Endoscopic eradication of Barrett's oesophagus with early neoplasia

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ENDOSCOPIC ERADICATION OF BARRETT’S OESOPHAGUS WITH EARLY NEOPLASIA
Endoscopic eradication of Barrett’s oesophagus with early neoplasia

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INTRODUCTION

The last decades, endoscopic treatment of early neoplasia in Barrett’s oesophagus has evolved as a valid and less invasive alternative to surgical resection in patients with a low risk of lymph node metastasis. Endoscopic resection (ER) is the cornerstone of endoscopic therapy, since it not only allows for curative removal of neoplastic lesions, but also enables an accurate histological diagnosis required to select patients with a low risk of lymphatic spread.

ER was pioneered in Japan, mainly for the resection of gastric lesions and squamous oesophageal neoplasia. In the Western countries, ER has increasingly been applied for the treatment of early gastro-oesophageal neoplasia, mostly associated with Barrett’s oesophagus. Promising results of ER for removal of early Barrett’s neoplasia published in the last years have made ER the treatment of choice for this indication. Technical developments and increasing possibilities for structured training in ER have resulted in more widespread implementation of ER.

In 2000 we started to use ER as monotherapy for neoplastic lesions in Barrett’s oesophagus, but we found that a significant number of patients required additional treatment for recurrent neoplasia in the remainder of their Barrett’s oesophagus. We then started studying different approaches to prevent recurrent lesions after focal ER of neoplasia. First, we evaluated the use of photodynamic therapy (PDT) with 5-aminolevulinic acid to eradicate all Barrett’s mucosa. This treatment protocol, however, was abandoned in 2003 when we found that most patients still had residual Barrett’s mucosa after PDT and a sustained remission of neoplasia was only achieved in 27% of patients. By then, we had gained more experience with widespread ER, which led to a treatment approach for which the whole Barrett’s segment was removed during subsequent ER sessions. This stepwise radical endoscopic resection protocol (SRER) was proven to be safe and effective in our hands, although the procedure was found to be technically demanding and associated with a significant rate of oesophageal stenosis. As a result, only patients with a Barrett’s segment shorter than 5 cm were deemed eligible for this protocol. In 2004, we therefore engaged in research on the use of a promising new ablation technique: stepwise circumferential and focal radiofrequency ablation (RFA) using the HALO system. Our group was the first worldwide to use this technique for treatment of high-grade intraepithelial neoplasia in Barrett’s oesophagus, as well as to use it in combination with ER of endoscopically visible abnormalities. Two pilot studies at our centre demonstrated that this approach was highly effective and safe for eradication of all neoplasia and all intestinal metaplasia.

After the single center studies performed in Amsterdam to evaluate SRER and RFA for treatment of early Barrett’s neoplasia, we studied both treatment approaches in a European multicentre setting. This unique collaboration between a number of leading European centres, allowed for inclusion of large numbers of patients from different backgrounds, treated by different endoscopists with expertise in the field of Barrett’s neoplasia. The results of those studies are described in this thesis.

OUTLINE OF THIS THESIS

Part A “Endoscopic Resection”

The first part of this thesis is focussed on endoscopic resection (ER) of early neoplasia in the upper gastrointestinal tract. Chapter 1 is a review on different ER techniques and their indications in the upper gastrointestinal tract. Prior to ER, endoscopic ultrasound (EUS) is often used for loco-regional staging of neoplasia. In Chapter 2 we evaluate the clinical value of EUS, next to endoscopic inspection and diagnostic ER, during work-up for endoscopic treatment of early oesophageal neoplasia in 131 patients. As described in Chapter 2, ER not only allows for removal of neoplastic lesions, but it also enables accurate histological staging of the removed lesion. In Chapter 3 we describe a randomized study comparing the safety and efficacy of the ER-cap and multiband mucosectomy (MBM) technique, for piecemeal ER of early Barrett’s neoplasia. After focal ER of neoplasia, there is a risk of 30% that patients develop metachronous lesions in the remainder of the Barrett’s oesophagus (BO). To prevent this, the remainder of the Barrett’s segment can be removed during subsequent ER sessions. In Chapter 4 we report on the combined experience of four European centers with this approach of stepwise radical endoscopic resection (SRER) in 169 patients.

Part B “Radiofrequency Ablation”

The second part of this thesis evolves around radiofrequency ablation (RFA), starting with Chapter 5, a review on the technical background of RFA and its role in the treatment of BO. Chapter 6 describes the first experiences at the Academic Medical Center in Amsterdam with RFA, with or without prior ER for visible lesions, in 44 patients with BO containing early neoplasia. To evaluate if the promising results of RFA could be reproduced in other centres, a multicentre study at 3 European sites was initiated. Chapter 7 reports on the results of this EURO-I study, which included 24 patients. Given the promising results of ER for focal lesions followed by RFA of the remaining Barrett’s segment, a randomized trial was performed to compared this approach to SRER in patients with early neoplasia in a BO <5 cm in length. The results of this study are reported in Chapter 8. For patients with a BO measuring up to 12 cm in length, a prospective cohort study in 13 European centres was initiated. This EURO-II study, described in Chapter 9, included 130 patients who were treated for early neoplasia in BO by a combination of ER and RFA. The efficacy of RFA to eradicate pre-existing genetic abnormalities in BO was studied by immunohistochemical staining and FISH. In addition, biopsy sampling depth and presence of buried Barrett’s in biopsies, keyhole biopsies and ER specimens from post-RFA neosquamous mucosa were evaluated. The results of this study are described in Chapter 10.

Chapter 11 evaluates the presence of post-RFA buried Barrett’s in biopsies from endoscopically normal neosquamous mucosa, and discusses artifacts that may lead to a false positive histological finding of buried Barrett’s.
PART I
Endoscopic Resection
Endoscopic resection of early oesophageal and gastric neoplasia

Roos E. Pouw, Jacques J. Bergman

Best Practice & Research Clinical Gastroenterology — 2008; 22: 929-943
ABSTRACT

The last decades, endoscopic treatment of early neoplastic lesions in oesophagus and stomach has evolved as a valid and less invasive alternative to surgical resection. Endoscopic resection (ER) is the cornerstone of endoscopic therapy. Next to the curative potential of ER, by removing neoplastic lesions, ER may also serve as a diagnostic tool. The relatively large tissue specimens obtained with ER enable accurate histological staging of a lesion, allowing for optimal decision making for further patient management. ER was pioneered in Japan, mainly for the resection of gastric lesions and squamous oesophageal neoplasia, and also Western countries have been increasingly implementing ER in the treatment of early gastro-oesophageal neoplasia, mostly associated with Barrett’s oesophagus. In this review we will give an overview of different techniques that have been developed and modified for ER of early gastro-oesophageal neoplasia, and we will discuss the indications for ER in the oesophagus and stomach.

INTRODUCTION

The last two decades endoscopic therapy has been proven a safe and effective alternative to surgery for the treatment of early gastro-oesophageal neoplasia. The cornerstone of endoscopic therapy is endoscopic resection (ER), which is not only a potential curative tool, but may also serve as an excellent diagnostic aid. ER was pioneered in Japan, mainly to resect early gastric cancers and early squamous lesions in the oesophagus. Also in Western countries endoscopic treatment has gained acceptance and has increasingly been implemented, mostly for the management of early neoplasia associated with Barrett’s oesophagus (BO). In contrast to surgical resection, which also allows for dissection of local lymph nodes, ER is limited to local removal of a lesion. Only patients with a minimal risk of lymph node metastases are, therefore, candidates for curative endoscopic treatment. This implies that optimal selection of patients for ER is of the utmost importance. Lesions limited to the mucosa (i.e. m1-m3) have a minimal risk of lymphatic involvement and may be treated endoscopically. Lesions infiltrating deep into the submucosa, poorly differentiated cancer, or the presence of lymphatic/vascular invasion are considered risk factors for lymph node metastasis and are, therefore, indications for surgical treatment. Next to detailed endoscopic inspection of a lesion and assessment of the infiltration depth with endoscopic ultrasound, the optimal staging tool is a diagnostic ER that provides a relatively large tissue specimen for accurate histological evaluation of these risk factors, on which further patient management can be based. A wide range of different ER techniques and tools have been developed and modified to facilitate ER, make the procedure safer, and to allow for removal of neoplasia in one piece. In this chapter we will discuss different ER techniques, novel developments in this area, and indications for ER in the oesophagus and stomach.

GENERAL ASPECTS OF ENDOSCOPIC RESECTION

En-bloc resection vs. piecemeal endoscopic resection

Most conventional ER techniques allow for en-bloc resection of lesions with a maximum diameter of 2 cm. Larger lesions require resection in multiple resections during a so-called “piecemeal” procedure. Piecemeal resections are technically more demanding, time-consuming, have a higher risk of complications, and are associated with a higher rate of local recurrence. Next to detailed endoscopic inspection of a lesion and assessment of the infiltration depth with endoscopic ultrasound, the optimal staging tool is a diagnostic ER that provides a relatively large tissue specimen for accurate histological evaluation of these risk factors, on which further patient management can be based. A wide range of different ER techniques and tools have been developed and modified to facilitate ER, make the procedure safer, and to allow for removal of neoplasia in one piece. In this chapter we will discuss different ER techniques, novel developments in this area, and indications for ER in the oesophagus and stomach.

Delineation and marking of the target lesion

To ensure radical resection of a suspicious lesion with a disease free margin, it is important to delineate the extent of a lesion with detailed endoscopic inspection using a high-quality endoscope. Advanced imaging techniques, such as chromoendoscopy (e.g. using acetic acid, indigo carmine, Lugol staining), zoom-endoscopy or narrow-band imaging may be helpful to assess the extent of a lesion. Since the endoscopic view during ER may be impaired by the use of distal attachment caps, submucosal lifting and bleeding, it is advisable to place coagulation markings 2-5 mm outside the lateral margins of the target lesion. The markings can be made with an argon plasma coagulation (APC) probe, the tip of an elec-
trococluation snare, or with a needle knife. Especially for lesions that require piecemeal resection, demarcation with markings may be useful to achieve complete resection with a tumour free margin.

**Submucosal lifting**

For some ER techniques, the mucosa needs to be lifted from the deeper wall layers by injection of a fluid into the submucosal layer. This submucosal lifting makes the mucosa more accessible for resection and it protects the deeper oesophageal wall layers for thermal injury and perforation. Furthermore, the type of submucosal lifting may provide useful information on the infiltration depth of a lesion. Kato et al. described four types of submucosal lifting in relation to the infiltration depth in a series of colorectal cancers. Type 1 lifting was described as complete, soft lifting that made the lesion stretch like a ‘dome’, and these lesions only showed superficial infiltration (max. T1sm1) upon histological evaluation. With type II lifting the lesion lifted completely, but hard, meaning that the form of the lesion was maintained. Lesions with type III lifting were mostly mucosal lesions, some were T1sm2, but all lesions could be resected radically. When the lesion could be lifted, but stayed behind compared to the surrounding mucosa (type III lifting), this was associated with submucosal infiltration, mostly T1sm2. If there was no lifting at all (type IV lifting), most colorectal lesions penetrated deep into the submucosa (T1sm3). Type IV lifting is, therefore, a contraindication for ER. In BO and in the stomach, however, is has to be noted that prior ulceration and inflammation may have resulted in scarring that impairs submucosal lifting.

**ENDOSCOPIC RESECTION TECHNIQUES**

**Strip-biopsy ER**

The strip-biopsy was the first technique described in relation to ER. For strip-biopsy a double-channel endoscope is used. After submucosal lifting of the target area, a polypectomy snare is opened over the lesion, and through the second working channel of the endoscope a grasping forceps is introduced to pull the mucosa through the opening of the snare. By pushing the snare down and closing it, the mucosa is captured in the snare and can be resected using electrocautery. Since the strip-biopsy technique does not require the use of a distal attachment cap visualization of the target lesion is optimal, and the centre of the lesion can be targeted precisely with the grasping forceps. By grasping the lesion, however, the tissue is damaged, impairing the histological evaluation, and the specimens obtained with strip-biopsy are usually small (10-15 mm) resulting in a low rate of radical en-bloc resections. In addition, strip-biopsy requires the use of a double-channel endoscope and an additional assistant. A number of different modifications to the strip-biopsy technique have been reported. For the resection of protruding lesions the double-snare strip-biopsy technique has been described, where a second snare is used for grasping the mucosa instead of a grasping forceps. Inoue et al. were the first to report on the use of a transparent overtube to provide better visualization of the lesion, decrease the risk of perforation, to make grasping of the lesion easier by suctioning the lesion into the tube, and to enable manipulation of the lesion to a better position. Another strip-biopsy modification described placement of 4 clips around the lesion, to enable easy and correct positioning of the snare around the lesion.

For resection of lesions that are difficult to approach, ER using a side-viewing endoscope, a special electrocautery snare, and a partial transparent cap has been reported. None of these modifications on the strip-biopsy technique are, however, widely used nowadays.

**Lift-and-snare technique (Fig. 1)**

With the lift-and-snare technique the target area is first marked, then lifted with submucosal fluid injection and subsequently resected by closing a polypectomy snare directly over the elevated lesion. The most commonly used snares for this technique are normal braided polypectomy snares. The braided, round wires of these snares have little friction with the mucosa, and since these snares are often relatively soft, the tip of the snare may lift off the mucosa when the proximal end is pressed against the mucosa during closure. To avoid the lesion to slip out of the snare during closure, it is important to correctly position the opened snare and to apply suction while closing the snare. To make capturing of lesions easier, special snares are available, for example snares with a short needle at the tip, to allow it to be anchored into the mucosa, to hold it from being lifted off when the proximal end of the snare is being pushed down. Other snares have small hooks attached to the wires on both sides of the loop to increase friction with the mucosa. Furthermore, stiff monofilament snares with a rectangular shape are available. The relative stiffness prohibits the distal tip of the snare from lifting up during snare closure, and the “sharper” edges make it easier to grasp the lesion. For flat type lesions or lesions in difficult locations, however, it remains difficult to capture the lesion and to achieve targeted, complete en-bloc resection. In the upper GI-tract, the lift-and-snare technique is mainly used in the stomach. For oesophageal lesions the lift-and-snare technique is, however, often not preferable, since most lesions in the oesophagus are located tangentially to the endoscope making positioning of the snare difficult. Giovannini et al. have used this technique for widespread mucosal resections in squamous and Barrett’s oesophagus using a needle-tipped polypectomy snare.

**Simple snare technique**

The simple snare, or bare snare, technique is comparable to the lift-and-snare technique, but without the submucosal lifting. The snare is simply placed over the target area and pressed against the mucosa that is drawn in the loop by applying suction via the endoscope. The snare is then closed and the mucosa is resected using electrocautery. For en-bloc resection of a lesion the simple snare technique may be less suitable, since it is difficult to position the centre of the lesion in the snare, and keep it there during closure of the snare. For resection of flat mucosa without focal lesions, however, the simple snare technique can be useful. The Hamburg-group has used a monofilament snare with a diameter of 0.4 mm, measuring 30x50 mm when opened, for widespread oesophageal ER, and despite the lack of submucosal lifting they did not encounter any perforations.
Ligate-and-cut technique (Fig. 3)

The ligate-and-cut technique is an easier alternative to the lift-suck-and-cut technique. For the ligate-and-cut technique a distal attachment cap, holding one or more rubber bands, is attached to the tip of the endoscope. The target lesion is sucked into the cap and by releasing a rubber band the mucosa is captured. This pseudo-polyp can then be resected with a snare. The ligate-and-cut technique a number of different accessories are available. The multiband mucosectomy device (Duette®, Wilson Cook, Limerick, Ireland) consists of a transparent cap that holds six rubber bands and allows for passage of a 7 Fr hexagonal snare through the accessory channel of the cranking device alongside the releasing wires, allowing resection after ligation without having to remove the endoscope.35,36 The re-usable Euroligator device (Euroligator; Mandel and Rupp, Erkrath, Germany) consists of a non-transparent cap that only holds one rubber band, and after ligation of the mucosa the endoscope needs to be removed in order to introduce a snare to resect the pseudo-polyp.36 An advantage of the ligate-and-cut technique over the lift-suck-and-cut technique is that no submucosal lifting is required, since the rubber band is not strong enough to hold in the deeper oesophageal wall layers. This makes the ligate-and-cut technique easier and quicker to apply, especially when used for piecemeal procedures.37 Despite the lack of submucosal lifting, the ligate-and-suck technique does not appear to be associated with a higher risk of complications as has been demonstrated in studies comparing both techniques.35,37
Endoscopic submucosal dissection

Endoscopic submucosal dissection (ESD) is a technique that overcomes the problem of piecemeal ER for larger neoplastic lesions, and allows for a better-targeted resection of a lesion. By using ESD the indication for ER is, therefore, extended to lesions with a diameter >2 cm. The concept of ESD is to incise the mucosa around a lesion, regardless how large, and then remove the mucosa by submucosal dissection using an electrosurgical knife instead of a snare. After careful delineation of a lesion, coagulation markings can be placed around the lesion using the tip of an electrosurgical knife, about 5 mm outside the margins of the lesion. The marked incision line and the mucosa within the markings are lifted by submucosal fluid injection. With an electrosurgical knife, the incision line can then be incised circumferentially around the lesion, while constantly repeating submucosal lifting to ensure a safe submucosal fluid cushion. When the incision around the lesion has been completed, the submucosa underneath the lesion can be dissected completely to remove the diseased part of the mucosa in one piece. A range of different techniques and types of electrosurgical instruments have been developed, of which the most widely used techniques are described below.

ESD using an IT knife

The insulated-tipped knife (IT knife) (KD-610L, Olympus, Tokyo, Japan) has a tip that is insulated with a small ceramic ball that prevents injury to the muscularis mucosa while dissecting the mucosa and submucosa. After submucosal lifting, a small mucosal incision is made with a standard needle knife, through which the ceramic ball of the IT knife can be introduced. The rest of the needle knife can then be used to make the circumferential mucosal incision, and subsequently to dissect the submucosa. Since the tip of the IT knife cannot be used for the dissection, all the cutting has to be performed with the blade that only allows for limited direction of the cutting.

ESD using a hook knife

The hook knife (KD-620LR, Olympus, Tokyo, Japan) has a ‘hooked’ tip of 1 mm in length that can be rotated to the optimal cutting direction. The hook of the knife can be used to hook onto the submucosal tissue, pull it, and then cut it, which allows for safe resection. If a transparent ESD cap is used, the tissue can be pulled into the cap, making resection even safer.

ESD using a flex knife

The flex knife (KD-630LR, Olympus, Tokyo, Japan) is a spiralled, multi-filament snare with a round tip and a flexible soft sheet. The flex knife can be adjusted in length and can be used to place markings, to make the initial circumferential incision, and to perform the submucosal dissection.

ESD using an ST hood

The small-calibre-tip transparent hood (ST hood) (DH-15GR, Fujinon, Saitama, Japan) can be placed on the tip of an endoscope and is used to open the incision line for better visualization of the submucosal layer during the submucosal dissection, making the procedure easier and safer. It can be used in conjunction with any of the aforementioned ESD knives.

ESD using a TT knife

The triangular tip knife (TT knife) (KD-640L, Olympus, Tokyo, Japan) has a small triangular metal tip that can be used for multiple purposes during ESD. The tip can be used to place markings, to perform the mucosal incision around the lesion, and to dissect the submucosa. The plate of the triangular tip allows for effective coagulation of blood vessels.

ESD using an MBM cap

A transparent ESD cap is used to place markings, to make the initial circumferential incision, and to perform the submucosal dissection. The MBM cap has been attached to the tip of the endoscope, and the lesion is targeted. By suctioning the lesion into the cap and releasing a rubber band, a pseudo-polyp is created. This pseudo-polyp is resected with an electrosurgical snare. After the resection the wound is inspected and no signs of bleeding or perforation are observed. Histological evaluation of the resection specimen showed high-grade dysplasia (H&E staining, original magnification 4x).

Endoscopic resection of early oesophageal and gastric neoplasia - CHAPTER 1
R-scope facilitated ESD in porcine models, and a small series of human cases. A drawback of the R-scope, however, is its relatively large diameter of 14.3 mm resulting from the incorporation of two movable instrument channels and the multibending system, which makes working in the retroflex position difficult. Further refinements of this therapeutic “R-scope”, however, may make it a useful tool for ESD.

**Use of a water-jet for submucosal lifting and dissection**

A novel development that allows for easy and effective submucosal lifting is the use of the Helix HydroJet® (Erbe Elektromedizin GmbH, Tübingen, Germany). The HydroJet can produce a focused water jet that may be used for dissection of interstitial tissue and parenchymatous organs. The precise water jet is able to separate parenchyma, while it leaves fibrous tissue, such as vascular walls and nerve tissue, unharmed. Studies in porcine models and in human oesophagectomy specimens have demonstrated that the HydroJet can successfully penetrate the mucosa to create a selective fluid accumulation in the submucosal layer. The use of HydroJet submucosal lifting for the ER and ESD has to be evaluated in clinical studies, but due to its selective dissecting properties it appears to optimize and facilitate the procedures.

