < .01). The median difference between groups was 4 minutes (95% confidence interval

![Figure 3. Flow chart illustrating patient screening, reasons for exclusion, and baseline characteristics for included patients.](image-url)

ER= endoscopic resection, HGIN/ER= high-grade intraepithelial neoplasia/early cancer, MBM= multiband mucosectomy, IQR= interquartile range, BE= Barrett’s esophagus.
Time per procedure also differed significantly between groups, with a median of 34 minutes (IQR 20-52) for MBM procedures versus a median of 50 minutes (IQR 30-70) for ER-cap procedures (P = .02; median difference between groups 13 minutes; 95% CI, 3.0-23.0), thus, a 32% reduction of procedure duration.

Complications
Minor bleeding during the ER procedure occurred in 22 patients (52%) treated with the ER-cap technique and in 17 patients (40%) treated with MBM (P = ns). None of these bleeding episodes were considered clinically relevant because all could be treated endoscopically during the same procedure by using conventional haemostatic techniques such as adrenalin injection, hemoclips, coagulation with the tip of a snare, APC, hot biopsy forceps, or a combination thereof. No delayed bleeding was reported.

In 4 patients, an acute perforation occurred: 3 (7%) in the ER-cap group and 1 (2%) in the MBM group (P = ns). The 3 complications occurring during ER-cap resection were graded as moderate, according to the aforementioned definitions. In 1 of 3 patients, the defect was treated by placement of a covered esophageal stent (Esophageal Choo Stent; Fujinon Medical Holland B.V., Veenendaal, The Netherlands) (Fig. 4). In 2 of 3 patients, the luminal defect was closed by placing clips (Resolution clips; Boston Scientific, Limerick, Ireland, UK) on the edges of the perforation and subsequently capturing the clips in an endoloop (Polyloop 30 mm; Olympus, Tokyo, Japan). The wound edges were

| Table 1. Results of piecemeal endoscopic resection for early Barrett neoplasia after randomization to either the ER-cap or MBM technique. |
|---------------------------------------------------|------------------|------------------|------|
|                        | ER-cap technique | MBM technique    | p-value |
|                        | (n=42)           | (n=42)           |       |
| Number of resections/procedure*               | 3 (2-7)          | 5 (3-7)          | ns    |
| Procedure time, min.*                        | 50 (30-70)       | 34 (20-52)       | 0.02  |
| Time/resected piece, min.*                   | 11 (8-15)        | 7 (5-10)         | <0.01 |
| Number of complications:                     |                  |                  |       |
| clinically not relevant bleeding during ER    | 22               | 17               | ns    |
| mild                                          | 0                | 0                | ns    |
| moderate (all perforations)                   | 3                | 0                | ns    |
| severe (perforation)                          | 0                | 1                | ns    |
| fatal                                         | 0                | 0                | ns    |
| Worst pathology in ER-specimens:              |                  |                  |       |
| ND-IM/LGIN†                                   | 8                | 5                | ns    |
| HGIN                                          | 13               | 6                | ns    |
| EC                                            | 21               | 31               | 0.03  |
| Max. diameter of specimens, mm*              | 20 (16-24)       | 18 (15-20)       | <0.01 |
| Min. diameter of specimens, mm*              | 15 (12-19)       | 13 (10-15)       | <0.01 |
| Max. thickness of ER specimen, µm *           | 2000 (1800-2300) | 2000 (1800-2800) | ns    |
| Max. thickness of submucosa, µm *             | 1000 (600-1200)  | 800 (500-1200)   | ns    |
| Costs disposables/procedure, euro’s*         | 322 (246-436)    | 240 (240-480)    | <0.01 |

* Presented as: median (IQR)
† As part of a stepwise radical endoscopic resection protocol to remove the whole BE with HGIN/EC.
approximated, and the defect was closed by tightening the endoloop. The approximation of the clips was optimized by placing a second endoloop underneath the first one (Fig. 4).

All 3 patients received immediate intravenous administration of antibiotics and acid suppressant therapy, an esophageal tube for suction, and were given nothing by mouth. All remained asymptomatic, did not show signs of leakage on contrast swallowing examination, and were discharged from the hospital after 9, 6, and 6 days, respectively.

The perforation in the patient undergoing MBM was graded as a severe complication. Because of peri-esophageal scarring after prior vagotomy surgery and later surgery for placement of an Angelchik antireflux prosthesis, endoscopic closure of the defect was not considered feasible, and the patient was referred for surgery.

One perforation occurred in the group treated by the endoscopist with extensive experience in ER (1/34, 3%) with the use of the ER-cap technique. Three perforations occurred during ER by an endoscopist training in ER (3/50, 6%) with the use of the ER-cap technique (n = 2) or MBM (n = 1) (P = ns).

Resection specimens
The histopathological findings are summarized in Table 1. In the ER-cap group, there were two cases with deep resection margins positive for cancer: in one patient the positive deeper resection margin involved the muscularis mucosae, and subsequent surgery

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Figure 4. Two cases of endoscopic treatment of a perforation caused by endoscopic resection (ER). Case 1, A: Esophageal perforation seen through an ER cap. B: The perforation was treated by placement of a covered esophageal stent. To be able to follow-up the location of the stent on radiography, we placed clips on the proximal end of the stent and on the mucosa at the level of the proximal end of the stent. C: After 2 months, the defect had completely healed. Case 2, D: Esophageal perforation seen through an ER cap. E: Clips were placed on the edges of the luminal defect and captured in an endoloop. The wound edges were approximated by closure of the endoloop. A second endoloop was placed underneath the first endoloop to optimize approximation of the wound edges. F: After 3 months, the defect had completely healed.
showed residual mucosal cancer (T1, N0, M2); in the second patient, the positive deeper resection margin involved the superficial submucosa, and the patient was subsequently treated with chemoradiotherapy because he was a poor candidate for surgery. In the MBM group, all resections were radical at the deep margins (P = ns).

Resection specimens obtained with the ER-cap technique had a larger diameter than MBM specimens (median 20 mm [IQR 16-24] × 15 mm [IQR 12-19] vs. 18 mm [IQR 15-20] × 13 mm [IQR 10-15]) (P < .01). ER-cap specimens were not significantly thicker than MBM specimens (median 2.0 mm [IQR 1.8-2.3] vs. 2.0 mm [IQR 1.8-2.8]) (P = ns) and did not contain a thicker layer of submucosal stroma (median 1000 µm [IQR 600-1200] vs. 800 µm [IQR 500-1200]) (P = ns).

Costs of disposables

Piecemeal ER with the MBM technique was significantly cheaper than with the ER-cap technique (€240 [IQR 240-480] vs. €322 [IQR 246-436], respectively) (P < .01). The median difference between groups was €44 (95% CI, €6.0-€82.0), thus, a 25% reduction of procedure costs.

DISCUSSION

This is the first randomized study comparing the ER-cap and MBM techniques for piecemeal ER of early neoplastic lesions in BE. Prior studies by May et al.18 and Peters et al.7 have demonstrated that the lift-suck-cut technique (ER-cap) and the ligate-and-cut technique (MBM) are highly effective, with low rates of severe complications for ER in BE. The sample-size calculation for this study was therefore based on the hypothesis that MBM for piecemeal ER might significantly reduce procedure time and costs, compared with those for ER-cap. Indeed, MBM proved to be significantly faster than ER-cap. Also, costs for disposables were significantly lower for MBM procedures. Besides the reduced costs for disposables, one may expect that the shorter procedure times with MBM would contribute to a reduction of total procedure costs for piecemeal ER compared with ER-cap procedures.