**Use of balloon-assisted techniques**

Another development that may allow for safer and easier endoscopic resection of early neoplasia is the use of balloon-assisted techniques. After submucosal lifting, a mucosal incision is made through which a balloon, e.g. a biliary retrieval balloon catheter, is inserted into the submucosal space. By inflating the balloon, the mucosa is mechanically separated from the deeper wall layers, without the need for electrocautery. By repeated inflation, a marked lesion can be completely loosened from the underlying muscularis propria, and this mucosal flap can then be resected with a needle knife. This approach has, however, only been evaluated in the porcine stomach, but may prove an easy and safe technique for ER in the future, and may overcome the problem of electrocautery artifacts that may impair histological evaluation.

**INDICATIONS FOR ENDOSCOPIC RESECTION**

**Early neoplasia arising in Barrett’s oesophagus**

Endoscopic resection for early neoplasia in BO has been proven safe and effective for lesions limited to the mucosa (m1-m3). The risk on lymph node metastasis in these patients is <2%, which is lower than the 30-day mortality risk after surgical oesophagectomy. Indications for ER in a BO are solitary lesions type 0-I, 0-IIa, 0-IIb or type 0-IIc, with a maximum diameter of 2 cm. If histopathological evaluation shows that a lesion was radically resected, limited to the mucosa (HGIN, m1, m2, m3), and if there were no signs of lymphatic or vascular infiltration, it can be assumed that the patient was curatively treated. In these patients additional treatment to eradicate the residual Barrett’s epithelium to prevent metachronous lesions is justified. Relative indications for ER of early Barrett’s neoplasia are infiltration in the superficial submucosa (T1sm1, <500 µm), poorly differentiated cancer (G3), and lesions with a diameter >2 cm. Treatment of these patients should be performed at expert centres, or under an IRB-approved study protocol. In case of infiltration beyond the superficial submucosa (>sm1), lymphatic or vascular infiltration, or undifferentiated cancer (G4), patients should not be treated endoscopically, but with alternative treatment modalities such as surgery, chemo- and/or radiotherapy.

**Early neoplasia arising in the squamous oesophagus**

Compared to Barrett’s neoplasia, ESCC invades the muscularis mucosae and submucosa at a particularly early stage, lymphatic invasion then occurs quickly, and distant metastases are seen in nearly 30% of patients. Indications to perform ER of ESCC are flat type lesions (type 0-IIa, 0-IIb, 0-IIc) that are mostly limited to the mucosa. Protruding (0-I) and ulcerated lesions (type 0-III) almost always invade the submucosa and should not be resected endoscopically. Lesions that are histologically confined to the epithelium and lamina propria (m1, m2) have a very low rate of local lymph node metastases (<5%) and are considered amenable for endoscopic treatment. Lesions invading into the muscularis mucosa (m3) or superficial submucosa (sm1), however, have an increased risk of lymph node metastases of approximately 10%. These lesions are, therefore, only relative indications for endoscopic treatment, e.g. in patients who carry an increased risk for severe complications of oesophagectomy.
PART I - Endoscopic Resection

Endoscopic resection of early oesophageal and gastric neoplasia - CHAPTER 1

FUTURE PROSPECTS OF ENDOSCOPIC RESECTION

For early neoplasia arising in Barrett’s oesophagus promising new ablation techniques, such as radiofrequency ablation (RFA), will adopt an important position in endoscopic treatment. RFA has been proven safe and highly effective in the eradication of Barrett’s epithelium and its associated dysplasia.1-3,22 Although ER will remain essential to remove visible lesions for diagnostic purposes and to render the mucosa flat, RFA may make widespread resections in BO unnecessary.3,22 Also for early squamous neoplasia, focal ER of visible lesions followed by RFA of residual flat type dysplasia may be a promising treatment modality.22 For other indications that still require widespread ER, techniques that allow for en-bloc resection, such as ESD, will replace the need for piecemeal resections. ESD is still technically demanding and, therefore, not widely practised outside Japan. New developments that facilitate easier ESD, however, may increase its clinical implementation. An important problem associated with ER is scarring of the oesophageal wall, resulting in stenosis.25-27 Medical interventions, however, will hopefully improve wound healing and decrease the high rate of oesophageal stricture after extensive ER. Although new developments have made ER technically easier, and despite promising new ablation techniques, it should be born into mind that endoscopic treatment is only part of the overall management strategy of patients with early gastro-oesophageal neoplasia. Next to being skilled in endoscopic treatment, an endoscopist must be experienced in detecting and delineating early neoplasia, and in selecting patients that are eligible for curative endoscopic treatment. In addition, adequate histopathological evaluation of ER specimens may be difficult. Thus, to ensure optimal patient care, endoscopic treatment of early gastro-oesophageal neoplasia should be centralised in centres with multidisciplinary experience in this field. Structured training aimed at improving endoscopic detection, endoscopic treatment and histological evaluation of ER specimens is, therefore, necessary (e.g. www.endosurgery.eu). By using a teaching-the-teachers model, such structured training programs may also arise in countries where endoscopic therapy is, as yet, non-existent.

SUMMARY

Over the last decades, ER of early gastro-oesophageal neoplasia in selected patients has been proven a safe and effective alternative to surgical resection. Adequate endoscopic work-up to identify patients that are eligible for endoscopic treatment is of the utmost importance, to select patients with a minimal risk of lymphatic involvement that can be cured by local endoscopic treatment. ER plays a pivotal role in this patient selection since it provides a large tissue specimen for accurate histological evaluation of risk factors for lymphatic involvement. To ensure optimal endoscopic work-up, histological evaluation and patient management, patients should be referred to centres with multidisciplinary expertise in this field. A number of different ER techniques are available. Developments such as the multi-band mucosectomy device make widespread ER easier, faster and possibly safer. Other developments, such as ESD, widen the indication for ER by allowing en-bloc resection of lesions with a diameter >2 cm. A number of promising new techniques are being developed all aimed at making ER easier to perform, safer, and to allow for en-bloc resection of larger lesions.

REFERENCES


Do we still need endoscopic ultrasound (EUS) in the work-up of patients with early oesophageal neoplasia? A retrospective analysis of 131 cases

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ABSTRACT

— Background: EUS is often used for locoregional staging of early oesophageal neoplasia. However, its value next to endoscopic inspection and diagnostic endoscopic resection (ER) may be questioned, since diagnostic ER allows for histological assessment of submucosal invasion, and other risk factors for lymph node metastasis (N+), e.g. poor differentiation/lymph-vascular invasion.

— Objective: Evaluate how often patients were excluded from endoscopic treatment of oesophageal neoplasia based on EUS.

— Design: Retrospective cohort study.

— Setting: Tertiary care institution.

— Patients: Patients with early oesophageal neoplasia.

— Interventions: EUS, diagnostic ER.

— Main outcome measurements: Number of patients excluded from endoscopic treatment based on EUS results.

— Results: 131 patients were included (98 men, age 66±13 yrs). In 105/131 patients EUS was unremarkable. In 25/105 (24%) patients diagnostic ER showed submucosal invasion (n=17), deep resection margins positive for cancer (n=2, confirmed at surgery), or poor differentiation/lymph-vascular invasion (n=6). In 26/131 patients EUS raised suspicion on submucosal invasion and/or N+. In the 14/26 patients with abnormal EUS, endoscopy was unremarkable. Diagnostic ER showed submucosal invasion in 7/14 (50%) patients, whereas no N+ risk factors were found in 7/14 (50%) patients, who subsequently underwent curative endoscopic treatment. After diagnostic ER, no risk factors for N+ were found in 3/12 (25%) patients.

— Limitations: Retrospective study.

— Conclusions: This study shows that EUS has virtually no clinical impact in the work-up of early oesophageal neoplasia and strengthens the role of diagnostic ER as a final diagnostic step.

INTRODUCTION

The last two decades, endoscopic therapy has proven its role in the management of early neoplasia (i.e. high-grade intraepithelial neoplasia (HGIN) or intramucosal cancer (IMC)) of the oesophagus and cardia. Endoscopic therapy offers a safe, effective and significantly less invasive alternative to surgical resection. Only neoplasia limited to the mucosal layer, which is associated with a minimal risk for lymph node metastasis, is indicated for endoscopic management. In the case of submucosal infiltration the risk of lymphatic involvement increases significantly and patients need to be referred for surgical resection. The work-up of patients who are considered for endoscopic treatment should therefore be aimed at identifying patients with neoplasia confined to the mucosa and thus with a low risk for lymphatic spread. Next to endoscopic inspection, endoscopic ultrasonography (EUS) is often used to evaluate the infiltration depth of a lesion and the presence or absence of suspicious lymph nodes. Although EUS is the most accurate technique for locoregional staging of oesophageal and cardiac cancer, several studies have demonstrated that EUS is a suboptimal technique to distinguish mucosal from submucosal lesions and to assess for positive lymph nodes in the case of early neoplasia. Diagnostic endoscopic resection (ER) may be used as a final step in the work-up for endoscopic treatment of early neoplasia. ER of a neoplastic lesion provides a relatively large tissue specimen, which allows for accurate histological staging of the infiltration depth, as well as other prognostic factors such as tumour differentiation grade and lymphatic and vascular involvement (Fig. 1).

In our center, ER is used in the work-up of virtually all patients with early neoplasia of the upper GI tract and since it provides more accurate information on infiltration depth than EUS, we questioned the value of EUS in this setting. Most studies have evaluated the accuracy of EUS for T and N staging. However, this does not allow assessing if EUS rightfully impacts on making appropriate decisions on who to treat endoscopically and who not. The aim of this retrospective study was therefore not to study the accuracy of EUS for T and N staging, but to evaluate how often the outcome of EUS changed the management approach of our patients with early oesophageal neoplasia.

Figure 1. Images obtained during work-up of an early Barrett’s cancer.
A: Type 0-IIa-IIc lesion, suspicious for submucosal invasion, but accessible for diagnostic ER. B: During EUS the lesion appeared to be infiltrating the submucosa. C: The lesion was removed by ER and histological evaluation showed a moderately differentiated adenocarcinoma, limited to the muscularis mucosae, without lymph-vascular invasion.
METHODS

Patient selection and data collection

For this study two reviewers independently performed a retrospective evaluation of all patients undergoing upper gastrointestinal EUS between May 2001 and June 2007, at the Academic Medical Center, Amsterdam, the Netherlands. Only patients undergoing EUS for staging of early oesophageal or cardia neoplasia who were considered for endoscopic treatment were included. Exclusion criteria were: (1) all other indications than staging of neoplasia; (2) prior treatment of oesophageal or cardia cancer; (3) no confirmation of HGIN/IMC in the ER-specimen or surgical resection specimen.

For all included patients relevant information was retrospectively retrieved from endoscopy, radiology, histology and surgery reports and recorded on standardized case report forms.

Endoscopic work-up

Endoscopic work-up was performed by endoscopists with experience in the field of early oesophageal neoplasia, using high-quality endoscopes (Olympus GIF-H180, GIF-Q240Z, GIF-Q260Z, or GIF-H260Z, Olympus Endoscopy, Tokyo, Japan), often supplemented with advanced imaging techniques such as chromoendoscopy, autofluorescence endoscopy, and/or narrow-band imaging. The type of lesion was reported, distinguishing squamous cell lesions, Barrett’s lesions and cardia neoplasia. The lesion size and typification according to the Paris classification were recorded: type 0-Ip being polypoid, 0-Ia sessile, type 0-IIa elevated, type 0-IIb flat, type 0-IIc depressed, and type 0-III excavated.18 In addition, it was reported if a lesion appeared to be suspicious for deep submucosal infiltration, and if it seemed to be accessible for ER, based on criteria such as lesion size, type, location, and movement of the lesion with peristalsis.

For the EUS examination a standard radial EUS-scope (GIF-UM130, GIF-UM160, XGF-UE140-AL5, GF-UE160-AL5, Olympus Europe), a high-frequency EUS 20-MHz catheter probe (UM-3-R, Olympus), or both were used. If a lesion could be visualized with EUS the infiltration depth was recorded as being mucosal, submucosal, doubtful or not assessable. Furthermore, the presence of suspicious lymph nodes was assessed and in the case of EUS-FNA, the number of punctured nodes and cytological results were recorded. For each of the above mentioned examinations, it was recorded if its results changed the management strategy by excluding patients from further work-up for endoscopic treatment, i.e. excluding patients from diagnostic ER and directly referring the patient for surgery.

Endoscopic resection

ER was performed as the final diagnostic step in the work-up for endoscopic treatment in all patients with endoscopically visible abnormalities, no matter how subtle. During detailed endoscopic inspection, the target lesion was delineated and marked with coagulation markings. ER was performed with the ER-cap technique after submucosal lifting, using either an 18 mm flexible oblique cap (ID206-5, Olympus GmbH, Hamburg, Germany) if there was a suspicion on submucosal infiltration, or a 16 mm hard oblique cap (MAJ-297/296, Olympus GmbH, Hamburg, Germany) if the lesion appeared to be mucosal. From November 2004, ER was also performed using the multi-band mucosectomy (MBM) technique (Duette®, Cook endoscopy, Limerick, Ireland), without prior submucosal lifting, for lesions that were not suspicious for submucosal infiltration. After complete endoscopic removal of the marked target area, all resection specimens were retrieved, pinned down on paraffin and fixed in formalin for histological evaluation.

Histological evaluation of ER specimens

ER specimens were sectioned in 2 mm slices, embedded in paraffin and at a minimum of 4 levels, 200 µm thick slices were cut, mounted on glass slides and routinely stained with hematoxylin and eosine. All slides were evaluated by a junior pathologist supervised by an experienced GI pathologist (FtK and MV). Presence of neoplasia and cancer was evaluated according to WHO classification, together with tumour infiltration depth, differentiation grade, presence of lymph-vascular infiltration and the radicality of the resection at the deep resection margin.

Patient management

The optimal treatment strategy for each patient was based on the outcome of the diagnostic ER procedure. If a diagnostic ER showed risk factors for lymphatic spread, i.e. submucosal invasion (sT1m3 cancer for patients with squamous cell dysplasia), poorly differentiated cancer (G3), lymph-vascular infiltration or tumour involvement at the deep resection margin, patients were considered for surgery. Patients in whom a diagnostic ER was not feasible, due to poor lifting or the inability to suction the lesion into the ER-cap, both possible signs of submucosal growth, were also considered for surgery. Patients who were not surgical candidates due to age, co-morbidity or who refused surgery were referred for chemoradiotherapy or were further managed endoscopically on a relative indication. The majority of patients with Barrett’s neoplasia, and no contraindications for endoscopic management after diagnostic ER, underwent additional treatment to eradicate all Barrett’s mucosa using either photodynamic therapy,21 stepwise radical ER,22,23 or radiofrequency ablation.24 After endoscopic treatment, all patients entered endoscopic follow-up. EUS during follow-up was not routinely performed if patients had no risk factors for lymph node metastasis (mucosal cancer, well/moderately differentiated cancer, no lymph-vascular infiltration and radical resection of neoplasia). Patients with risk factors for lymph node metastasis who were treated endoscopically on a relative indication, all underwent EUS in addition to endoscopic inspection with biopsies during follow-up.

Outcome parameters

1. The frequency in which patients were excluded from endoscopic treatment based solely on the outcome of EUS.
2. The frequency with which EUS detected recurrence of neoplasia during follow-up.

Statistical analysis

Statistical analysis was performed with SPSS 12.0.1 Software for Windows. For descriptive statistics mean (±SD) 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 student t test and the Mann-Whitney test were used.
RESULTS

Patients

Between May 2001 and July 2007 a total number of 1,027 patients underwent oesophageal EUS. We found 131 patients eligible for this study (98 men, mean age 66 ± 12.6 years). Early neoplasia of the cardia was diagnosed in 7 patients; neoplasia arising in Barrett’s oesophagus in 114 patients and 10 patients had early oesophageal squamous cell neoplasia.

Endoscopy and EUS findings during work-up

Normal EUS

All 131 patients underwent endoscopic work-up and EUS. In 105/131 (80%) patients EUS did not show any suspicion on deep submucosal invasion or suspicious lymph nodes. All these 105 patients underwent ER of their endoscopically visible lesion and in 25/105 (24%) patients, the ER specimens showed submucosal invasion (n=17), poor differentiation and/or lymph-vascular invasion (n=6), or deep resection margins positive for cancer (n=2; subsequent surgery: T1sm1N0, T3N0) (Fig. 2).

Abnormal EUS

In 26/131 (20%) patients, abnormalities were found during EUS investigation: suspected submucosal invasion (n=14), suspicious lymph nodes (n=9), or both (n=3). In order to investigate the relative contribution of EUS over the preceding endoscopic inspection, cases were separated into 2 groups: abnormal EUS and unremarkable endoscopy, or abnormal EUS and abnormal endoscopy.

In 14 patients with an abnormal EUS, endoscopic inspection was unremarkable and did not raise any doubts on the attainability of curative endoscopic treatment. The EUS abnormalities in these 14 patients consisted of: suspected submucosal invasion (n=8), suspicious lymph nodes (n=5) or both (n=1). In the 6 patients with suspicious lymph nodes EUS-FNA was performed, which did not show malignant cells in 4 patients. In two patients atypical cells were found, originating from an undiagnosed small cell lung cancer and known chronic lymphatic leukemia. Diagnostic ER confirmed submucosal invasion in 7/14 (50%) patients. However, in 7/14 (50%) patients with an abnormal EUS, no submucosal invasion or other risk factors for lymph node metastasis were found upon diagnostic ER [Fig. 2]. These 7 patients were successfully treated endoscopically without any signs of recurrent neoplasia after a median follow-up of 42 [IQR] months.

In the other 12 patients with an abnormal EUS (suspected submucosal invasion (n=6), suspicious lymph nodes (n=4), both (n=2)), endoscopic inspection was also abnormal and had already raised doubts on attainability of endoscopic treatment, either because the lesion was not accessible or too widespread for ER, or because it appeared to be invading the submucosa. These doubts on attainability of endoscopic treatment were confirmed in 8/12 (67%) patients by diagnostic ER that showed submucosal invasion (n=4), poorly differentiated cancer/lymph-vascular invasion (n=2), non-lifting sign in an 87-yr old patient who was subsequently treated with radiotherapy, and by surgery in one patient (TsmN1M0). In the 6 patients with suspicious lymph nodes EUS-FNA was performed, which did not show malignant cells in 5 patients. In one of these patients with a negative EUS-FNA, subsequent surgery showed tumour localization in 4 of 16 resected lymph nodes (TsmN1M0, see above). In 1/12 (8%) patient in whom endoscopic treatment was considered doubtful given severe pre-existing stenosis, EUS-FNA showed malignant cells. The patient was referred for oesophagectomy (T2N0M0) yet none of the 16 resected lymph nodes showed metastasis. Three of the 12 patients (25%) with both an abnormal EUS and abnormal endoscopy, did not have risk factors for lymph node metastasis in the diagnostic ER specimens, and were further treated endoscopically with no signs of recurrence of neoplasia after a median follow-up of 30 months (Fig. 2).

**EUS during follow-up**

A total of 53 patients underwent EUS in addition to endoscopic inspection during follow-up. Median follow-up time from the removal of the neoplasia until the last endoscopy or the last EUS investigation was 39 (IQR 22-56) months and 25 (IQR 14-47) months, respectively. A median of 8 (IQR 6-10) endoscopies and 2 (IQR 1-3) EUS investigations were performed during follow-up in these 53 patients. Recurrence of neoplasia occurred in 10/53 patients (19%), all in patients with an initial diagnosis of Barrett’s neoplasia. Recurrences occurred in areas of residual Barrett’s mucosa in 8 patients, in a recurrent island of Barrett’s mucosa after eradication of all Barrett’s mucosa by photodynamic therapy in one patient, and at the cardia after radical endoscopic resection of all Barrett’s mucosa in one patient. All 10 intra-oesophageal recurrences were detected primarily during endoscopic inspection. EUS was abnormal in 3/10 patients: the first patient had T1/2N1Mx upon EUS, repeat ER showed poorly differentiated cancer and the patient was referred for surgery (T1N1M0); the second patient had T2N1Mx upon EUS, biopsies showed poorly differentiated cancer and the patient was referred for surgery (T3N1M0); the third patient had TxN1Mx upon EUS, repeat ER showed HGIN, EUS-FNA showed no malignant cells. In 7/10 patients with a recurrence detected during endoscopy, EUS was normal and repeat-ER confirmed HGIN (n=3) or T1m2 cancer (n=4). In 4 patients with no signs of recurrence upon endoscopy, EUS-FNA was performed to sample suspicious lymph nodes, which did not show malignant cells in any of the cases. No recurrence of neoplasia was detected solely by EUS and missed during endoscopic inspection.

**DISCUSSION**

EUS is still routinely used during work-up for endoscopic therapy in most centers, despite disappointing sensitivity and specificity for infiltration depth and lymph node metastasis demonstrated by several studies. It is therefore sought to evaluate from a clinical and practical point of view how often the outcome of EUS, after endoscopic inspection, changed patient management and excluded patients from diagnostic ER during work-up for possible endoscopic management.

Although EUS is an accurate technique for staging of oesophageal and cardia cancer, a number of studies have demonstrated that the resolution of standard EUS is not sufficient to distinguish mucosal from submucosal invading lesions in the case of early neoplasia. Even when using high-frequency EUS mini-probes, the discrimination between mucosal and submucosal lesions is only 80% accurate. Especially in Barrett’s oesophagus the heterogeneous tissue architecture with crypts and villi, the mucosal inflammation and often doubled muscularis mucosae, impede accurate EUS assessment. Furthermore, EUS evaluation of neoplastic lesions located in the distal oesophagus and cardia may be complicated due to the anatomical conditions at the oesophago gastric junction. The diagnostic accuracy of EUS for N-staging in oesophageal cancer ranges between 68% and 86%, EUS-guided fine-needle aspiration (EUS-FNA) of suspicious lymph nodes has been shown to increase the specificity of EUS N-staging and can increase the accuracy of EUS N-staging up to 90% in advanced oesophageal carcinomas. In our study 12 patients underwent EUS-FNA, which showed malignant cells in 3 patients: one patient had a known chronic lymphatic leukemia, another patient had an undiagnosed small cell lung cancer. The third patient with malignant cells upon EUS-FNA underwent surgery, which showed T2N0M0 cancer. This false positive finding was probably caused by contamination of the EUS-FNA needle by puncturing through the neoplastic lesion. Although this should always be avoided, it may be difficult to avoid puncturing through neoplastic mucosa, especially in the case of suspicious lymph nodes within the peri-tumoural region. In these cases, it may be recommendable to remove the neoplasia first by endoscopic resection, to be able to sample the lymph node without contamination by the tumour. In addition, another patient who was referred for surgery to resect a lesion that was too widespread for endoscopic treatment and who had a negative EUS-FNA, did have 4/16 positive lymph nodes in the oesophagectomy specimen.

In this study, 105 patients had a normal EUS without signs of submucosal growth or lymph node metastasis. After diagnostic ER, however, 17 patients did have submucosal invasion, 2 patients had deep resection margins positive for cancer with T1sm1 and T3 cancer at subsequent surgery, and 6 patients had poorly differentiated cancer and/or lymph-vascular invasion. Thus, based on the diagnostic ER, 25 of the 105 patients with a normal EUS (24%) had risk factors for lymph node metastasis that would have been missed without histological correlation of the diagnostic ER. Normal EUS should thus not be considered enough to engage in endoscopic ablation therapy (e.g. photodynamic therapy [PTD], radiofrequency ablation [RFA]) without diagnostic ER of all visible abnormalities first for accurate staging of the disease. Furthermore, 14 patients without doubts on attainability of ER upon endoscopic inspection, had signs of submucosal invasion or lymph node metastasis upon EUS. Diagnostic ER confirmed submucosal invasion in 7 patients. The other 7 patients, however, did not have submucosal invasion or other risk factors for lymph nodes metastasis in the ER specimens. Thus, only abnormal EUS is not enough to refer a patient for surgery without a diagnostic ER first, since half of the patients may still be eligible for curative endoscopic treatment. Lastly there was a group of 12 patients in whom endoscopic inspection raised doubts on the feasibility of ER, in addition to an abnormal EUS. As already described above, EUS-FNA in one patient resulted in a false positive diagnosis of tumour spread, and another patient undergoing surgery did have lymph node metastases in the resection specimen that were missed during EUS-FNA. The other 10 patients underwent diagnostic ER, after which 3 patients still had an indication for curative endoscopic treatment. Thus, even if both endoscopic inspection and EUS are abnormal, it is recommendable not to directly proceed to surgery but to perform a diagnostic ER first, provided that the lesion is accessible for a safe ER. In this respect, it is also noteworthy to mention that none of the diagnostic ER procedures in this study resulted in a severe complication. The results of this study strengthen our opinion that the optimal work-up for endoscopic treatment of early oesophageal and cardia neoplasia should consist of detailed endoscopic inspection to evaluate the macroscopic appearance of a lesion and to evaluate if a lesion is accessible for ER. If the endoscopic appearance of a lesion does not raise suspicion on deep submucosal infiltration, the lesion may be removed by ER. The resected specimen then allows for accurate histological evaluation of infiltration depth and other prognostic factors. Patients with mucosal lesions can be managed by further endoscopic treatment or follow-up, whereas a diagnosis of submucosal infiltration, poorly or undifferentiated cancer,
lymph-vascular invasion or unradical resection at the deep resection margin, warrants surgical treatment. We think that this approach allows for optimal selection of patients for endoscopic management, omitting the additional step of EUS, which often does not result in a clear-cut differentiation between mucosal and submucosal lesions, lacks assessment of other prognostic factors, and which has a poor positive predictive value for the presence of lymph node metastasis.

Follow-up with EUS next to endoscopic inspection was performed in 53 patients. Recurrence of neoplasia occurred in 10 of these 53 patients, all detected primarily during endoscopic inspection. None of the recurrences was solely detected by EUS. This suggests that after successful endoscopic treatment, endoscopic inspection is the most important modality to detect intra-oesophageal recurrences that can then be biopsied or removed by ER for histological confirmation. Although we cannot conclude this from our results, it may suffice to reserve EUS for those patients with a higher risk for lymph node metastasis based on biopsy or ER-specimen histology, instead of performing routine follow-up EUS after endoscopic therapy.

This study has several limitations that need to be discussed. First, this was a retrospective study and therefore patient inclusion may have suffered from selection bias. However, by having two independent researchers screening all patients undergoing any type of EUS within the pre-determined time frame to identify patients undergoing EUS for work-up of early oesophageal or cardia neoplasia, we tried to minimize selection bias. Secondly, endoscopic assessment and EUS were often not performed as independent investigations and we can therefore not exclude the possibility that the outcomes of endoscopy and EUS have influenced each other. However, since we did not attempt to compare the diagnostic accuracy of endoscopic inspection versus EUS, but aimed to evaluate the clinical value of EUS next to endoscopic inspection, this back-to-back use of EUS and EUS as routinely practiced in most centers, may not be very relevant for the outcomes of this study. Furthermore, the numbers in the some of the subgroups were relatively small and conclusions based on these small groups should therefore be considered carefully. Lastly, all endoscopies, EUS and ER procedures were performed by endoscopists with extensive experience in this field. Extrapolation of the results to centers with less experience in the management of early neoplasia should be performed with caution.

In conclusion, in this retrospective study in 131 patients with early oesophageal and cardia neoplasia, we found that the additional value of EUS during work-up including ER and follow-up was very limited. In none of the patients EUS alone changed the treatment policy. In addition, the results of this study strengthen the role of diagnostic ER as a final step in the work-up for endoscopic treatment.
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 oesophagus. 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 × 13 mm vs 20 × 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 oesophagus (BO) 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 Compared with surgical oesophageal resection, ER of early neoplastic lesions is less invasive.3,4 Different ER techniques have been developed, of which the ER-cap technique, first described by Inoue et al.,5 is the most widely used in the oesophagus. This technique involves the use of a specially designed transparent cap with a distal ridge, allowing placement of an endoscopic 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.6 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 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:

- BO 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 BO >5 cm), (2) as part of a stepwise radical endoscopic resection protocol of the whole BO in multiple sessions, (3) or (3) removal of visible abnormalities before additional ablative 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.

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, and, with 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.

**Figure 2. Piecemeal ER of early Barrett’s neoplasia by using the endoscopic resection-cap technique.**
A: Endoscopic view of a BO with a type 0-IIa-IIc lesion14 at the 1 to 4 o’clock position. B: View in the retroflexed position, after delineation of the lesion with argon plasma coagulation markings. C: The lesion is lifted by submucosal injection of diluted adrenaline. D: A snare is prelooped in a distal rim in the cap. E: The mucosa is captured in the snare by suctioning the mucosa into the cap and closing the snare, and it can then be resected by using electrocautery. F: After two resections, the delineated area is completely removed, without signs of bleeding or perforation in the resection wound.

**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.15 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/dL, 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);10
- 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 BO.1 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).
RESULTS

Patients

During 365 ER procedures for HGIN/early cancer in BO, 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.

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 [CI], 2.3-6.0 minutes). 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 adrenaline 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 oesophageal stent [Oesophageal 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 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 oesophageal 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-oesophageal 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 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 chemoradiation 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.

<table>
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<th>Parameter</th>
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<th>MBM technique [n=42]</th>
<th>p-value</th>
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<tr>
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Table 1. Results of piecemeal endoscopic resection for early Barrett neoplasia.

* Presented as: median [IQR]
† As part of a stepwise radical endoscopic resection protocol to remove the whole BD with HGIN/EC.
D I S C U S S I O N

This is the first randomized study comparing the ER-cap and MBM techniques for piecemeal ER of early neoplastic lesions in BO. Prior studies by May et al. and Peters et al. 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 BO. 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. 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. 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 et al., 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 may also 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.

A C K N O W L E D G E M E N T

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REFERENCES


Stepwise radical endoscopic resection for eradication of Barrett’s oesophagus with early neoplasia in a cohort of 169 patients

Roos E. Pouw, Stefan Seewald, Joep J. Gondrie, Pierre H. Deprez, Hubert Piessevaux, Heiko Pohl, Thomas Rösch, Nib Soehendra, Jacques J. Bergman

Gut — 2010; 59: 1169-1177
ABSTRACT

— Background & Aims:
Endoscopic resection is safe and effective to remove early neoplasia (i.e., high-grade intramucosal neoplasia/early cancer) in Barrett's oesophagus (BO). To prevent metachronous lesions during follow-up, remaining BO can be removed by stepwise radical endoscopic resection (SRER). Aim was to evaluate the combined experience in four tertiary referral centers with SRER to eradicate BO with early neoplasia.

— Design:
Retrospective cohort study.

— Setting:
Four tertiary referral centers.

— Participants:
169 patients (151 males, age 64 years (IQR 57-71), BO 3 cm (IQR 2-5)) with early neoplasia in BO ≤ 5 cm, without deep submucosal infiltration or lymph node metastases, treated by SRER between Jan'00-Sept'06.

— Intervention:
Endoscopic resection every 4-8 weeks, until complete endoscopic and histological eradication of BO and neoplasia.

— Results:
According to intention-to-treat analysis complete eradication of all neoplasia and all intestinal metaplasia by the end of the treatment phase was reached in 97.6% (165/169) and 85.2% [144/169] of patients, respectively. One patient had progression of neoplasia during treatment and died of metastasized adenocarcinoma (0.6%). After median follow-up of 32 months (IQR 19-49), complete eradication of neoplasia and intestinal metaplasia was sustained in 95.3% [161/169] and 80.5% [136/169] of patients, respectively. Acute, severe complications occurred in 1.2% of patients, and 49.7% of patients developed symptomatic stenosis.

— Conclusions:
SRER of BO ≤ 5 cm containing early neoplasia appears to be an effective treatment modality with a low rate of recurrent lesions during follow-up. The procedure, however, is technically demanding and is associated with oesophageal stenosis in half of the patients.

INTRODUCTION

In patients with Barrett’s oesophagus (BO) containing high-grade intraepithelial neoplasia (HGIN) or early cancer (EC), oesophagectomy used to be the treatment of choice.1-3 However, since lymph node involvement occurs rarely with these early lesions (0% for HGIN and less than 3% for early cancer),4-7 treatment with less invasive endoscopic techniques has emerged and has been shown to be an effective and safe alternative for selected patients.8-11 The cornerstone of endoscopic treatment is endoscopic resection (ER) of focal lesions, which provides a relatively large tissue specimen for accurate histological assessment. Focal ER, however, is associated with recurrent lesions elsewhere in the BO in 25-33% of patients during follow up if the residual Barrett’s mucosa is left untreated.6-11 To minimize this risk of recurrence, not only the neoplasia but all Barrett’s mucosa can be removed by stepwise radical endoscopic resection (SRER). In SRER the whole BO is resected by subsequent ER sessions resulting in complete removal of the BO with histological correlation of the whole Barrett’s segment. SRER has been shown to effectively eradicate neoplastic BO in relatively small-sized single center studies.12-20 The aim of the current multi-center study was to retrospectively evaluate SRER in four European tertiary referral centers for early Barrett’s neoplasia, all using a prospective treatment protocol. This study aimed at evaluating the safety and efficacy of SRER in a significantly larger cohort with a longer follow-up period than reported thus far.
PART I - Endoscopic Resection

PATIENTS AND METHODS

Data collection
All participating centers used a comparable protocol for SRER treatment. In two centers, data were prospectively entered into a dedicated database whereas the other two centers documented their findings in endoscopy and pathology reports. For the purpose of this study, all centers were visited by two researchers experienced in the field of endoscopic treatment of early Barrett’s neoplasia. Standardized case record forms were used to extract relevant data from the prospective databases and/or endoscopy and pathology reports. The collected data was then entered into a central database for further evaluation. After a first analysis, all centers were revisited by the same researcher to update follow-up data for included patients, collect missing data, and to review charts of all patients that underwent ER at the participating centers again to ensure that no patients were inadvertently excluded.

Selection criteria
For this retrospective multi-center cohort study, all patients that underwent an ER at the University Hospital Eppendorf (Hamburg), Charité - Campus Virchow (Berlin); Cliniques Universitaires Saint-Luc (Brussels) and Academic Medical Center (Amsterdam), from January 2000 till September 2006, were reviewed. Patients were included if they met all of the following criteria:

1. Maximum estimated BO length of 5 cm, with intestinal metaplasia (IM) upon biopsy;
2. A histological diagnosis of HGIN or invasive cancer in biopsies or ER-specimens;
3. In the case of a diagnostic ER (always performed during the first ER session), specimens could not show any of the following criteria: invasion >T1sm1, poorly/undifferentiated cancer (G3/G4), lymph-vascular invasion, irradical deep resection margins (Note: T1sm1 cancer with G1/G2 differentiation and no lymph-vascular invasion, was considered a relative indication for endoscopic treatment, if patients had serious contraindications for surgery or refused surgery. Both endoscopic and surgical options were discussed with these patients before treating them with SRER);
4. No signs of lymph node or distant metastasis on endosonography or computed tomography;
5. Patients were considered eligible for complete eradication of their BO by means of SRER.

Patients were excluded if after focal ER of visible lesions they were not additionally treated by SRER, but with endoscopic ablation (e.g. APC, PDT or RFA), or if residual BO was kept under surveillance.

Treatment protocol
In the case of visible lesions suspicious for submucosal invasion, patients first underwent a diagnostic ER to assess their eligibility for further endoscopic treatment by means of SRER. If there was no suspicion on deep submucosal infiltration on endoscopy or endosonography, 50% of the circumference of the BO, including the most suspicious lesion, was removed during the first SRER session. Subsequent SRER sessions were performed with an interval of 4-8 weeks until endoscopic eradication of all BO mucosa and neoplasia was considered complete. Histological eradication of IM and neoplasia was confirmed by biopsies from the neosquamous mucosa and immediately distal to the neo-squamocolumnar junction (neo-SCJ) (Fig. 1). Patients were prescribed high-dose proton pump inhibitor therapy (esomeprazole/omeprazol 40 mg BID) as maintenance medication during the entire treatment phase and after completion of the protocol.

Figure 1. Endoscopic images of BO with early cancer treated by SRER.
A: C1M4 BO with at the 10 o’clock position a 0-IIa-IIc-lesion. B, C: Acute bleeding during diagnostic ER of the lesion treated by placement of a clip. D: Resection specimen pinned down on paraffin. E: Prior to the second ER session, six weeks after the diagnostic ER a scar is observed at the 9 o’clock position. F: Resection wound after the SRER session. G: View on the resection wound with the endoscope in the retrograde position. H: Complete regeneration of squamous mucosa after SRER and 4 dilatation sessions for symptomatic stenosis. I: Extensive biopsies from the neo-squamous mucosa during follow-up.
**Endoscopic resection procedures**

SRER procedures were generally performed on an outpatient basis using propofol sedation, or conscious sedation with midazolam and fentanyl. Therapeutic procedures were performed using standard therapeutic video endoscopes (Olympus GIF-1T140/160, Olympus Europe, Hamburg, Germany). ER was performed using either the ER-cap technique, with oblique caps (diameter 12.8/14.8/18-mm, MAJ-296/297 or D206-5, Olympus Europe, Hamburg, Germany), or multi-band mucosectomy (Duette™ Multi-Band Mucosectomy kit, Cook Endoscopy, Limerick, Ireland). In all cases, the area to be resected was delineated with coagulation markings, followed by ER of the target area using one of the above mentioned techniques that have been described in detail elsewhere. All ER-specimens were retrieved, pinned on cork or paraffin and fixed in formalin.

**Additional ablation**

APC was used to ablate small areas of BO mucosa that could not be resected (e.g. tissue bridges between adjacent resections, areas difficult to reach due to stenosis), or to ablate the neo-SCJ in some patients. A 2.3-mm forward spraying APC probe (Erbe APC 300, Erbe Elektromedizin GmbH, Tubingen, Germany) was used (power 80-99 W, argon-flow 1.6-2.0 L/min).

**Histopathological evaluation**

Formalin-fixed biopsies and ER-specimens were processed to hematoxylin & eosin stained slides for routine histological evaluation by experienced GI-pathologists. Biopsies and ER-specimens were evaluated for the presence of (sub)squamous intestinal metaplasia and neoplasia, graded according to the WHO classification. Furthermore, tumour infiltration depth, differentiation grade, lymph-vascular involvement and radicality at the deep resection margins were assessed in ER-specimens as described elsewhere.

**Follow-up**

Follow-up (FU) started after complete removal of all endoscopically visible columnar epithelium in the oesophagus, confirmed histologically by biopsies. All patients underwent at least the first follow-up endoscopy in the center where they were treated and a strict biopsy protocol was applied with random four-quadrant biopsies distal to the neosquamous junction and for every 1-2 cm of the neosquamous epithelium. Subsequent FU endoscopies were performed every 3-6 months during the first year with six monthly or annual endoscopic FU thereafter. FU endoscopy consisted of standard video endoscopy combined with chromoendoscopy using Lugol staining or narrow-band imaging to detect recurrent columnar epithelium. Targeted biopsies were obtained from visible columnar-like epithelium in the oesophagus, and randomly from neosquamous mucosa and immediately distal to the neo-SCJ.

**Endpoints**

**Primary endpoints were assessed at the end of the treatment phase and at the end of the follow-up:**

1. Complete eradication of all HGIN/cancer, defined as absence of HGIN/cancer in biopsies (complete response of neoplasia, CR-N);
2. Complete eradication of all BO, defined as absence of IM in biopsies obtained from neosquamous mucosa and immediately distal to the neo-SCJ (complete response of IM, CR-IM).

**Secondary endpoints were:**

1. Number of treatment sessions and need for additional treatment during the treatment phase to achieve complete eradication of neoplasia and complete endoscopic removal of all BO;
2. Number of patients that required surgery;
3. Histological outcome of subsequent ER procedures;
4. Complications during the treatment phase, defined as ‘acute’ (during procedure), ‘early’ (0-48hrs) and ‘late’ (>48hrs). Complications were only recorded if they were clinically significant and graded as ‘mild’ (unplanned hospital admission, hospitalization <3 days, hemoglobin drop <3g/dL, no transfusion), ‘moderate’ (4-10 days hospitalization, <4 units blood transfusion, need for repeat endoscopic intervention, radiologic intervention), ‘severe’ (hospitalization >10 days, ICU admission, need for surgery, >4 units blood transfusion, in the case of stenosis: >5 dilatations, stent placement or incision therapy) or ‘fatal’ (death attributable to procedure <30 days or longer with continuous hospitalization);
5. Need for re-treatment during follow-up.

**Statistical analysis**

For intention-to-treat analysis the primary endpoints at the end of the treatment phase and at the end of follow-up were accounted for in all patients who were included for this study. Patients lost to follow-up were considered a failure for both primary endpoints. For patients who discontinued treatment or follow-up, CR-N and CR-IM were defined as the exit from the study and in case these endpoints were unknown, they were considered a failure. The treatment approach used to achieve and sustain CR-N and CR-IM was a secondary endpoint. For the per-protocol analysis at the end of the treatment phase, patients who were lost for further treatment or who discontinued treatment for unrelated reasons were censored. For per-protocol analysis at the end of the follow-up phase, patients who were lost to follow-up were censored and for patients who discontinued follow-up CR-N and CR-IM were defined at the exit from the study. Patients undergoing surgery during the treatment or follow-up phase were considered a failure. For descriptive statistics, mean ±SD was used in case of a normal distribution of variables and median (25% - 75%) was used for variables with a skewed distribution. Where appropriate, the student t test, Mann-Whitney U test, or Mantel-Haenszel test was used.
RESULTS

Patients
From a total of 341 patients undergoing ER in BO at the four centers from January 2000 till September 2006, 172 patients were excluded. Exclusion reasons: BO >5 cm (n=91); histological evaluation of diagnostic ER-specimens showing contraindications for endoscopic treatment (n=27); no signs of HGIN/EC in biopsies or ER-specimens (n=18); ER-monotherapy followed by surveillance (n=15), extensive APC (n=2), PDT (n=8) or RFA of residual BO (n=11).
A total of 169 patients (151 males, median age 64 years (57-71), median BO 3 cm (2-5)) were included. Visible lesions were identified in 127 patients (78%) and the worst histological grade prior to ER was HGIN in 88 patients and EC in 54 patients. Twenty-seven patients had no histological diagnosis based on biopsies prior to ER, but underwent immediate diagnostic ER of focal lesions that were endoscopically suspicious for neoplasia (all showing HGIN/EC in resected specimens).
EUS was performed in 86 patients, of which 12 underwent EUS-FNA (fine-needle aspiration) without any signs of malignancy.