Besides procedure time and costs, an important outcome parameter was the rate of radical resection of HGIN and/or early cancer. Complete endoscopic removal of neoplasia was reached in all 42 patients treated with MBM and in 40 patients treated with ER-cap (P = ns), which illustrates that both techniques are very effective in this respect. ER-cap resulted in resection specimens with a significantly larger median diameter than those of MBM. A prior study by Matsuzaki et al.19 demonstrated that larger ER specimens also result in deeper resections. In this study, however, the maximum median thickness of the resected specimens and the median amount of resected submucosal stroma did not differ significantly between ER-cap and MBM techniques. One may conclude, based on the comparable specimen thickness and the fact that two resections in the ER-cap group were not radical at the deep resection margin, that the larger resection specimens obtained with ER-cap may not have much clinical relevance in the comparison with MBM.
However, it should be stressed that in this study, we used ER-cap and MBM only in patients who were selected to have lesions that were not suspicious for submucosal invasion, based on endoscopic appearance and endosonography. Furthermore, a standard, hard, oblique cap (inner Ø 12 mm) was used for ER-cap resections in this study. We did not study the use of the larger, soft, oblique cap (inner Ø 16 mm) that is available for ER-cap resections. Matsuzaki et al.\textsuperscript{19} have previously demonstrated that this large-caliber cap results in deeper resections compared with the standard cap used in this study (mean 1.54 mm vs. 1.08 mm; \( P < .01 \)). This may be important to consider in patients who have contraindications for surgery and have lesions that are suspicious for submucosal invasion. In these patients, the initial ER may be the best chance for curative removal of the neoplasia. To increase the chances of completely removing the neoplasia, it may therefore be preferable to use the large, flexible cap in these cases.

In addition to our hypothesis that ER-cap and MBM would be equally effective, as discussed previously, we anticipated that both techniques would be equally safe. Although no significant differences were observed in the rate and severity of complications, there were 3 perforations in the ER-cap group and 1 in the MBM group. Although this difference was not statistically significant, it makes it unlikely that the lack of submucosal lifting with MBM increases the risk of perforation compared with that of the ER-cap technique. The safety of MBM was in concordance with results of a prospective series published by Herrero \textit{et al.}\textsuperscript{20} which showed no perforations in 243 consecutive MBM procedures. However, because the sample size was not calculated to find a significant difference in complications, the numbers in both treatment groups may have been inadequate to detect statistically significant differences in complications between MBM and ER-cap techniques.

Compared with the ER-cap technique, the MBM technique was significantly faster, because it did not require repeated submucosal lifting for each resection or positioning of the snare in the rim of the cap and keeping it in place. However, despite its relatively easy application, a drawback while using MBM may be the decreased visibility through the distal attachment cap holding the rubber bands. Not only do the bands narrow the endoscopic view, but the black rubber also absorbs the light that is emitted by the endoscope. A good pre-procedural plan with delineation of the target area by placement of markers before placing the MBM cap is therefore necessary to achieve complete endoscopic removal of a lesion.

For this study, the first patients were treated by an endoscopist with extensive experience with the use of the ER-cap and MBM techniques. However, to be able to extrapolate the results of this study to centers with less experience in ER, we extended the study to a multicenter setting, and the last 50 ER procedures were performed by endoscopists with little experience in ER. These endoscopists participated in a training program on endoscopic detection and treatment of early upper GI neoplasia. In patients treated by the experienced endoscopist and patients treated by endoscopists in training, MBM proved equally safe and effective as ER-cap, but it was faster and cheaper. The learning curve for MBM also may be shorter compared with that of ER-cap, because it combines the commonly known techniques of variceal band ligation and polypectomy. Our data did not allow us to ascertain whether the advantages of MBM were more
pronounced for the expert cases than for the cases treated by endoscopists in training. Conceptually, however, the benefits of MBM should have a greater impact for the latter group, given that MBM is easier to learn.

In conclusion, the data of this randomized study suggest that MBM and ER-cap have a comparable success rate for radical resection of early Barrett’s neoplasia in selected patients. Despite the lack of submucosal lifting, MBM was not associated with more perforations or bleeding. In addition, MBM was significantly faster and cheaper compared with ER-cap. MBM may therefore be preferred for piecemeal ER of Barrett’s mucosa containing early neoplasia.
Chapter 3

REFERENCES