Primary endpoints
Intention-to-treat analysis at the end of the treatment phase
A total of 169 patients were included, there were no deaths during the treatment phase. Five patients discontinued SRER treatment due to the following non-related reasons: withdrawal of consent (n=1), poor mental state (n=1), cardiovascular co-morbidity (n=1), second primary cancer detected during the treatment phase (n=2). Status at time of discontinuation in these five patients was: CR-N (n=5) and CR-IM (n=0). Three patients were lost for further treatment and were considered as a failure for both endpoints. A total of 161/169 patients (95%) finished the SRER protocol. Complete eradication of neoplasia (CR-N) was reached in 160/161 patients (99%), with small remnants of endoscopically visible Barrett’s mucosa in ten patients (6%) and a once-only histological finding of IM in seven patients (4%) during the first follow-up in biopsies immediately distal to the neo-SCJ (n=2, 1%) and underneath neosquamous mucosa (n=5, 3% “buried Barrett’s”).
According to an intention-to-treat analysis the overall primary endpoints after the treatment phase for the whole cohort of 169 patients were: CR-N 165/169 (97.6%) and CR-IM 144/169 (85.2%) (Fig. 2). Of the four patients in whom CR-N was not reached, three were lost to follow-up as described above and one patient, initially treated for HGIN, was a failure of the endoscopic treatment protocol. This patient had a C2M3 BO with inconspicuous HGIN in biopsies. After 4 SRER sessions, with HGIN as the worst histology in ER specimens, a rim of BO mucosa with HGIN persisted at the gastro-oesophageal junction. The area was treated with APC, since ER was impeded by scarring after prior ERs. However, a visible lesion developed in this area and repeat-ER showed a partially removed, poorly differentiated mucosal carcinoma. Subsequent surgery revealed residual HGIN and a lymph node metastasis at the celiac trunk. Seventeen months after surgery the patient was diagnosed with abdominal metastasis and died 12 months later.

Per-protocol analysis at the end of the treatment phase
For the per-protocol analysis patients who were lost for further treatment (n=3) or who discontinued treatment for unrelated causes (n=5) were censored. Patients undergoing surgery (n=3) were considered a failure for both endpoints. By per-protocol analysis the primary endpoints at the end of the treatment phase were: CR-N 158/161 (98.1%) and CR-IM 141/161 (87.6%).

Intention-to-treat analysis at the end of the follow-up phase
A total of 160 patients entered the follow-up phase after eradication of neoplasia. Median follow-up time from the start of the treatment until the last follow-up endoscopy was 32 (19-49) months, and 27 months (12-42) from the last therapy session to the last FU endoscopy. Patients underwent a median of 4 (2-5) follow-up endoscopies. Follow-up was discontinued in 10 patients due to unrelated death (n=7), advanced age (n=2), or a second primary cancer detected during the follow-up phase (n=1). Status at the time of discontinuation of these 10 patients was: CR-N (n=10) and CR-IM (n=7). Four patients were lost to follow-up and were considered failures for both endpoints. By the time of the last data-review, 146 patients were alive and under follow-up, of which nine patients underwent re-treatment during the follow-up phase (see below). Complete eradication of neoplasia (CR-N) was sustained in 146/146 patients (100%) whereas sustained eradication of all IM (CR-IM) was accomplished in 129/146 patients (88%); seven patients (5%) had a small island (<5 mm) of non-dysplastic Barrett’s mucosa and in 10 patients (7%) local IM was found in biopsies obtained immediately distal to the neo-SCJ at the last follow-up endoscopy. The overall primary endpoints at the end of the follow-up phase were: CR-N 156/160 (97.5%) and CR-IM 136/160 (85.0%).
Per-protocol analysis at the end of the follow-up phase
For the per-protocol analysis patients who were lost for further treatment (n=4) were censored and for patients who discontinued follow-up (n=10) CR-N and CR-IM were defined at the exit from the study. Patients undergoing surgery (n=2) were considered a failure for both endpoints. By per-protocol analysis the primary endpoints at the end of the follow-up phase were: CR-N 152/154 (98.7%) and CR-IM 134/154 (87.0%). The overall primary endpoints at the end of treatment and follow-up phase according to per-protocol analysis were: CR-N: 152/157 (96.8%) and CR-IM: 134/157 (85.4%).
Secondary Endpoints

Number of treatment sessions and need for additional treatment during the treatment phase

Patients underwent a median number of 2 (2-3) ER sessions, with a median number of 4 (2-6) resections per session. ER-cap technique was used in 44%, multiband mucosectomy in 23%, simple snare technique in 22% and a combination of these techniques in 8% of procedures (Table 1).

Additional ablation with APC was performed in 103 patients (61%), either during the ER procedure, or at a separate APC session (n=55 patients, median of 1 additional APC session). In 57 patients APC was used to ablate small islands (<10 mm²), in 20 patients areas between 10-20 mm² were ablated and in 26 patients APC was performed for areas >20 mm². Overall, patients underwent a median of 3 (2-4) endoscopic treatment sessions, including all ER and additional APC sessions.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Hamburg</th>
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<th>Brussels</th>
<th>Berlin</th>
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Table 1. Overview of the different endoscopic resection (ER) techniques used in the participating centres.

Number of patients that required surgery

A total of 5 patients underwent surgical oesophagectomy (5/169, 3%). Two patients were referred for surgery to remove persisting neoplasia that could not be removed endoscopically; one of these patients, with intra-abdominal tumour recurrence, has been described above; the other patient had a T1m3 G1 cancer in a C0M1 BO removed by ER, however residual HGIN could not be removed by ER due to pre-existing scarring resulting from reflux ulceration. Two patients were treated surgically for a perforation caused by ER (n=1) or by dilatation of a stricture (n=1). Finally, one patient in whom a C2M4 BO with a T1m2 G1 cancer was completely removed during 2 SRER sessions, had recurrence of neoplasia during follow-up (see below). Repeat-ER showed a radically removed T1sm1 cancer, for which the patient was referred for subsequent surgery. However, no tumour rest or positive lymph nodes were found in the surgical resection specimen.

Histological outcome

Table 2 displays the worst histological outcome at the subsequent ER sessions. The worst overall diagnosis in ER specimens per patient was a radically removed T1sm1 cancer in 7 patients (4%); mucosal cancer in 69 patients (41%); HGIN in 72 patients (43%); LGIN in 10 patients (6%); and no neoplasia in 11 patients (7%). The latter 21 patients with LGIN/no dysplasia in their ER specimens, all had confirmed HGIN/cancer in biopsies obtained during endoscopic work-up.

During subsequent ER sessions, histological evaluation did not reveal worse tumour characteristics as diagnosed during the first ER session (i.e. no submucosal invasion, no G3/G4 differentiation, no lymph-vascular invasion).
### Complications during the treatment phase

Four acute complications occurred during 415 ER procedures, all perforations (1.0% of procedures, 2.4% of patients). Two perforations were considered severe: one was treated surgically; the other by placement of a covered stent (Oesophageal Choo Stent, Fujinon Medical Holland B.V., Veenendaal, Netherlands) and ICU admission. Two perforations were graded as moderate: one was closed with clips and the patient was hospitalized for 5 days, the other was hospitalized for 7 days with conservative treatment.

A moderate, early complication occurred in four patients (1.0% of procedures, 2.4% of patients), all delayed bleedings treated by repeat endoscopy and placement of hemoclips (n=3). Late complications, all symptomatic stenoses, developed in 84 patients (49.7%). The rate of oesophageal stenosis was related to the length of the resected BO (Mantel-Haenszel test; p=0.002). Using either Savary bougienage or balloon dilatation, all patients were adequately treated by a median of 3 (IQR 2-6) dilatation sessions, supplemented by placement of a stent (n=2) or incision therapy (n=4). In 56 (67%) patients the stenosis was graded as moderate: one was closed with clips and the patient was hospitalized for 5 days, the other was hospitalized for 7 days with conservative treatment.

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### Need for re-treatment during follow-up

Three patients underwent repeat ER for recurrence of HGIN (n=2) and T1sm1 cancer (n=1); in all cases located immediately distal to the neo-SCJ without a clearly visible Barrett’s segment in two patients and with a small columnar-lined tongue in one patient. Six patients were additionally treated for visible columnar epithelium in the tubular oesophagus with IM upon biopsy; using APC (n=2), repeat ER (n=1) or a biopsy forceps (n=3). In all cases these were small islands (<5 mm), likely overlooked during the treatment phase.

### DISCUSSION

Until recently, oesophagectomy was considered the standard therapy for patients with HGIN/EC in Barrett’s oesophagus. Although modern series in centers of excellence have reported a mortality of almost 0% for HGIN and <5% for EC,2,3 oesophagectomy remains an invasive procedure and less invasive endoscopic alternatives have therefore been considered.

Endoscopic resection is the cornerstone of endoscopic therapy, since it provides a relatively large tissue specimen for histopathological evaluation, enabling proper selection of patients for subsequent endoscopic versus surgical therapy.8,11,23 In the case of submucosal invading lesions (T1sm), the significant risk for lymphatic involvement (15-30%) warrants surgical oesophagectomy with resection of surrounding lymph nodes.6,7 However, in selected patients with HGIN or EC limited to the mucosal layer (T1m), the risk of lymphatic involvement is minimal, and ER in these patients has been reported to have a 5-year disease specific survival of 98 %.6,4 In addition, endoscopic treatment is associated with little complications, most patients are treated in an outpatient setting and the functional integrity of the oesophagus is preserved.6,11 After focal ER of HGIN/EC, the residual BO still holds the potential of malignant degeneration, and metachronous lesions occur in 30% of patients.10,13 Additional treatment of the residual BO after focal ER is therefore advocated, for example using stepwise radical endoscopic resection (SRER). In this retrospective multi-center study we evaluated the results of SRER for BO containing HGIN/EC in four tertiary referral centers.

The protocol involved stepwise resection of the whole BO in multiple endoscopic sessions and was limited to patients with a BO >5 cm in length. A total of 169 patients were included in the study and according to intention-to-treat analysis complete eradication of all neoplasia was reached in 97.6% of patients. Three of the four failures were due to patients who were lost to follow-up during the treatment phase and were considered failures according to the intention-to-treat analysis. In one patient (0.6%), however, neoplasia progressed under treatment with eventually a fatal outcome. The SRER-protocol proved to be relatively safe: acute, severe complications occurred in 1.2% of patients and the vast majority of patients were treated with endoscopic procedures only, generally in an outpatient setting, with oesophagectomy being performed in 2.9% of patients.

Complete eradication of all histological IM was reached in 85.2% of patients, with only small remnants of visible Barrett’s mucosa in ten patients and seven patients without any endoscopic signs of residual BO had “hidden” IM in biopsies from neosquamous mucosa or immediately distal to the neo-SCJ at the first follow-up endoscopy. Due to our strict criteria, these patients were considered failures for the CR-IM endpoint although we feel that the extent of residual BO might be considered negligible.

A prior study from Amsterdam has demonstrated that the neosquamous mucosa that regenerates after SRER is free of oncogenic abnormalities as present in the pre-treatment BO.24 This may be reflected in the low recurrence rate during follow-up: three patients (1.8%) developed recurrence of neoplasia that was effectively treated in all cases. After median follow-up of 32 months, intention-to-treat analysis resulted in a sustained complete eradication of neoplasia in 95.3% of patients. The recurrence rate for neoplasia after SRER is thus much lower than the 25-33% that can be expected after mono-therapy with ER.10,11 Also bearing in mind that these series have reported their outcomes per-protocol and...
not according to the intention-to-treat principle as in our current study. For comparison, per-protocol analysis of our study showed sustained complete eradication of neoplasia in 98.7% of patients.

SRER is, however, technically demanding and a number of issues need to be discussed. Firstly, after SRER it may be difficult to assess if the distal end of the BO has been completely resected, since it is almost impossible to endoscopically distinguish residual Barrett’s from gastric mucosa at the level of the gastric folds. It is therefore important that the ER extends deep enough into the cardia since additional resections at a later stage may prove difficult due to scarring. Completeness of SRER should ideally be confirmed by biopsies taken immediately distal to the neo-SCJ. Despite absence of endoscopically visible BO, focal IM was incidentally found in a small number of patients during follow-up and since all three recurrences of neoplasia occurred in the neo-SCJ area, this may be a relevant finding. Other groups have also reported on the issue of neoplasia developing in the cardia months to years after complete removal of the Barrett’s segment.25,32 However, the reason for these undesirable events is still unknown and it is therefore recommendable to focus on the neo-SCJ during follow-up, by detailed endoscopic inspection in the antegrade as well as retrograde position and by obtaining four-quadrant biopsies from this area.

Histological IM underneath neosquamous mucosa (buried Barrett’s) was diagnosed in a small number of patients at a single follow-up endoscopy. The clinical relevance of buried Barrett’s is still unclear. Despite anecdotal reports of subsquamous cancers that supposedly originated from areas with buried Barrett’s after APC and PDT,25 some believe that the risk of malignant degeneration in buried glands is negligible since they are protected from the harmful effects of gastro-oesophageal refluxate.24 In this series, the clinical relevance of buried glands may also be questioned since they were only diagnosed early during follow-up, without being reproduced at a later stage. In 21 patients no HGIN/EC was found in any of the ER-specimens during SRER treatment, despite that these patients all had HGIN/EC in pre-treatment biopsies, confirmed after revision by a second pathologist. There are three possible explanations for this discrepancy: first, patients did not have HGIN/EC and the diagnosis was misinterpreted by multiple expert pathologists; second, the very small foci of HGIN/EC were effectively removed by biopsies; third, residual HGIN/EC removed during SRER was overlooked either due to histological misinterpretation or because the area of the ER specimen containing neoplasia was not assessed by the pathologist. However, irrespective of the reason for this discrepancy, the decision to treat the patients with SRER was made according to standard guidelines. In countries were endoscopic management of BO neoplasia is less well accepted, these patients would likely have been considered for surgery.

Symptomatic oesophageal stenosis occurred in almost half of the patients and in 33% of these patients the stenosis met our definitions of a severe complication because they required >5 dilatation sessions, additional incision therapy, stent placement or dilatation resulted in a perforation. The stenosis rate was correlated with the length of the resected BO and although we anticipate that SRER may also be successful in patients with BO >5 cm, we feel that this currently is the upper limit for safe and effective SRER. To decrease the high stenosis rate after SRER, studies on methods to prevent stricturing after ER would be helpful, for example placement of biodegradable stents, injection of steroids, “prophylactic” dilatation during the healing phase, or application of autologous stem cells.26,28 Recent studies combining focal ER of neoplasia with RFA of residual BO have shown high efficacy rates for eradication of BO and associated neoplasia, with absent or low rates of stenosis even when used for long-segment BO.14 Yet, a downside of all ablation techniques is the lack of histological correlation. This may pose patients at unnecessary risk for lymph node metastases if a lesion with submucosal invasion, poor differentiation or lymph-vascular invasion is not diagnosed as such, and ablated. In this respect, the total histological correlation of the SRER protocol may be considered an important advantage. In the current study, however, the most suspicious area within the BO was identified during endoscopic work-up and removed during the first SRER session. In all patients, the histological findings of the first SRER procedure corresponded with the overall worst histopathology of the patient: all T1sm1 cancers were identified as a suspicious lesion and removed during the first procedure and no G3/G4 cancers or lymph-vascular invasion were diagnosed at subsequent ER sessions. This may suggest that after thorough endoscopic work-up and ER of the most involved area with histological correlation, the remaining BO can be safely treated with ablation therapy without significant risk of leaving submucosal lesions undiagnosed and under-treated. A randomized comparison of SRER and focal ER supplemented with RFA for residual BO is currently underway.37

This study has a number of limitations that need to be addressed. Firstly, the endoscopists at the four study centers had extensive experience in endoscopic detection and work-up for early neoplasia in BO, and in performing safe and effective piecemeal resections in BO. The safety and efficacy results of SRER reported in this study may, therefore, be different in centers where endoscopic treatment of early Barrett’s neoplasia is practiced less frequently. Secondly, this was a retrospective study without a predetermined mutual protocol and without central histopathological assessment. We acknowledge that review by a single pathologist would have increased the homogeneity of our cohort. However, all four centers have a tertiary referral function for early Barrett’s neoplasia, which ensures a certain level of histopathological expertise.

Lastly, despite prospective registration of SRER data, the retrospective inclusion of patients may have resulted in exclusion of patients that were initially considered for SRER treatment, but who did not finish treatment because of adverse effects, e.g. poor healing, stricturing, or otherwise. However, according to the intention-to-treat principle these cases should be included in the cohort. After the initial data analysis it was therefore decided to re-visit all centers to review not only patients treated with SRER, but to review all patients undergoing an ER between Jan 2000–Sept 2006. By surveying the course of all ER patients at each center, we aimed at minimizing the number of intention-to-treat patients that were missed during the first data collection.

In summary, focal endoscopic resection of Barrett’s neoplasia has been proven safe and effective with 5-year survival rates up to 98%.6 To minimize the 30% risk of recurrent neoplasia in the residual Barrett’s segment during follow-up,1,11 SRER can be used to remove all Barrett’s mucosa at risk for malignant progression, with histological correlation. The results of this study demonstrate that SRER of BO ≤5 cm containing HGIN/EC is an effective treatment modality to remove all neoplasia and all Barrett’s mucosa, with a low rate of recurrent lesions during follow-up. However, the high rate of oesophageal stenosis in almost half of the patients is a significant drawback of this approach that needs to be overcome by developments to prevent post-ER stricturing.
REFERENCES


PART II
RADIOFREQUENCY ABLATION
Radiofrequency ablation for total Barrett’s eradication: a description of the endoscopic technique, its clinical results and future prospects
ABSTRACT

Stepwise circumferential and focal radiofrequency ablation using the HALO system is a novel and promising ablative modality for Barrett’s oesophagus. Primary circumferential ablation is performed using a balloon-based bipolar electrode, while secondary treatment of residual Barrett’s epithelium is performed using an endoscope-mounted bipolar electrode on an articulated platform. It has been used as a single modality treatment or in combination with other therapies. Recent studies suggest that this ablation technique is highly effective in removing Barrett’s mucosa and its associated dysplasia without the known drawbacks of photodynamic therapy or argon plasma coagulation such as oesophageal stenosis and subsquamous foci of intestinal metaplasia (a.k.a. “buried Barrett’s”). In this review paper we will explain the technical background of radiofrequency ablation using the HALO system, give a summary of its current status and speculate on possible future applications.

INTRODUCTION

Given the morbidity and mortality that may be associated with oesophagectomy, less invasive endoscopic treatment modalities have emerged to treat high-grade dysplasia (HGD) and intramucosal cancer (IMC) in Barrett’s oesophagus (BO). Endoscopic resection (ER) of focal lesions allows for histological correlation enabling optimal patient selection.1 Patients with submucosal invading lesions should be referred for surgery because they have a 15-30% risk of positive local lymph nodes whereas this risk is minimal in patients with IMC.2,3 ER, however, only removes a focal area from the BO keeping the patient at risk for metachronous lesions during follow-up.4 To prevent this, ER has been combined with ablative therapy, such as photodynamic therapy (PDT) or argon plasma coagulation (APC), to remove residual (dysplastic) Barrett’s mucosa.5-9 PDT and APC, however, have significant shortcomings. First, they often do not result in complete ablation of the whole BO.5-9 Second, studies have shown that oncogenetic alterations, as present in BO prior to ablation, can still be found in areas of residual BO and these may be associated with recurrence of neoplasia.10 Third, foci of intestinal metaplasia (IM) may be hidden underneath the neosquamous mucosa after treatment (a.k.a. “buried Barrett’s”) and some fear that these areas may progress to cancer without being detected endoscopically due to their “hidden” nature.11,12 Lastly, PDT and APC are associated with complications of which oesophageal stenosis is the most relevant.5-9

Step-wise circumferential and focal radiofrequency ablation (RFA) using the HALO system is a relatively new endoscopic treatment modality for BO.13-15 Recent studies suggest that this ablation technique is highly effective in removing Barrett’s mucosa and associated dysplasia without the aforementioned drawbacks of other ablation techniques.16-22 In this review we will explain the technical background of RFA, give a summary of its current status and speculate on possible future applications.

Technical Background

The HALO system comprises two distinct ablation systems: the HALO360 system for primary circumferential RFA and the HALO90 system for secondary focal RFA or primarily as treatment for short segment BO. Prior to circumferential RFA, a sizing catheter with a 4-cm long non-compliant balloon at its distal end is used for measuring the inner oesophageal diameter. Upon activation via a foot-switch the sizing balloon is inflated by the HALO360 energy generator, and the mean oesophageal inner diameter is automatically calculated for the entire length of the 4-cm long balloon.

The HALO360 ablation catheter holds a balloon at its distal end, with a 3-cm long bi-polar electrode on its outer surface (Fig. 1). The HALO360 ablation balloon is available in five outer diameters (22, 25, 28, 31 and 34 mm). Via a foot-switch the ablation catheter is inflated and upon activation RF energy is delivered to the electrode. Extensive dosimetry studies have shown that for circumferential ablation two applications of RF energy at 10 or 12 J/cm² and 40 W/cm² are the most effective regimens to ablate the full thickness of the epithelium.13-15 Focal RFA of BO may be conducted with the HALO90 system that consists of an endoscope mounted ablation catheter and an energy generator similar to the HALO360 generator, but without the pressure:volume system (Fig. 1). The electrode-surface is 20 mm long and 13
mm wide, allowing for selective focal ablation. Currently, a "double x double" 12 or 15 J/cm² and 40 W/cm² ablation regimen is advised to reach effective eradication of IM. For both HALO ablation devices, a HALO³⁶⁰⁰ energy generator automatically delivers RF energy to the electrode upon activation via a footswitch. Due to the combination of high-power density, and a preset energy density, ablation results in uniform tissue penetration depth (~1,000 µm) that is not operator dependent. RFA using the HALO system, therefore, results in controlled destruction of the columnar epithelial layer, the lamina propria, and part of the muscularis mucosae, while the submucosa typically remains uninjured.¹³-¹⁵.

The HALO³⁶⁰⁰ and HALO⁹⁰ ablation procedures
Stepwise circumferential and focal ablation of a BE, generally starts with a circumferential ablation procedure using the HALO³⁶⁰⁰ system (Fig. 2), which comprises the following steps:

1) Recording oesophageal landmarks:
After spraying the oesophageal wall with acetylcysteine (1%) and flushing it with plain water to remove excessive mucus, the top of the gastric folds and the maximum proximal extent of the BO (including isles) are recorded for reference during the sizing and ablation procedure. Then a stiff guide-wire (e.g. Amplatz extra stiff 0.035", Cook, Denmark, Europe) or metal wire is introduced and the endoscope is removed.

2) Sizing oesophageal inner diameter:
The sizing procedure is generally performed as a "blind" procedure using the one-cm scale on the catheter shaft for reference. However, in special cases (e.g. localized narrowing) endoscopic visual control may be useful to assure that sizing is performed at the required level. The measurement cycle is started with the catheter positioned 5 cm above the maximum proximal extent of the BO (the distal end of the balloon is then located one cm above any Barrett’s mucosa), and measurement is repeated for every cm of the targeted portion of the oesophagus, advancing the balloon distally with 1 cm linear increments.

3) Selecting the appropriate HALO³⁶⁰⁰ ablation catheter:
Based on the oesophageal inner diameter measurements an appropriate HALO³⁶⁰⁰ ablation catheter is selected, which is smaller than the smallest measured diameter. In patients who underwent prior ER the ablation catheter should be selected conservatively.²³

4) First circumferential ablation pass:
The HALO³⁶⁰⁰ catheter is introduced, followed by the endoscope. Under endoscopic visualization the proximal margin of the electrode is placed one cm above the maximum proximal extent of the BO. The balloon is inflated, and via a footswitch the electrode is then activated. Moving from proximally to distally the balloon is repositioned, allowing a small overlap with the previous ablation zone of 5-10 mm, until the entire BO has been ablated.

5) Cleaning procedure in between ablation cycles:
After the first ablation pass the ablation catheter is removed and the electrode surface is cleaned from coagulum with a wet gauze. A soft distal attachment cap (e.g. Model MB-046, Olympus, Tokyo, Japan) is fitted on the tip of the endoscope, and the soft extending rim of the cap can be used to slough off the coagulum from the ablation zone (Fig. 2). Additional forceful spraying of plain water through a spraying catheter using a high-pressure pistol (e.g. Alliance™, Boston Scientific, Limerick, Ireland, UK) can be used to ‘blast’ off residual coagulum. Although the extensive cleaning procedure requires extra procedure time, it has been proven to increase the efficacy of the first ablation session from 90% surface regression to 95%.²¹,²²,²⁴

6) Second ablation pass:
After the cleaning procedure, the entire BO is ablated a second time.
A minimum of eight weeks after the first circumferential ablation treatment, patients are re-scheduled. In case of residual circumferential BO >2 cm or multiple isles or tongues, patients are treated with a second circumferential ablation. In case of an irregular Z-line, small tongues, circumferential extent <2 cm, or diffuse isles, patients are treated with focal ablation using the HALO® system (Fig. 3), following the steps below:

1) Introduction of the HALO® catheter:
The HALO® electrode is fitted on the tip of the endoscope and positioned at the 12 o’clock position in the endoscopic video image. When the laryngeal cavity is visualized the tip of the endoscope is deflected slightly downward allowing the leading edge of the catheter to be passed behind the arytenoids. The patient is asked to swallow and the endoscope is gently advanced. In about 8% of cases introducing the HALO® catheter may prove difficult. In those cases a Zenker diverticulum should be excluded, and a biopsy forceps, a guide-wire or a spraying catheter may be used as a guide to enter into the proximal oesophagus (Fig. 4).

2) First ablation pass:
Residual Barrett’s epithelium is positioned at the 12 o’clock position in the endoscopic video image. The electrode is brought into close contact with the mucosa, deflected upward, and activated. While keeping the electrode into place it is immediately activated again, resulting in a ‘double’ application of energy. Ablation of the entire Z-line with the HALO® device is recommended, to ensure eradication of IM at the gastro-oesophageal junction.

3) Cleaning procedure:
After all residual BO has been ablated, the coagulum is carefully pushed off the oesophageal wall with the leading edge of the electrode, followed by cleaning of the electrode outside the patient and cleaning of the ablation zone with forceful spraying of water as described above.

4) Second ablation pass:
Using the ablation zones from the first ablation pass for orientation, all ablated areas are treated with a double application of energy again.

Ablation can be repeated every 2-3 months, until all BO has been eradicated visually, and then confirmed histologically. Most patients will need one circumferential ablation session and 1-2 focal ablation sessions to eradicate all dysplasia and IM.

Figure 2. Endoscopic appearance of a circumferential ablation procedure using the HALO® system.
A: C6M7 BO with HGD. B: The HALO® catheter is introduced and inflated at the upper end of the Barrett’s segment. C: After the first application of energy the whitish coagulum resulting from the ablation shows after the catheter is deflated and advanced distally. D: After ablation of the whole Barrett’s segment and cleaning of the electrode and ablation zone, the catheter is reintroduced for a second ablation pass. E: The second ablation pass results in a tan colored ablation zone. F: Treatment effect after two circumferential ablation passes.

Figure 3. Endoscopic appearance of a focal ablation procedure using the HALO® system.
A: Antegrade view of a C6M7 BO 6 weeks after primary circumferential ablation. B: Residual isles of Barrett’s mucosa. C: Corresponding image with NBI. D: Ablation effect immediately after HALO® ablation (distal end of the catheter visible at 12 o’clock). E: Endoscopic appearance after the first ablation pass (2x15 J/cm²) and cleaning of the ablation zones. F: After the second ablation pass (2x2 15 J/cm²) the ablation zones have a tan-colored appearance.

Figure 4. Difficult introduction of the HALO® catheter may be facilitated by using a biopsy forceps.
A: Leading edge of the HALO cap is visible just proximal to the arytenoids. B: A biopsy forceps is blindly advanced behind the arytenoids into the oesophagus. C: The endoscope is angulated downward, causing the leading edge of the HALO® catheter to touch the biopsy forceps. D: After gently advancing the endoscope, using the biopsy forceps for guidance, the proximal oesophagus is entered.
Post-treatment care
After RFA proper acid suppressant therapy is very important to minimize patient discomfort, and to allow the oesophagus to heal optimally and regenerate with squamous epithelium. Patients should be prescribed high-dose proton-pump inhibitors as maintenance medication. Additional H2-receptor antagonists and sucralfate can be prescribed, there is, however, no scientific evidence that this improves healing. After RFA, patients are advised to adhere to a liquid diet for 24 hours that they may gradually expand to a soft and normal diet to their own discretion. Patients may experience symptoms of chest discomfort, sore throat, difficulty or pain with swallowing and/or nausea, which usually improve each day. Proposed analgesic measurements are viscous lidocain, liquid acetaminophen with or without codeine, and anti-emetic medication. If necessary, patients may use acetaminophen suppositories. Use of NSAIDs is not advisable. Some patients may present with severe chest pain and fever, observation and conservative management with an optimal anti-secretory and analgetic regimen usually suffices in these cases.

Follow-up regimen
Two to three months after the last treatment the absence of residual Barrett’s epithelium is examined by endoscopic inspection. The use of high-resolution endoscopes with Lugol’s staining [2%] or preferably NBI is important to detect even small areas of residual IM (Fig. 5). A strict biopsy protocol should be applied with 4 quadrant biopsies immediately distal (<5 mm) to the neosquamocolumnar junction and every 1-2 cm of the neosquamous epithelium (Fig. 6). Since no long-term follow-up data after RFA are available thus far it is recommended to schedule patients for follow-up endoscopy two and six months after the last treatment and then annually.

Position of RFA for Barrett’s eradication
RFA after ER of visible lesions containing IMC or HGD:
Patients with visible abnormalities in a BO containing IMC or HGD may be treated with RFA, but only after ER of the IMC or visible lesion. First, ER allows for optimal histopathological staging of a lesion, enabling selection of patients with IMC and a low risk of lymph node involvement, for endoscopic treatment.1,21,22 Second, RFA should be performed on an endoscopically flat mucosa to ensure that the uniform ablation depth, as uniquely effected by the HALO system, truly reaches as deep as the muscularis mucosae.

RFA for flat HGD:
Barrett’s patients with HGD seem to be ideal candidates for RFA, since eradication of their dysplastic BO may prevent development of IMC. Proper selection of these patients is, however, of the utmost importance. Patients should have no visible lesions: these require ER for optimal staging and treatment. We have also required absence of cancer in biopsies (4Q/1-2cm) obtained during at least 2 high-resolution work-up endoscopies within 2 months prior to RFA and no studies have yet evaluated the use of RFA for flat IMC.

RFA for LGD:
The natural course of LGD in BO is a controversial issue. Recent publications, however, have shown that after a consensus diagnosis of LGD, patients are indeed at an increased risk of malignant degeneration, suggesting that eradication of all BO at risk may prevent development of cancer.23 Recent US studies on the use of RFA for LGD have shown excellent results.
RF therapy for non-dysplastic BE

The risk of progression to cancer in patients with non-dysplastic BE is small and no objective markers are yet available to identify patients with an increased risk of developing cancer. Although RF is a promising ablation modality for BO, there are still some unclear issues that need to be studied further, especially relating to its long-term efficacy. Treatment of patients with non-dysplastic BO with RF is, therefore, still controversial. Since the risk of progression to cancer in patients with non-dysplastic BO is small, randomized trials to evaluate if RF reduces the risk of developing cancer are difficult to perform given the required sample size. Hopes are set for future development of biological markers for risk-stratification to decide which patients with non-dysplastic BO are at risk for malignant progression and would benefit from RF.

Overview of clinical trials

After initial dosimetry studies in the porcine oesophagus and human oesophagus prior to oesophagectomy, a number of prospective clinical studies were initiated to evaluate the safety and efficacy of RF in the whole spectrum of BO patients: non-dysplastic BO, LGD, HGD and IMC. In the AIM-trial reported by Sharma et al., 102 patients with non-dysplastic BE were included and treated with RF. The first phase of the study (AIM-I) was a dosimetry phase (n=32) to evaluate the dose-response and safety of circumferential ablation by one application of RF energy ranging from 6-12 J/cm². There were no dose-related adverse events, and for the second phase of the trial (AIM-II), the effectiveness phase (n=70), two applications of 10 J/cm² were delivered for circumferential ablation. In the AIM-II trial complete eradication of IM at 12 months was achieved in 48/70 subjects (70%), using only the HALO™ system for circumferential ablation. The HALO™ device for focal ablation became available halfway during the first human trials. Fleischer et al. described the use of the HALO™ device for additional ablation in patients from the AIM-II trial with residual BE. At 30 months follow-up, this resulted in complete clearance of IM in 97% of patients by intention to treat analysis. None of the patients from the AIM-trial presented with oesophageal stenosis, and no buried Barrett’s glands were found in any of the >4000 neosquamous biopsies obtained during follow-up.

In a prospective trial by Sharma et al. that included 10 patients with confirmed LGD, RF resulted in 100% clearance of dysplasia and 90% clearance of IM at two-year follow-up, again without any oesophageal strictures or buried Barrett’s glands. A prospective cohort of 63 patients with LGD (n=39) and HGD (n=24) at the Mayo Clinic with a median follow-up of 24 months, Sharma et al. reported an overall complete response for IM of 79% and complete response for dysplasia of 89%. For the LGD cohort, complete response for IM was 87% and 95% for dysplasia. For the HGD cohort, complete response for IM was 67% and 79% for dysplasia.

For ablation of BO in patients with LGD or HGD, the strongest evidence that RF reduces the risk of malignant progression comes from the randomized sham-controlled trial by Shaheen et al. that was conducted in 19 USA centers. Although it has not been completely published yet, the 1-year interim results of this high-profile quality study provide convincing evidence that RF is effective in eradicating IM and dysplasia in patients with LGD and with flat HGD. By intention to treat analysis, a total of 101 patients with HGD (n=43) and LGD (n=58) were included and randomized to RF treatment or sham (2:1). At 12 months, 85% of patients treated with RF had clearance of dysplasia (sham: 24%, p<0.001), and 77% had clearance of IM (sham: 0%, p=0.001). In the sham arm, 18.9% of patients had progression of dysplasia: 3/19 from LGD to HGD and 4/18 from LGD to EC. In the RF arm 4.7% of patients had progression of dysplasia: 2/39 from LGD to HGD and 1/25 from HGD to EC. Five patients presented with an oesophageal stricture (6%), all resolved with a mean of 2 endoscopic dilatations. There were no related deaths or perforations. Gondrie et al. reported on a total of 23 patients with HGD and/or IMC, of which 13 underwent ER of IMC and visible lesions prior to RF. After a median of 1.5 circumferential and 2.6 focal ablation sessions, and additional ‘escape’ ER in two patients, complete eradication of all dysplasia and IM was achieved in all patients (100%). There were no adverse events, or buried glandular mucosa in any of the 839 biopsies obtained during follow-up. Only one patient presented with dysphagia that resolved after one endoscopic dilatation. An important observation from the studies by Gondrie et al. is the possibility to resect areas of Barrett’s mucosa that persist after multiple RF sessions with the ligate-and-cut technique, without the need for submucosal lifting.

This may be a significant advantage compared to other endoscopic ablation techniques that typically result in submucosal scarring, which makes escape treatment with ER complicated.

Compared to the 0-56% stricter rate associated with other endoscopic ablation techniques, the minimal rate of oesophageal stenosis reported in the trials discussed above, is encouraging. A study by Beaumont et al., comparing measurements of oesophageal inner diameter, motility and compliance before RF treatment and 2 months after the last ablation session, showed no significant differences, grounding the observation that RF does not impair the functional integrity of the oesophagus.

Gondrie et al. demonstrated that stepwise circumferential and focal ablation of BE with HGD results in restoration of normal appearing neosquamous mucosa without any of the oncogenetic abnormalities as present before treatment, using fluorescence in situ hybridization analyses of brush cytology specimens obtained from the BO prior to ablation and from the neosquamous epithelium after RF. These important findings were confirmed by Finkelstein et al., suggesting that the neosquamous tissue holds no residual malignant potential.

Unanswered questions and directions for future research

Since RF is a relatively new technique, there are some unanswered questions that will hopefully be answered by ongoing and future research. First, since the HALO™ technology only became available halfway during the first human trials the optimal energy settings to eradicate dysplasia and IM have not been completely unraveled. Currently, different energy settings and ablation regimens are applied for focal ablation,
cm² and “double x double” 15 J/cm² ablation. Furthermore, very small residual isles (<2 mm) may just as well be targeted with APC, which may be quicker, cheaper, and equally effective for this indication as ablation with the HALO® system.

Second, though RFA may appear to be a very appealing new technique for BE ablation, it has to be stressed that ER remains the cornerstone of endoscopic treatment for HGD and IMC as was discussed above. Combining ER of visible lesions with RFA of residual BO, therefore, seems to be the ideal treatment modality for patients with early BO neoplasia. Thus far, however, there is only limited data on the combination of ER with RFA. In an evaluation by Pouw et al. circumferential RFA seemed safe in case no prior ER was performed. However, mucosal lacerations were observed in patients who had prior ER >33% of the circumference and >2.5 cm in length, and who underwent ablation with a catheter that exceeded the smallest measured inner oesophageal diameter. The few cases of oesophageal stenosis after RFA all occurred in patients with ER >50% of the circumference and >2 cm in length. Based on these observations, it is advisable to limit the extent of ER to <50% of the circumference and <2 cm in length, and to conservatively select the ablation catheter (e.g. if the smallest measured diameter is 29 mm, a 28-mm balloon would be appropriate in case of no prior ER; prior ER, however, warrants the selection of a 25-mm balloon). It is expected that ongoing clinical studies will provide more information to optimize this promising combination of ER with RFA.

Another area that requires further research is the optimal approach to RFA of the gastro-oesophageal (GO) junction. The often tortuous course of the distal oesophagus and widening into a hiatal hernia may make it difficult to bring the electrode of the HALO® catheter into good circumferential contact with the mucosa at the GO-junction. This may result in insufficient ablation of the BO at this level and given the difficulty to endoscopically differentiate Barrett’s mucosa from gastric mucosa, a rim of untreated BE may persist at the top of the gastric folds. To prevent this, we advise to ablate the full circumference of the GE-junction using the focal HALO® device. Histological confirmation is, however, mandatory to ensure complete clearance of IM. Despite this approach, however, focal non-dysplastic IM can be detected in biopsies obtained immediately distal to the neosquamocolumnar junction. The clinical relevance of this finding remains unclear. One may argue that these patients, with an initial diagnosis of HGD or IMC, are still not completely cured from their underlying disease. IM of the cardia, however, is found in up to 25% of normal subjects and in those cases it is not considered a premalignant condition. Non-dysplastic IM in biopsies distal to the neosquamocolumnar junction does, therefore, not require additional treatment, whereas IM with LGD or HGD should be treated. Fourth, we would like to address the issue of “buried Barrett’s glands” after ablation. The clinical relevance of “buried Barrett’s” is still uncertain, but of concern is the possibility of occult malignant progression of the buried glands, as has been suggested by incidental reports of adenocarcinoma arising underneath neosquamous epithelium after ablation therapy. Thus far, no truly buried Barrett’s has been detected in patients who had complete eradication of all IM after RFA. Since this finding is in discordance with the rate of subsquamous IM (0-53%) found after other ablative techniques, some argue that the biopsies do not sample the neosquamous epithelium deep enough to reliably evaluate the presence of buried Barrett’s glands. Ongoing studies evaluating sampling depth and presence of buried glands in biopsies and ER specimens from neosquamous epithelium after RFA should enlighten this issue. In this respect, the artifacts that may lead to a wrongful diagnosis of buried Barrett’s should also be addressed. Biopsies from neosquamous epithelium near the neosquamocolumnar junction may lead to sampling of the transition from neosquamous to columnar epithelium. This may lead to a histological finding of glandular mucosa underneath the neosquamous epithelium, which may mistakenly be interpreted as buried Barrett’s. The same holds when a biopsy is taken from presumably neosquamous epithelium, while there is in fact a small isle of IM that was not detected endoscopically. Tangential sampling of the isle and tangential sectioning of the biopsy may then also result in an erroneous finding of buried Barrett’s. A diagnosis of buried Barrett’s glands should, therefore, only be made if the endoscopist is positive that there were no BE isles after detailed inspection with NBI, and if the biopsies are not obtained at the level of the neosquamocolumnar junction, as was the case in a case report of a single patient, single biopsy “buried gland”.

Fifth, it is questionable if every endoscopist should be trained in RFA. Although this novel ablation technique is relatively easy to apply, RFA is just one aspect in the whole spectrum of endoscopic management of BO patients. Selection of patients with a proper indication for RFA involves thorough endoscopic work-up, the possibility to safely perform ER, and accurate histological evaluation of tissue specimens for the presence of risk factors for lymph node metastasis. We think it would be desirable if RFA were centralized in centres with multidisciplinary expertise in this field. To realize this, adequate training courses (e.g. www.endosurgery.eu), aimed at the whole spectrum of endoscopic management, are mandatory to maintain the status of endoscopic treatment as a valid and safe alternative to surgical treatment in the management of early Barrett’s neoplasia.

An important question that remains is from where the neosquamous epithelium originates. Different hypotheses have arisen over the last years, involving outgrowth from existing pools of squamous cell progenitors, repopulation from adjacent areas with squamous epithelium, or multipotent progenitor cells. To fully understand the process of squamous repopulation after RFA further studies are required, since more insight in the source of the neosquamous epithelium may enlighten if replacing BO with neosquamous epithelium by RFA, indeed reduces the risk of developing cancer.

Furthermore, identification of factors that are associated with a good or poor response after RFA may enable prediction of what patients will respond well and will only need 1-2 ablation sessions, and what patients will respond with poor healing and will need multiple treatment sessions. In those rare patients that respond poorly to RFA, measurements that improve post-RFA healing would be valuable. Other developments should be aimed at the use of imaging technology to inspect residual isles and the gastro-oesophageal junction, to assess if these contain IM and thus require immediate treatment during the same endoscopy session.

Summary
Current data suggest that RFA is an encouraging modality for eradication of BO with many appealing aspects. RFA has been proven to be highly effective in eradicating IM and its associated dysplasia, it has a low complication rate, preserves the oesophageal functional integrity, is relatively easy to apply, and the regenerating neosquamous epithelium is free of the pre-existing oncogenetic alterations. There are, however, still some unanswered ques-
tions concerning the optimal use of the HALO™ catheter, the optimal combination of ER with RFA, the presence of buried Barrett’s glands following RFA, and if the effect is maintained on the long run. For patients with IMC and HGD, RFA appears to be a less invasive and valid alternative to PDT, APC or oesophagectomy, be it after thorough endoscopic work-up and ER of IMC and visible lesions. For patients with LGD or non-dysplastic BO, RFA treatment is more debatable but in our opinion justified in selected cases. Further clinical studies, long-term follow-up data after RFA, and development of biological markers to predict malignant progression of IM, however, will enlighten which patients should be treated with RFA for BO eradication.

REFERENCES


Eradication of Barrett’s oesophagus with early neoplasia by radiofrequency ablation, with or without endoscopic resection

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ABSTRACT

Background:
Radiofrequency ablation is safe and effective for complete eradication of non-dysplastic Barrett’s oesophagus (BO). Aim was to report the combined results of two published and two ongoing studies on radiofrequency ablation of BO with early neoplasia, as presented at SSAT presidential plenary session DDW 2008.

Methods:
Enrolled patients had BO ≤12 cm with early neoplasia. Visible lesions were endoscopically resected. A balloon-based catheter was used for circumferential ablation and an endoscope-based catheter for focal ablation. Ablation was repeated every 2 months until all Barrett’s epithelium was endoscopically and histologically eradicated.

Results:
Forty-four patients were included (35 men, median age 68 years, median BO 7 cm). Thirty-one patients first underwent endoscopic resection [early cancer (n=16), high-grade dysplasia (n=12), low-grade dysplasia (n=3)]. Worst histology remaining after resection was high-grade dysplasia (n=12), low-grade (n=32), or no (n=2) dysplasia. After ablation, complete histological eradication of all dysplasia and intestinal metaplasia was achieved in 43 patients (98%). Complications following ablation: mucosal laceration at resection site (n=3) and transient dysphagia (n=4). After 21 months follow-up (IQR 10-27), no dysplasia had recurred.

Conclusions:
Radiofrequency ablation, with or without prior endoscopic resection for visible abnormalities, is effective and safe in eradicating BO and associated neoplasia.

INTRODUCTION
Barrett’s oesophagus (BO) is a condition characterized by a change of the normal squamous oesophageal lining into a columnar epithelium containing specialized intestinal metaplasia (IM), due to longstanding exposure to gastro-oesophageal refluxate. BO is the best-recognized risk factor for the development of oesophageal adenocarcinoma, and patients diagnosed with non-dysplastic BO are, therefore, advised to undergo endoscopic surveillance with biopsies every one to three years. By histological evaluation of these biopsies, malignant progression to low-grade dysplasia (LGD), high-grade dysplasia (HGD) or early cancer (EC) may be detected. Early neoplasia (i.e., HGD and/or EC) can be treated by surgical oesophagectomy. Given the morbidity and mortality that may be associated with oesophagectomy, less invasive endoscopic alternatives have been considered. Endoscopic resection (ER) is the cornerstone of endoscopic therapy, since it provides a relatively large tissue specimen for histopathological evaluation, enabling proper selection of patients for subsequent endoscopic versus surgical therapy.

Selected patients with HGD or EC limited to the mucosal layer (T1m) have a minimal risk of lymphatic involvement, and ER in these patients has been reported to have a 5-year disease specific survival of 95%. Patients with submucosal invading lesions (T1sm), however, have a 15-30% risk of lymphatic involvement, warranting surgical oesophagectomy with resection of surrounding lymph nodes. After focal ER of HGD/EC, the residual BO still holds the potential of malignant degeneration, and metachronous lesions occur in 30% of patients. Additional treatment of the residual BO after focal ER is therefore advocated, and different treatment modalities have been proposed for this end. The residual BO may be completely removed with stepwise radical endoscopic resection (SRER). This approach allows for histopathological evaluation of the entire BO segment and removes all oncogenic alterations that are present in the pre-treatment BO. SRER, however, is technically demanding, only amendable for patients with a BO <5 cm and has a significant stricture rate. Ablating the residual BO with argon plasma coagulation (APC) or photodynamic therapy (PDT) has also been described, but these techniques do not always result in complete eradication of all Barrett’s epithelium, pre-existing oncogenic alterations may still be found in residual areas of BO, and both techniques are associated with issues of variable ablation depth and safety. Furthermore, after APC and PDT, areas of IM may become hidden underneath the newly formed squamous epithelium after ablation (a.k.a., “buried Barrett’s”), and some fear that these buried glands may progress to dysplasia and adenocarcinoma without being detected endoscopically.

Stepwise circumferential and focal radiofrequency ablation (RFA) using the HALO system is a novel and promising ablative modality. Primary circumferential ablation is performed using a balloon-based bipolar electrode, while secondary treatment of residual BO is performed using an endoscope-mounted bipolar electrode on an articulated platform. Studies involving circumferential ablation were initially conducted in the porcine animal model and in humans prior to oesophagectomy, in order to determine dosing and technique parameters. Subsequently, RFA has been proven safe and effective for the eradication of dysplasia and IM in a number of clinical trials involving patients without dysplasia, with LGD or HGD, and after ER of EC and visible lesions. In addition, no buried Barrett’s glands have been found in over 4000 neosquamous biopsies obtained during follow-up.
oncogenetic abnormalities as present in the pre-treatment BO are absent in the regenerated neosquamous epithelium after RFA,[28] and the functional integrity of the oesophagus is not affected by RFA.[29] In this paper we will present the results reported in Abstract 215, that was selected for oral presentation during the SSAT presidential plenary A session, at the Digestive Disease Week 2008, San Diego, CA, U.S.[30] We will review our results, as available up until November 30, 2007, of stepwise circumferential and focal ablation in 44 patients with Barrett’s oesophagus and HGD/EC who were consecutively treated in four different, IRB-approved, study protocols at the Academic Medical Center, Amsterdam, the Netherlands.

MATERIALS AND METHODS

Patient selection

Starting July 2005, patients between 18 and 85 years old, were consecutively included in a series of IRB-approved clinical protocols evaluating the effect of RF ablation on BO with early neoplasia, and conducted at the Academic Medical Center, Amsterdam, the Netherlands. Patients were eligible if they had endoscopically visible BO (≤12 cm) with HGD or EC diagnosed at two separate endoscopies by an experienced gastrointestinal pathologist (FK). Any visible endoscopic abnormalities, or EC without a clear lesion detected by biopsies, were removed with ER prior to ablation, per the protocol. In case of prior ER, histological evaluation of the specimen could not show vertical resection margins positive for cancer (R+), deep submucosal invading cancer (T1sm1), poorly or undifferentiated cancer (G3, G4), or presence of lymphatic/vascular invasion (V+). Patients with oesophageal stenosis at baseline and patients with invasive cancer in biopsies obtained after ER but prior to RF ablation were also excluded. Our four serial and unique study protocols were as follows:

1. The first prospective study on circumferential RF ablation of HGD/EC in patients with a median BO segment of 5 cm (IQR 5-7) using the HALO360 ablation catheter, with prior en-bloc ER of visible lesions and EC. Halfway through this study, the focal HALO90 ablation device became available.[25]

2. The second prospective study on RF ablation for the treatment of HGD and EC in patients with a median BO length of 7 cm (IQR 6.5-8) had a study protocol similar to the first study. Based on the experiences from the first trial, however, the protocol for this second trial had been optimized by thorough cleaning of the ablation zone and electrode surface in between ablation cycles, and the focal HALO90 device was available from the start of the study. In addition, also patients with prior piecemeal ER of visible lesions were included.[27]

3. The first, ongoing, European multicenter trial to evaluate the safety and efficacy of RF ablation in patients with a Barrett’s segment up to 12 cm long, with early neoplasia, with or without prior ER.[31]

4. An ongoing prospective randomized multicenter trial comparing SRER and RF ablation for the eradication of dysplasia and IM in patients with a BO <5 cm containing early neoplasia.

Endoscopic procedures and medication

All endoscopic procedures were performed on an outpatient basis using intravenous conscious sedation comprised of midazolam and/or fentanyl. After the procedure, patients were clinically observed for 2-4 hours before they were discharged. All patients were prescribed high-dose proton pump inhibitors (i.e., esomeprazole 40 mg bid) as a maintenance dosage during the entire study period. Sucralfate suspension 5 mL (200 mg/mL) qid and ranitidine 300 mg before bedtime were prescribed for two weeks after each therapeutic endoscopy. In case of post-procedural discomfort, patients were allowed to take acetaminophen 500 mg (max. 6/24 h), and if this did not suffice diclofenac suppositories 100 mg bid were permitted.

Endoscopic ablation systems (Chapter 5 Fig. 1)

Both ablation systems that were used (HALO Ablation Systems, BÄRRX Medical Inc., Sunnyvale, California, U.S.) have 510(k) clearance by the Food and Drug Administration in the U.S. and the CE Mark for Europe for the treatment of Barrett’s oesophagus. The HALO Ablation system comprises two distinct ablation systems: the HALO90 system for primary circumferential ablation and the HALO360 system for secondary focal ablation. The HALO90 system includes an energy generator, ablation catheters, and sizing catheters. The HALO360 energy generator delivers radiofrequency (RF) energy to the electrode, and has an integrated pressure-volume system to inflate the sizing balloon and automatically measure the inner oesophageal diameter. The sizing balloon catheter consists of a 4-cm non-compliant balloon that is used for measuring the inner oesophageal diameter of the targeted portion of the oesophagus, prior to circumferential ablation. The sizing catheter is introduced over a guide-wire and it balloon is inflated in an automated manner to 4 psi (0.28 atm). Based on the baseline balloon volume:geometry and the volume needed to inflate the balloon to 4 psi, the mean oesophageal inner diameter is calculated. Measurement is repeated moving distally, for every centimeter of the targeted oesophagus, until an increase in diameter indicates the transition to the stomach or hiatal hernia. The HALO90 ablation catheter has a balloon at its distal end that is completely encircled by 60 electrode rings that alternate in polarity, over a length of 3 cm. The HALO90 balloon is available in five outer diameter sizes (22, 25, 28, 31 and 34 mm). Extensive dosimetry studies in the porcine oesophagus and human oesophagus prior to surgical oesophagectomy have shown that for circumferential ablation two applications of RF energy at 10 - 12 J/cm2 and 40 W/cm2 is the most effective regimen to ablate the full thickness of the epithelial layer, without injuring the submucosa. Focal ablation of residual BO tissue was performed with the HALO360 system. The HALO360 system consists of the focal ablation catheter and an energy generator. The bipolar electrode array of the HALO360 catheter is 20 mm long and 13 mm wide and is mounted on an articulated platform that can be attached to the tip of an endoscope with a flexible strap. The electrode array geometry and spacing is identical to that of the balloon-based electrode.

Endoscopic work-up

Prior to ablation, all patients underwent at least two high-resolution endoscopies with narrow band imaging (NBI) (GIF-Q240Z, Lucera 260 system, Olympus, Tokyo, Japan or GIF-H180, Excera Il-system and a high-definition monitor, Olympus Europe, Hamburg, Germany) to
Endoscopic resection procedures
All visible lesions and EC were removed with endoscopic resection (ER) prior to ablation. The objective of the ER was twofold. Firstly, ER allowed for histological evaluation and staging, enabling optimal selection of patients eligible for endoscopic treatment. Secondly, ER of visible lesions ensured that the subsequent ablation could be performed on an endoscopically flat mucosa. ER was performed using the ER-cap technique (Olympus GmbH, Hamburg, Germany) after submucosal lifting, or the multi-band mucosectomy (MBM) technique (DuetteTM, Cook Endoscopy, Limerick, Ireland). Lesions with a diameter <2 cm were resected en-bloc, larger lesions were resected in multiple pieces (piecemeal procedure). All resected specimens were retrieved, pinned down on paraffin, and fixed in formalin for histopathological evaluation.

Endoscopic ablation procedures
For primary circumferential ablation the oesophageal wall was sprayed with acetylcysteine (1%) and flushed with plain water, to remove excessive mucous. After recording the oesophageal landmarks (i.e., top gastric folds, maximum extent of BO) the endoscope was removed, leaving a guide-wire (Amplatz extra stiff 0.035”, Cook, Denmark, Europe) behind. A sizing balloon was introduced and the inner oesophageal diameter was measured for every centimeter of the targeted BO segment, moving from proximally to distally. Based on the measurements, an ablation catheter with an appropriate outer diameter was selected. The ablation catheter was introduced over the guide-wire, followed by the endoscope to allow the ablation procedure to be performed under endoscopic guidance. The electrode was placed one centimeter above the maximum proximal extent of the BO, the balloon was inflated and the electrode activated (12 J/cm², 40 W/cm²). This resulted in a 3-cm long, circumferentially ablated segment. Depending on the length of the BO segment, the ablation catheter was advanced and, allowing an overlap of 5-10 mm, repositioned distal to the first ablation zone. Ablation was repeated until the entire length of the BO segment had received one application of energy. Then, the ablation zone and electrode surface were cleaned. In the first eleven patients cleaning was performed by advancing the ablation balloon into the stomach where it was inflated, and flushed with water through the endoscope to rinse off excessive coagulum.26 The ablation zone was also rinsed with water through the spraying channel of the endoscope. For the next 12 patients,27 the ablation catheter was removed and the electrode surface was cleaned outside the patient. The ablation zone was more rigorously cleaned compared to the first trial, by forcefully spraying water through a spraying catheter using a pressure pistol (Alliance™, Boston Scientific, Limerick, Ireland, UK). In the following patients cleaning was optimized by the use of a soft distal attachment cap fitted on the tip of the endoscope that was used to slough off most of the coagulum from the ablation zone, prior to forceful rinsing with water through a spraying catheter. After the cleaning procedure, the entire ablation zone was ablated a second time, using the same energy settings. For secondary focal ablation with the HALO90 system, the mucosa was sprayed with acetylcysteine (1%) and flushed with plain water. The HALO90 electrode was fitted on the tip of the endoscope, introduced, and used for targeted ablation of residual Barrett’s epithelium. The squamocolumnar junction was routinely ablated when the HALO90 electrode was introduced to ablate residual isles or tongues. The HALO90 system only became available at the end of the first trial, and the energy settings were escalated from 2x 12 J/cm² to 2x2x 12 J/cm² and eventually to 2x2x 15 J/cm² at 40 W/cm². All areas were ablated with cleaning of the electrode and ablation zone in between ablation cycles, as previously described for the circumferential ablation procedure.

Treatment protocol
After a minimum of six weeks after any ER, patients were treated with primary circumferential ablation using the HALO360 system. After six to eight weeks patients were scheduled for endoscopy to assess the treatment effect. Depending on the extent of residual BO, patients underwent a second HALO360 procedure, or secondary focal ablation using the HALO90 system. In the first study protocol all patients were treated with a second circumferential ablation using the HALO360 system, regardless of the extent of the residual BO, since the HALO90 system for focal ablation was only introduced halfway through the study.38 Additional ablation was repeated every 6-8 weeks and a maximum number of 2 circumferential and 3 focal ablation sessions were allowed to achieve complete eradication of all intestinal metaplasia. Persisting IM after the maximum number of ablations could be endoscopically resected using the MBM technique. Two months after the last treatment session, the endoscopic eradication of IM was assessed during endoscopy using high-resolution endoscopes with Lugol’s staining (2%) or narrow-band imaging. To assess the histological clearance of IM, biopsies were obtained from four quadrants just distal to the neo-squamoscolumnar junction, and every 1-2 cm from the neosquamous epithelium over the full length of the initial BO segment.

Follow-up
Patients were scheduled for follow-up endoscopy two, six and twelve months after the last treatment session, and then annually. High-resolution endoscopes with narrow-band imaging facilities were used to thoroughly inspect the oesophagus for recurrence of IM,
and 4 quadrant biopsies were obtained for every 1-2 cm of the neosquamous epithelium over the original BO length, and immediately distal to the neo-squamocolumnar junction. Patients initially treated for EC underwent EUS every 12 months to exclude the presence of lymph node metastases.

Histopathological review

All biopsies and ER specimens were embedded in paraffin, mounted on glass slides and routinely stained with hematoxylin and eosin. For the purpose of the described studies, all slides were reviewed by an expert GI-pathologist (FK). The ER specimens were evaluated for the presence of dysplasia according to the revised Vienna classification, tumor infiltration depth, tumour differentiation grade, presence of lymphatic or vascular infiltration, and the radicality of the resection at the deep resection margins. Biopsies were evaluated for the presence of IM, LGD, HGD or EC and in case of neosquamous biopsies the presence of glandular mucosa underneath the neosquamous epithelium was assessed.

Ethical considerations and statistical analysis

The Medical Ethics Committee at our institute approved all aforementioned study protocols, and written informed consent was obtained from all included patients. Statistical analysis was performed with SPSS 12.0.1 Software for Windows. For descriptive statistics mean (± SD) was used in case of a normal distribution of variables, and median (IQR) was used for variables with a skewed distribution. Where appropriate, the student t-test and the Mann-Whitney test were used.

RESULTS

Patients

A total of 44 patients was enrolled in the different study protocols, and all had finished treatment by November 30, 2007: 35 men, median age 68 (IQR 57-75) years, median Barrett’s length C5M7 (IQR C2-7, M4-9). Eleven patients were included in the first published trial on RF ablation, 12 patients in the second published trial, and 9 patients in the ongoing European multicenter trial, and 12 patients were randomized to RF ablation in the ongoing randomized trial comparing RF ablation with SRER. A total of 36 ER procedures were performed in 31 patients prior to ablation. Nineteen were performed with the ER-cap technique after ablation, 26 patients in the second published trial, and 9 patients in the ongoing European multicenter trial, and 12 patients were randomized to RF ablation in the ongoing randomized trial comparing RF ablation with SRER.

Eradication of dysplasia and intestinal metaplasia (Fig. 1)

Complete histological eradication of dysplasia and complete endoscopic and histological clearance of IM was achieved in 43 patients (98%), after a median of 1 (IQR 1-2) circumferential ablation, 2 (IQR 1-2) focal ablation sessions, and escape ER in 3 patients. These three patients had small areas of residual columnar epithelium that persisted after the maximum number of allowed ablation sessions. These areas were resected using the MBM technique, and showed LGD (n=2) and HGD (n=1) upon histological evaluation. In one patient the proposed treatment protocol failed (2%). After 2 ER sessions, one circumferential and two focal ablations, a persisting area of suspicious looking columnar epithelium was observed and resected en-bloc using the MBM technique. Histology showed a T1sm1 adenocarcinoma, radically resected at the deep resection margins (RO). Two months after the escape ER, however, a suspicious 5-mm isle was identified. Additional resection of this area failed due to scarring resulting from the prior ER sessions. Since the patient strongly opposed against surgical treatment, the area was ablated with APC (forced coagulation 60 W, gas flow 1.6 L/min, ERBE Vio System, Erbe Elektromedizin GmbH, Tübingen, Germany). Two subsequent follow-up endoscopies with extensive biopsies and EUS showed no signs of recurrent dysplasia or IM.

Adverse events

In five patients a complication occurred during ER (16%): there were four mild bleedings that could be easily managed with endoscopic hemostatic techniques, and there was one oesophageal perforation. The perforation was treated conservatively by placement of clips (resolution clips, Boston Scientific, Limerick, Ireland, UK), and a covered oesophageal stent was placed.
A session, at the Digestive Disease Week 2008. A total of 44 consecutive patients with BO epithelium only one (0.07%) showed buried glandular mucosa. junction at a single follow-up endoscopy. In 1475 biopsies obtained from neosquamous endoscopic signs of BO during follow-up. Five patients had focal IM detected in biopsies the upper end of the initial C9M10 Barrett’s segment; none of the other 43 patients showed one patient, a 1-mm BO island was identified 16 months after the last treatment, located at Follow-up

During a median follow-up of 21 [10–27] months no recurrence of dysplasia was observed. In one patient, a 1-mm BO islet was identified 16 months after the last treatment, located at the upper end of the initial C9M10 Barrett’s segment; none of the other 43 patients showed endoscopic signs of BO during follow-up. Five patients had focal IM detected in biopsies obtained immediately distal to an endoscopically normal appearing neo-squamocolumnar junction at a single follow-up endoscopy. In 1475 biopsies obtained from neosquamous epithelium only one (0.07%) showed buried glandular mucosa.

**DISCUSSION**

This manuscript reviews our interim results of RF ablation for BO with early neoplasia from four different study protocols at the Academic Medical Center, Amsterdam, the Netherlands, and was written to accompany our oral presentation during the SSAT presidential plenary A session, at the Digestive Disease Week 2008. A total of 44 consecutive patients with BO containing HGD and/or EC had finished treatment by November 30, 2007. Of these, 23 patients were treated in the first two pilot studies worldwide to evaluate the use of stepwise circumferential and focal ablation of BO with HGD/EC after prior ER of any visible abnormalities and EC. The other 21 patients were included for the first European multicenter study on RF ablation of BO up to 12 cm containing HGD/EC or in an ongoing study comparing stepwise radical endoscopic resection (SRER) with RF ablation in patients with early neoplasia in BO <5 cm. In all four studies, it was protocolized that any visible lesions and EC had to be removed with ER prior to ablation to enable histological evaluation for accurate staging of the infiltration depth and tumour differentiation, and to ensure that subsequent RF ablation could be performed on an endoscopically flat mucosa. In the first study, six of the eleven patients had undergone an en-bloc resection of a visible lesion. No significant oesophageal scarring was observed in these patients, and no complications such as mucosal injury or dysphagia occurred after ablation treatment. In the other three studies, patients with prior piecemeal ER or multiple ER sessions were included, and mucosal injuries (n=3) and dysphagia (n=4) were observed for the first time. The four patients presenting with dysphagia had all undergone widespread ER and/or were treated with a relatively large diameter ablation catheter compared to the measured oesophageal diameter. To prevent complications resulting from ER scarring, it is in our opinion that one should limit the extent of ER of visible lesions to 50% of the circumference and 2 cm in length. In addition, the HALO90 ablation catheter size should be selected conservatively in cases of prior ER, preferably one size smaller than the catheter that would be selected based on the oesophageal inner diameter measurements. No oesophageal stenoses were observed in patients without a prior ER who were exclusively treated with ablation therapy. These results are in concordance with the U.S. multicenter AIM-study (ablation of intestinal metaplasia), where no strictures were reported in 100 patients treated with RF ablation.25 The absence of submucosal scarring as a result of RF ablation was also illustrated by our ability, in 3 patients, to remove focal areas of persistent Barrett’s mucosa after multiple ablation sessions using the multiband mucosectomy technique, without the need for submucosal lifting in three patients. This is a significant advantage compared to other endoscopic ablation techniques, after which escape treatment using ER is usually difficult as a result of submucosal scarring. In the 1475 biopsies obtained from neosquamous epithelium during follow-up, only one biopsy showed focal intestinal metaplasia hidden underneath the newly formed squamous epithelium. This biopsy was obtained at the upper end of an initial C9M10 Barrett’s segment, at the same level where at a following endoscopy a small 1 mm isle was identified with narrow-band imaging that may have been left untreated and unobserved at the preceding endoscopies. The fact that no buried glands were found in 8 biopsies obtained at this level during other follow-up endoscopies, and the absence of any IM in an ER specimen to remove the 1 mm isle, suggest that the biopsy with buried IM may have sampled this minute isle tangentially, rather than sampling truly buried Barrett’s glands. Although this hypothesis cannot be confirmed, the 0.07% of submucosal IM still compares favorably to the 53% rate of buried glands reported after other ablation techniques.26-27 Our findings were in concordance with the absence of buried glands in 3007 neosquamous biopsies after RF ablation in the 100 patients described by Sharma et al.28 Further studies on the adequacy of biopsies from the neosquamous epithelium after RF ablation should, however, clarify this issue further. Ablation at the GO-junction using the HALO360 catheter may be difficult, since the often tortuous course of the distal oesophagus and widening into a hiatal hernia, present in most BO patients, may impede good circumferential contact of the electrode with the mucosa at this level. In addition, endoscopically differentiating cardia mucosa from Barrett’s mucosa at the top of the gastric folds after ablation treatment may be difficult. Therefore, all patients were treated with ablation of the GO-junction using the HALO360 catheter. The HALO90 device allows for targeted, focal ablation, and was used to completely ablate the full circumference of the GO-junction to ensure that there was no small rim of residual Barrett’s mucosa left untreated at the transition of the columnar...
epithelium into the neosquamous epithelium. Despite this approach, focal IM was diagnosed in five patients (11%) in a single biopsy obtained just distal to a normal appearing neosquamocolumnar junction at a single follow-up endoscopy, not reproduced at following endoscopies. The clinical relevance of this finding may be debated. Since all patients had an initial diagnosis of HGD or EC, one may argue that finding residual IM in the cardia during follow-up means that the IM had not been completely eradicated and that the patients were not completely cured from their underlying disease. IM of the cardia, however, can be detected in up to 25% of patients with a normal appearing squamocolumnar junction and is not considered a premalignant condition in those cases.31 In addition, we think that the patchy nature of this finding, and the fact that all patients will remain under endoscopic follow-up given their initial diagnosis of HGD/EC, does not justify additional treatment. As described in the “Materials and Methods” section, the treatment protocol for the second trial was improved based on the experiences from the first trial. These improvements were reflected in the median number of treatment sessions required to achieve complete eradication of intestinal metaplasia. Although the median BO length was longer in the second trial (7 cm [IQR 6.5-8] vs. 5 cm [IQR 4-7]), the mean number of ablation sessions was lower (3.4 vs. 4.2 sessions). The three most significant changes in the protocol were as follows: firstly, the HALO® catheter for secondary focal ablation only became available halfway through the first trial. Most patients had by then already undergone a second circumferential ablation session, regardless of the amount of residual BO, whereas in the second trial the HALO® device could be used to treat isles or tongues persisting after the first circumferential ablation. Secondly, the energy settings used for focal ablation were escalated from two ablations at 12 J/cm², to two times two ablations at 12 J/cm² (“double-double”), to double-double 15 J/cm² when the device became available during the first trial. In the second trial, the double-double 12 J/cm² dose was used initially, but in four patients a step up to double-double 15 J/cm² ablation was required to eradicate all IM. Since this “double-double 15 J/cm²” approach proved effective without causing significant side-effects, this dose is currently used in the ongoing studies. Thirdly, in the first study the electrode surface of the HALO® catheter was cleaned by inflating the balloon in the stomach and flushing it with water prior to the second ablation pass, without significant cleaning of the ablation zone. In the second trial the electrode surface was cleaned with a stomach and flushing it with water prior to the second ablation pass, without significant side-effects, this dose is currently used in the ongoing studies. Following additional procedure minutes, meticulous cleaning of the electrode and ablation zone after the first pass improves the efficacy of RF ablation and should always be performed. The thorough cleaning protocol has, therefore, been incorporated in current trials.

CONCLUSION

Stepwise circumferential and focal radiofrequency ablation of Barrett’s epithelium with high-grade dysplasia or early cancer, with or without prior endoscopic resection of focal lesions, is highly effective in achieving complete eradication of dysplasia and intestinal metaplasia, without any serious adverse events. This novel treatment modality, therefore, appears to be a favorable alternative to oesophagectomy, radical endoscopic resection, argon plasma coagulation or photodynamic therapy.

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Efficacy of radiofrequency ablation combined with endoscopic resection for Barrett’s oesophagus with early neoplasia
ABSTRACT

— Background & Aims:
Radiofrequency ablation (RFA) is safe and effective for eradicating intestinal metaplasia and neoplasia in patients with Barrett’s oesophagus. We sought to assess the safety and efficacy of RFA in conjunction with baseline endoscopic resection for high-grade intraepithelial neoplasia (HGIN) and early cancer.

— Methods:
This multicentre, prospective cohort study included 24 patients (mean age 65 years, median Barrett’s oesophagus 8 cm), with Barrett’s oesophagus ≤12 cm containing HGIN or early cancer, from 3 European tertiary-care medical centres. Visible lesions were endoscopically resected, followed by serial RFA. Focal escape endoscopic resection was used if Barrett’s tissue persisted despite RFA. Complete response, defined as all biopsies negative for intestinal metaplasia and neoplasia, was assessed during endoscopy with 4-quadrant biopsies every 1 cm of the original Barrett’s segment 2 months after the last treatment.

— Results:
Twenty-three patients underwent pre-RFA endoscopic resection for visible lesions; 16 had early cancer and 7 HGIN. The worst residual histology, pre-RFA (after any endoscopic resection) were: HGIN (n=10), low-grade intraepithelial neoplasia (n=11), and intestinal metaplasia (n=3). Eradication of neoplasia and intestinal metaplasia was achieved in 95% and 88% of patients, and after additional escape endoscopic resection in 2 patients in 100% and 96%, respectively. Complications after RFA included melena (n=1) and dysphagia (n=1). After additional follow-up (median 22 months [IQR 17.2-23.8]) no neoplasia recurred.

— Conclusions:
This European multicentre study demonstrated that early neoplasia in Barrett’s oesophagus can be effectively and safely treated with RFA, in combination with prior endoscopic resection of visible lesions.

INTRODUCTION
Barrett’s oesophagus (BO), defined as a columnar-lined oesophagus and biopsy demonstrating specialized intestinal metaplasia [IM], is the most important risk factor for the development of esophageal adenocarcinoma. Patients with known BO undergo endoscopic surveillance to detect neoplasia (i.e. high-grade intraepithelial neoplasia [HGIN] or early cancer [EC]) at a curable stage. Patients with HGIN or with EC confined to the mucosa (T1m) may be treated endoscopically due to low risk for lymph node metastasis at time of diagnosis, but more advanced cancers are surgical indications. The cornerstone of endoscopic treatment of early BO neoplasia is endoscopic resection (ER), which allows for removal of visible lesions and assessment of tumour infiltration depth and differentiation. After focal ER, however, the residual Barrett’s mucosa remains at risk for malignant transformation and cancer recurrences are found in 30 % of patients during follow-up. To prevent such metachronous lesions, endoscopic approaches have been studied in an attempt to eradicate the residual Barrett’s mucosa, e.g. radical ER, photodynamic therapy (PDT), and argon plasma coagulation (APC). These treatment options, however, do not always result in complete clearance of IM and associated intraepithelial neoplasia and are limited by other shortcomings such as oesophageal stenosis, photosensitivity, and submucous foci of IM, a.k.a. “buried Barrett’s”.

A newer endoscopic ablation technique is radiofrequency ablation (RFA), which has promising safety and efficacy results published for non-dysplastic BO, low-grade intraepithelial neoplasia (LGIN), and HGIN. Single-centre studies have effectively combined focal ER of visible lesions with RFA for residual Barrett’s mucosa in patients with HGIN and EC. The aim of the present study was to further evaluate the safety and efficacy of this combined modality approach in BO patients with HGIN or EC in a European multicentre setting.

MATERIALS AND METHODS
Study Design
This is a prospective cohort trial conducted at three tertiary-care medical centres in Europe. The ethical committee of each institution reviewed and approved the protocol and the patient informed consent form. The trial was registered at www.trialregister.nl (NTR1434). A central study coordinator monitored all procedures and entered data into a central database. Patients were eligible if they met all inclusion criteria: age 18-85 years; BO length ≤12 cm; HGIN or EC in BO segment on two endoscopies in prior 6 months; visible lesions removed with ER prior to RFA; no signs of metastasis on endoscopic ultrasonography (EUS) or CT-scan. Patients were excluded if they met any exclusion criterion: pre-RFA ER with cancer at the vertical resection margin, >T1sm invasion, poor differentiation or worse, or angiolymphatic invasion; oesophageal stenosis preventing passage of 11.3 mm endoscope; persistent visible lesions after ER and pre-RFA; invasive cancer on biopsies after ER and pre-RFA. To ascertain eligibility, patients underwent two high-resolution endoscopies with documentation of BO landmarks according to the Prague classification system.26 In the case of visible lesions, these were removed with ER followed by two additional endoscopies with 4-quadrant 1 cm
biopsies to exclude residual cancer and residual non-flat lesions. EUS was performed in all patients to rule out exclusionary lesions. In the case of cancer on biopsy or ER, a CT-scan of thorax and upper 1/3rd of the abdomen was performed to rule-out metastatic disease.

Endoscopic interventions

All procedures were performed on an outpatient basis using intravenous midazolam, fentanyl, pethidine, propofol, or a combination thereof. All patients were prescribed high-dose proton pump inhibitor therapy (esomeprazole 40 mg BID) during the entire study period, supplemented with sucralfate suspension 5 mL (200 mg/mL) QID and ranitidine 300 mg before bedtime for two weeks after any therapeutic endoscopy.

ER of any non-flat lesions was performed using the ER-cap technique, the multiband mucosectomy (MBM) technique or endoscopic submucosal dissection, at the discretion of the physician.

The ablation systems used in this trial have 510(k) clearance by the Food and Drug Administration in the U.S.A. and the CE Mark for Europe for the treatment of BO (BÂRRX Medical Inc., Sunnyvale, California, U.S.A.). For circumferential RFA, a sizing catheter was introduced over a guide-wire to measure the inner oesophageal diameter of the oesophagus. A HALO360 ablation catheter of appropriate outer diameter was introduced over the guide-wire followed by the endoscope in a side-by-side manner. The electrode was positioned 1 cm above the proximal extent of the BO, the balloon inflated, and energy delivered (12 J/cm², 40 W/cm²) resulting in circumferential ablation of a 3 cm segment. The ablation catheter was repositioned distal to the prior ablation zone, allowing minimal overlap, and ablation was repeated until the entire BO was ablated. The ablation catheter was removed to clean the electrode surface, while a soft distal attachment cap (Model MB-046, Olympus, Tokyo, Japan) was mounted on the endoscope and used to clean the ablation zone. Residual debris was then removed by forceful spraying of water through a spraying catheter with a pressure pistol (Aliance™, Boston Scientific, Limerick, Ireland, U.K.). After cleaning, the ablation catheter was re-introduced and the BO segment was treated a second time. For secondary focal ablation, each island or tongue of residual Barrett’s mucosa was aligned with the focal HALO60 catheter, the endoscope deflected to bring the electrode into contact, and RF energy delivered twice in succession (15 J/cm², 40 W/cm²). All areas were cleaned by pushing the coagulum off with the leading edge of the device. The electrode was removed to clean its surface, and then reintroduced to re-treat all areas twice more in succession. In all patients, the gastro-oesophageal junction was treated circumferentially with the HALO90 catheter at least once (2x2 15J/cm²), to ensure eradication of IM at this level.

Patient Flow

Baseline visible lesions were removed by ER, followed by primary RFA at least 6 weeks after the ER and within 3 months after the last biopsy session. Additional RFA sessions were thereafter scheduled every 8 weeks until complete endoscopic eradication was achieved. A maximum of 2 circumferential and 3 focal RFA sessions was allowed. Any residual Barrett’s epithelium persisting after the maximum number of allowable RFA sessions was removed with a focal ER as escape therapy. Two months after the last treatment session, the original extent of BO was biopsied every 1 cm, including immediately (<5 mm) distal to the neo-squamocolumnar junction. If patients had reached complete resolution of intestinal metaplasia and complete resolution of neoplasia, they were scheduled for follow-up endoscopy and four-quadrant biopsies at 6 and 12 months after the last treatment session, and annually thereafter (Fig. 1).

Figure 1. Flow-chart of the study protocol.

Outcome variables
The primary endpoints were histology-based (biopsies obtained 2 months after last therapeutic intervention). A complete response was defined as all biopsies negative for IM (CR-IM) and neoplasia (CR-neoplasia), separately reported. Secondary endpoints: disease progression, adverse events, and durability of CR-IM and CR-neoplasia at last biopsy available.

Histological Analysis
All ER specimens and biopsies from baseline and follow-up were evaluated by the central study pathologist (FKK), an expert in gastrointestinal pathology. ER specimens were evaluated for neoplasia according to the WHO classification,27 tumour infiltration depth, differentiation, presence of lymphatic or vascular infiltration and completeness of resection at the vertical margin. Biopsies were evaluated for presence of IM, LGIN, HGIN and EC, and follow-up biopsies from neo-squamous epithelium were evaluated for the presence of subsquamous areas of IM.

Statistical analysis
Statistical analysis was performed with SPSS 16.0.2 Software for Windows. For descriptive statistics mean (± standard deviation (SD)) 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 student t test and the Mann-Whitney test were used.

RESULTS
Enrolment and baseline characteristics
Twenty-four patients were included, 20 men, mean age 65±9.8 years, median BO length C6M8 [IQR C2-9, M4-10]. Twenty patients had a hiatal hernia [median 2 cm [IQR 2-3]]. Sixteen patients had a baseline diagnosis of cancer; eight patients had HGIN as the worst histological finding at two work-up endoscopies.

Baseline Endoscopic Resection
Twenty-three patients (96%) underwent a total of 25 ER sessions prior to RFA (2 patients had 2 ER sessions). Of the 25 ER sessions, 12 were performed with the ER-cap technique, 12 with the MBM technique and 1 with endoscopic submucosal dissection. The worst histological grade based on ER was EC in 16 patients [T1m2, n=8; T1m3, n=6; T1sm1, n=2] and HGIN in 7 patients. The worst grade of residual intraepithelial neoplasia in biopsies obtained from the remaining BO during at least two high-resolution endoscopies after any ER, was HGIN in 10 patients [in the absence of any visible lesions], LGIN in 11 patients, and three patients had IM without neoplasia.

Number of treatment sessions
Patients underwent a median of 1 [IQR 1-1] primary circumferential and 1 [IQR 1-2] secondary focal RFA sessions. Escape ER was necessary in 1 patient to remove a resistant 8 mm Barrett’s island (LGIN). Escape ER was necessary in a second patient to remove 4 small BO foci (IM, no neoplasia) due to difficulty in introducing the focal RFA device. Overall, patients required a median number of 3 [IQR 3-4] therapeutic interventions (including any ER before and after RFA), during a median period of 6.4 (IQR 5.5-11.3) months.

Primary Outcome Variables
Eradication of intraepithelial neoplasia and IM
In 20/21 patients with residual LGIN/HGIN in their BO after ER and prior to ablation, CR-neoplasia was achieved with RFA (95%). Escape ER for an 8mm island of LGIN resulted in CR-neoplasia in all 21 patients (100%). CR-IM was achieved with RFA in 21/24 patients (88%). After escape ER in two patients [see above], CR-IM was reached in 23/24 patients (96%). The failure (n=1) had a C10M10 non-neoplastic Barrett’s segment after ER of a T1m2 carcinoma at baseline, and did not regenerate neosquamous epithelium readily after RFA. Thus, he was removed from the trial.

Secondary Outcome Variables
Progression
During follow-up, there were no new cancers and no histological progression of disease.

Adverse events
ER related complications:
One severe complication, an oesophageal perforation, occurred after baseline ER (cap technique), treated non-surgically with clips and a covered stent. This patient underwent a second ER session 3 months after the perforation and went on to have RFA 2 months after the second ER, without further complication, and is now CR-IM at 2, 6 and 12 months. RFA related complications: No severe complications occurred after RFA. Two complications (8%) were graded as being of moderate severity since they required additional endoscopic procedures: one patient presented with melena 2 weeks after focal ablation. Upon endoscopic inspection, no active bleeding was observed, but two visible vessels in the ablated area were preventively clipped. A second patient developed dysphagia after the first RFA. He had undergone widespread ER prior to RFA and had a mucosal laceration after circumferential RFA. The stenosis was resolved with 5 endoscopic dilatation sessions, after which ablation treatment could be resumed. Mucosal laceration was observed in five patients (21%) after circumferential RFA, all occurring within the mucosal scar region of a prior ER (Fig. 2). All patients remained asymptomatic and no therapeutic interventions were required. These lacerations were therefore graded as mild complications.

Durability of complete response
After a median follow-up of 22 [IQR 17.2-23.8] months after the last treatment, and a median of 3 [IQR 3-4] follow-up endoscopies per patient, no recurrence of neoplasia was observed.

Long-term complete response for IM was maintained in 20/24 included patients (83%). In one patient with baseline C9M10 and CR-neoplasia and CR-IM at 2 months, a tiny (0.5x3 mm) glandular island with IM upon biopsy was found at 6 months follow-up endoscopy [Fig. 3]. The island was located distal to a reflux stenosis and was only detected after inspection with NBI. In 2 patients at 6 months, focal non-neoplastic IM was found in one biopsy each, obtained distal to the gastro-oesophageal junction. In neither patient was this finding reproduced at 12 months. No buried Barrett’s was found in a total of 1,201 neosquamous biopsies obtained during follow-up.
Figure 2. Endoscopic images of circumferential RFA, complicated by non-transmural mucosal laceration.
A: C11M12 BO. B: At 32 cm from the incisors a suspicious lesion was observed. C: The lesion was removed in 2 pieces. Histology showed HGIN. D: Prior to circumferential RFA oesophageal scarring was observed at the resection site. E, F: During circumferential RFA, inflation of the balloon catheter caused non-transmural mucosal laceration, due to overstretching at the level of the ER-scar. G: Three months after primary RFA, two residual Barrett’s islands were detected with NBI. H: The islands were treated with focal RFA. I-L: Two months after the last ablation, complete endoscopic eradication of neoplasia and IM was reached and the oesophagus was covered with normal appearing neosquamous epithelium, as seen with white light endoscopy and NBI.

Figure 3. Endoscopic images of a small island of glandular mucosa detected during follow-up.
A, B: C9M10 BO with proximal reflux stenosis, containing diffuse HGIN after ER for early cancer. C, D: Residual Barrett’s mucosa was completely converted to neosquamous mucosa by 1 circumferential and 1 focal RFA. No recurrence of neoplasia or IM was found at two months follow-up. E, F: At 6 months follow-up NBI revealed a tiny island of columnar epithelium just distal to the reflux stenosis and biopsy showed IM.
DISCUSSION

There is no generally accepted management strategy for patients with early neoplasia in BO. However, studies in which focal ER for neoplasia was followed by surveillance of residual BO have reported a 20–30% rate of metachronous lesions,14 whereas studies in which the whole BO was eradicated after focal ER only reported recurrence in up to 6% of patients.15 Based on these data, we believe that combining ER with complete ablation of residual Barrett’s mucosa is the preferable treatment approach, and in this first multicenter European trial we therefore evaluated RFA in conjunction with ER for the treatment of BO containing neoplasia and early cancer. The primary endpoint of complete eradication of all residual neoplasia after ER was achieved in 21 of 21 patients (CR-neoplasia 100%). The primary endpoint of complete eradication of all residual IM after ER was achieved in 23 of 24 patients (CR-IM 96%). These results comport with those from two recently published, single-centre studies in which RFA was used in conjunction with ER for HGIN and EC to achieve 100% CR-IM and 100% CR-neoplasia.16–18 These favourable outcomes collectively endorse a central role for RFA in the treatment of patients with flat-type neoplasia in a BO. All but one patient in this study underwent baseline ER for visible lesions. This emphasizes that in our opinion ER should be performed not only for nodules, but for any mucosal irregularity or area with suspicious glandular patterns no matter how subtle, even if prior biopsies do not show cancer but HGIN or lower. Not only is baseline ER important to render the mucosa flat for subsequent ablation, it also provides an adequate specimen for histopathological analysis and has been shown to change the histological diagnosis on the basis of prior biopsies in 49% of patients.19 Such a rigorous baseline evaluation is of the utmost importance to enable selection of patients who are eligible for further endoscopic treatment with RFA, i.e. patients without deep submucosal invading cancer (≥T1sm1), or poorly differentiated cancer, versus those that require surgical intervention. In the case of minimal submucosal infiltration (T1sm1), recent studies have suggested that the risk of lymph node metastasis is very low.20 Two patients had minimal submucosal invasion diagnosed in the ER specimen. Due to the absence of other risk factors for lymph node metastasis (poor differentiation grade, irradical resection, lymphatic/vascular infiltration), surgical as well as endoscopic treatment options were elaborately discussed with the patients, and both decided to be treated endoscopically within the study protocol.

In a prior clinical study,21 we allowed only single-piece baseline ER prior to RFA. In a subsequent study,22 we relaxed this restriction to allow multiple piece ER (median 2 specimens [IQR 2–3]) prior to RFA. No mucosal lacerations occurred and a stricture occurred in only one patient after 2 ER sessions and RFA. In the present study, based partially on prior results, we did not initially restrict the extent of baseline ER. During the first half of this study, we noted 5 mucosal laceration events within the ER scar zone during circumferential RFA and one stenosis; and we related this to extensive baseline ER and scarring. None of the lacerations required intervention or caused complaints and, therefore, they were regarded as mild complications. However, since lacerations may provoke severe bleeding or oesophageal perforation, the investigator group added a restriction to the baseline extent of ER (max. 50% of circumference, 2 cm length, and 1 session). We also used a more conservative approach in selecting an ablation catheter balloon size, erring on the small size. After this modification, no further lacerations or stenoses were observed.

One patient was removed from the trial and considered a failure due to poor healing of the oesophagus after circumferential RFA. At baseline, the patient had an EC resected with ER, leaving a residual C1OM10 Barrett’s segment with no neoplasia. The patient had previously undergone a Nissen fundoplication and post-operative 24 pH-metry was normal off proton-pump inhibitor. Yet, despite a maximum acid suppressant regimen [esomeprazole 80 mg BID, ranitidine 300 mg AN, sucralfate 5 ml QID] the oesophagus failed to readily regenerate with neosquamous epithelium. It is unclear what factors were at play in this particular patient’s poor healing, but it highlights the fact that tailored management may be necessary in some cases.

In three patients (83%) with complete eradication of IM and neoplasia, IM was found in biopsies during follow-up, which raises the question if endoscopic surveillance will remain necessary after complete eradication of IM and neoplasia has been reached. Currently, endoscopic follow-up after successful eradication BO by RFA is recommended at regular intervals based on baseline histological diagnosis, since no long-term follow-up data is as yet available. In 2/3 patients with IM during follow-up, focal non-dysplastic IM was found in a single biopsy just distal to the neo-squamo-columnar junction at a single follow-up endoscopy and in neither patient was this finding reproduced during following endoscopies. Since all patients had an initial diagnosis of HGIN/EC, one may argue that residual IM in the cardia reflects incomplete cure of the underlying disease. However, IM of the cardia can be detected in up to 25% of patients with a normal appearing squamo-columnar junction and is not considered premalignant in those cases.23 The clinical relevance of this finding is thus unclear, but in our opinion non-dysplastic IM in biopsies distal to the neo-squamo-columnar junction does not require additional treatment, whereas IM with LGIN or HGIN should be treated. However, long-term follow-up data of these patients may clarify how focal IM in the cardia will behave after RFA. One patient had a 0.5x3 mm island of columnar epithelium detected, with IM upon biopsy, during follow-up endoscopy at 6 months, despite being CR-IM at 2 months. The island was only observed after inspection with NBI. We hypothesize that we missed this island visually on prior follow-up endoscopy due to its limited size and position just distal to a reflux stenosis. It is in our opinion therefore recommended to use high-resolution endoscopy, NBI, Lugol’s chromoendoscopy or comparable techniques during follow-up after RFA, to exclude the presence of residual BO tissue, especially small islands. As described in this manuscript, selection of patients for endoscopic treatment involves thorough endoscopic work-up, the possibility to safely perform ER, and accurate histological evaluation of tissue specimens for presence of risk factors for lymph node metastasis.24 Since all patients in this study underwent endoscopic work-up and RFA in specialized tertiary-care centres, the high reported safety and efficacy should be extrapolated to general practice with care. In our opinion, it may be advisable to centralize RFA of BO patients with HGIN/EC in centres with multidisciplinary experience in this field (i.e. expertise with endoscopic imaging and ER, access to oesophageal surgery, expert GI histopathology) and that have participated in dedicated RFA training courses at expert centres. One strength of this study is the use of a single expert gastrointestinal pathologist for all biopsy specimens and high-quality information on the primary endpoints of endoscopy and histopathology. The study has several limitations, including a lack of a control group, lack of long-term follow-up, and a relatively small study size. However, the study demonstrates the feasibility and safety of a combined approach of ER followed by RFA for the treatment of BO with early neoplasia.
observation of all RFA procedures and primary endpoint biopsy procedures by the central study monitor to ensure protocol adherence, and patient follow-up that extends an 22 months beyond the last therapeutic intervention. One weakness of this study is that many of the baseline ER procedures were performed prior to patient enrolment during confirmation of eligibility, and therefore were not supervised by the study coordinator. However, all centers used standardized techniques and data collection, and all ER specimens were reviewed by the study pathologist. Also, we allowed focal escape ER in certain rare circumstances, which could favourably bias our outcomes. Midway through the study we necessarily adjusted the extent of permissible baseline ER. This restriction appears to be associated with a lower risk of complications (namely, mucosal laceration), but more patients are needed to make this determination. Lastly, a relatively small number of patients was included in this study and treated in three expert centres. We have therefore initiated a second multicentre study in 12 European centres that uses the inclusion criteria defined in this study and aims at including a minimum of 100 patients. The results of this first European multicentre study demonstrate that patients with early neoplasia arising in BO can be effectively and safely treated with RFA, in combination with prior ER of visible lesions.

REFERENCES

Stepwise radical endoscopic resection versus radiofrequency ablation for Barrett’s oesophagus with high-grade dysplasia or early cancer: a multicentre randomized trial

Frederike G. van Vilsteren, Roos E. Pouw, Stefan Seewald, Lorenza Alvarez Herrero, Carine M. Sondermeijer, Mike Visser, Fiebo J. W. ten Kate, Karl C. Yu Kim Teng, Nib Soehendra, Thomas Roesch, Bas L. Weusten, Jacques J. Bergman

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ABSTRACT

Objective: After focal endoscopic resection (ER) of high-grade dysplasia (HGD) or early cancer (EC) in Barrett’s oesophagus (BO), eradication of all remaining BO reduces the recurrence risk. The aim of this study was to compare the safety of stepwise radical ER (SRER) versus focal ER followed by radiofrequency ablation (RFA) for complete eradication of BO containing HGD/EC.

Methods: A multicentre randomised clinical trial was carried out in three tertiary centres. Patients with BO <5 cm containing HGD/EC were randomised to SRER or ER/RFA. Patients in the SRER group underwent piecemeal ER of 50% of BO followed by serial ER. Patients in the ER/RFA group underwent focal ER for visible lesions followed by serial RFA. Follow-up endoscopy with biopsies [four-quadrant/2 cm BO] was performed at 6 and 12 months and then annually. The main outcome measures were: stenosis rate; complications; complete histological response for neoplasia [CR-neoplasia]; and complete histological response for intestinal metaplasia [CR-IM].

Results: CR-neoplasia was achieved in 25/25 (100%) SRER and in 21/22 (96%) ER/RFA patients. CR-IM was achieved in 23 (92%) SRER and 21 (96%) ER/RFA patients. The stenosis rate was significantly higher in SRER (88%) versus ER/RFA (14%; p<0.001), resulting in more therapeutic sessions in SRER (6 vs 3; p<0.001) due to dilations. After median 24 months follow-up, one SRER patient who had recurrence of EC, requiring ER.

Conclusions: In patients with BO <5 cm containing HGD/EC, SRER and ER/RFA achieved comparably high rates of CR-IM and CR-neoplasia. However, SRER was associated with a higher number of complications and therapeutic sessions. For these patients, a combined endoscopic approach of focal ER followed by RFA may thus be preferred over SRER.

INTRODUCTION

In Barrett’s oesophagus (BO), the normal squamous oesophageal epithelium has been replaced by a columnar epithelium containing specialised intestinal metaplasia (IM), as a result of chronic gastro-oesophageal reflux.1 BO is an important risk factor for the development of oesophageal adenocarcinoma, a cancer with a rapidly rising incidence in the western world.2 High-grade dysplasia (HGD) and early cancer (EC) limited to the mucosa in BO can be effectively treated by endoscopic means, without the need for surgery. Endoscopic resection (ER) is the basis of endoscopic treatment. ER allows for removal of focal lesions within the BO and provides a specimen for histological evaluation, which is imperative for optimal patient management: patients with HGD/EC can be managed endoscopically given their low risk of local lymph node involvement, whereas patients with lesions invading deep into the submucosa should be considered for oesophagectomy and lymph node dissection.3 However, after focal ER alone, up to 30% of patients will develop metachronous lesions in the residual BO segment during follow-up.4 Therefore, complete eradication of the residual BO is recommended.5,6 Currently, the two most promising strategies for complete BO eradication are stepwise radical endoscopic resection (SRER) and radiofrequency ablation (RFA). SRER is a technique in which the complete BO segment is removed in consecutive ER sessions. Retrieval of the entire BO segment for histological assessment is ideal, as it may permit referral to surgery for advanced lesions. Single-centre studies have shown excellent results of SRER for HGD/EC with eradication of all neoplasia in 76-100% and complete eradication of all intestinal metaplasia [CR-IM] in 68-100%.7-14 An important limitation of SRER is the rate of complications, such as oesophageal stenosis requiring dilatation, which occurs in up to 56% of cases.15,16 RFA is a new technique for endoscopic eradication of BO that is characterised by controlled and uniform thermal ablation. Several studies have shown that RFA, with and without prior ER, is an effective treatment for HGD/EC, with eradication of all neoplasia in 81-100% and complete removal of IM in 74-100%.17-20 RFA has demonstrated a favourable safety profile, with oesophageal stenosis occurring in only 0-6% of cases.16-20 RFA, however, does not yield a histological specimen; therefore, it is imperative that all visible abnormalities are removed by focal ER prior to RFA to guarantee optimal staging and to ensure that RFA is applied to flat mucosa only.21 The aim of this prospective randomised clinical trial was to compare the safety and efficacy of endoscopic treatment of BO (<5 cm) containing HGD/EC using either SRER or RFA after focal ER of visible abnormalities.

PATIENTS AND METHODS

Selection criteria

Patients were eligible if they met the following criteria: (1) age between 18 and 85 years; (2) BO length <5 cm; (3) HGD and/or EC in BO in specimens obtained at two separate endoscopies; (4) no signs of deep submucosal invasion, regional lymph node involvement or distant metastases on endoscopic ultrasonography (EUS) and CT of thorax and abdomen (in the case of EC); (5) no prior endoscopic treatment of BO other than a single prior ER for staging; (6) in the case of a prior diagnostic ER, specimens with a negative deep resection margin, no deep submucosal invasion (<T1sm2), no lymphatic/vascular invasive growth and no poorly or undifferentiated cancer (G3-G4); and (7) written informed consent.
Endoscopic investigation and staging
Pre-assessment consisted of two high-resolution endoscopies [GIF-Q160/GIF-Q260FZ/ GIF-H180, Olympus, Hamburg, Germany] with targeted biopsies of visible lesions and four quadrant biopsies from every 1-2 cm of the Barrett’s segment.21 Oesophageal landmarks were recorded according to the Prague C&M classification.22 Visible lesions were classified using the Paris classification.23 EUS was performed for T- and N-staging, and suspicious lymph nodes were sampled by fine needle aspiration. In the case of carcinoma, a CT scan of thorax and abdomen was made for M-staging. Only patients with lesions that were deemed ‘suspicious for submucosal invasion’ by the endoscopist, based on the macroscopic appearance, underwent a focal staging ER prior to randomisation. In all other patients with visible lesions, ER was performed after randomisation: in the SRER arm the lesion was removed together with the first 50% of the BO segment in the same session (to minimise the number of ER sessions and to avoid a more difficult ER at a later stage due to scarring at the site of the lesion), whereas in the RFA arm only a focal ER of the lesion was performed for staging and to render the mucosa flat prior to RFA.

Endoscopic resection
In both study groups, the ER-cap technique and the multiband mucosectomy (MBM) technique were used as described previously.24,25 Additionally, the use of the ‘simple snare’ technique was allowed.11

Stepwise radical endoscopic resection (SRER)
In SRER, the Barrett’s segment was removed in consecutive sessions at 6-8 week intervals, with a maximum of four sessions, inclusive of the baseline ER (where applicable) [Chapter 4 Fig. 1]. In the initial SRER session, piecemeal ER of 50% of the circumference of the entire Barrett’s segment was performed, inclusive of the visible abnormality if not yet removed in a diagnostic ER session.26 For short segment BO (length of circumferential BO [L] ≤ 1, maximal BO length [M] ≤ 3), SRER in a single session was allowed. In cases where small bridges of residual BO were left in situ between ER wounds, these were preferably removed with additional ER, but argon plasma coagulation (APC) of tissue bridges during SRER was also allowed (60-80 W for Erbe ICC200; 30-40 W for Erbe Vio; APC-probe 2200A, Erbe Elektromedizin, Tübingen, Germany). If visible Barrett’s mucosa was present after the maximum allowable SRER sessions, patients underwent escape treatment with RFA. Escape treatment with APC or hot biopsy forceps for areas of residual BO (<5 mm) was allowed to avoid an additional ER or RFA, or when ER was not possible.

Radiofrequency ablation (RFA)
Patients randomised to RFA underwent focal ER of visible abnormalities followed by RFA after 6-8 weeks, when the residual BO contained at the utmost HGD upon biopsy (Chapter 6 Fig. 1). RFA was performed using the HALO system (BÂRRX Medical, Sunnyvale, California, USA) as previously described.9,10 Primary circumferential ablation was performed using the HALO100 balloon catheter, with a double RFA delivery (12 J/cm², 40 W/cm²) and a cleaning step in between two ablation passes to remove coagulum from the ablation zone and electrode surface. At subsequent RFA sessions, the HALO100 device was used for focal ablation of residual Barrett’s tongues and islands <2 cm in length, and to ablate the squamocolumnar junction (Z-line) circumferentially at the gastric folds. The HALO90 catheter consists of a small electrode that is fixed to the tip of the electrode. Focal RFA was delivered twice to each area [15 J/cm², 40 W/cm²], followed by a cleaning step and a second ablation pass, again delivering RFA twice.17 RFA was repeated every 2-3 months until complete endoscopic eradication of BO was achieved. In cases where BO persisted after four RFA sessions (<2 HALO90 procedures), escape ER was performed using the MBM technique. For minute islands of unsuspicious BO (<5 mm), hot biopsy forceps treatment was allowed when this avoided an additional RFA session or escape ER.

Preprocedural and postprocedural care
All endoscopic procedures were performed on an outpatient basis using conscious sedation with midazolam and fentanyl, or pethidine, or monitored anaesthesia with propofol.27 After endoscopic treatment, patients were observed in the endoscopy unit for 4 hours before being discharged to home with detailed instructions. During the study period, patients were administered esomeprazole 40 mg twice daily, with addition of ranitidine 300 mg at bedtime and sucralfate suspension 5 ml (200 mg/ml) four times a day for 14 days after every treatment session. Patients were allowed to take acetaminophen 500 mg [maximum 3 g per day] for postprocedural pain, or diclofenac suppositories 100 mg [maximum 200 mg per day] if not responding to acetaminophen.

Follow-up
After visible eradication of all BO was achieved, biopsies were taken from every four-quadrant/2 cm of the neosquamous epithelium throughout the original BO segment and immediately distal (<5 mm) to the neo-Z-line. When histological assessment of the biopsies showed complete eradication of IM [CR-IM] and early neoplasia [CR-neoplasia], patients were scheduled for follow-up high-resolution endoscopy with narrow band imaging and four-quadrant/2 cm biopsies at 6 months, 12 months and annually thereafter. Standard EUS was performed at 12 months of follow-up to exclude local lymph node metastasis. The duration of follow-up was defined as the time between the first treatment session and the most recent follow-up endoscopy.

Histological evaluation
All biopsies and ER specimens were routinely processed and were evaluated by a gastrointestinal pathologist. For the purpose of this study, all pre-treatment biopsies, ER specimens and biopsies obtained at the first follow-up endoscopy were reviewed by a local expert pathologist at each centre with extensive experience in Barrett’s neoplasia. The study pathologists were blinded to study group assignment. Biopsies were assessed for the presence of IM and grade of dysplasia using the revised Vienna classification (IM without dysplasia, indefinite for dysplasia [ID], LGD, HGD or cancer).26,27 ER specimens were evaluated for infiltration depth, vertical resection margins, tumour differentiation or grade of dysplasia, and lymphatic/vascular invasive growth. During follow-up, biopsies of neosquamous epithelium were assessed for the presence of IM at or below the surface [subsquamous intestinal metaplasia or buried Barrett’s].
**Outcome parameters**

Based on previous studies we expected that SRER and ER/RFA would be equally effective in the removal of neoplasia and IM, yet that SRER would result in a higher rate of oesophageal stenosis as compared with RFA.\textsuperscript{16,17,30} The primary outcome parameter was the rate of symptomatic oesophageal stenosis. Secondary outcome parameters were:

1. CR-neoplasia, defined as absence of any neoplasia, including LGD and ID in all biopsies obtained at the first follow-up endoscopy.
2. CR-IM, defined as absence of IM in all biopsies, including biopsies obtained immediately distal to the neo-Z-line obtained at the first follow-up endoscopy.
3. Rate of complications other than stenosis.
4. Number of treatment sessions required to achieve CR-neoplasia and CR-IM inclusive of escape treatment and treatment for complications.
5. Proportion of patients with CR-neoplasia at the last follow-up endoscopy.
6. Proportion of patients with CR-IM at the last follow-up endoscopy.
7. Need for additional treatment for recurrent neoplasia during follow-up.

To classify stenosis and other complications, the following definitions were used: ‘acute’, during the procedure; ‘early’, <48 h; ‘late’, >48 h; graded as ‘mild’, unscheduled hospital admission, hospitalisation <3 days, haemoglobin (Hb) drop <3 g/dl, no need for transfusion; ‘mild’, hospitalisation 4–10 days, <4 units blood transfusion, need for repeat endoscopic treatment including dilation; ‘severe’, hospitalisation >10 days, intensive care unit (ICU) admission, need for surgery, >4 units blood transfusion or, in the case of stenosis, >5 dilations, stent placement or incision therapy; ‘fatal’, death attributable to the procedure <30 days or longer with continuous hospitalisation. Only events requiring any intervention were scored.

**Ethical considerations, sample size and statistics**

The study protocol was approved by the local medical ethics committee of each study centre (NTR1337, http://www.trialregister.nl). Written informed consent was obtained from all patients. Patients were randomised in each centre according to a computer-generated randomisation sequence per centre, which was concealed from the researchers who screened and enrolled patients by the use of sequentially numbered, sealed opaque envelopes. All procedures were attended by a study monitor (CS) who prospectively collected all relevant data on standardised case record forms, and data were entered into a dedicated database. Sample size calculations were based on the assumption that SRER would result in a higher oesophageal stenosis rate compared with RFA. No differences in CR-neoplasia, CR-IM, or severe complications were expected based on previous studies.\textsuperscript{16,17,30} To confirm the hypothesis that SRER results in a significantly higher stenosis rate, with estimated stenosis rates of 5\% for SRER\textsuperscript{20,21} and 4\% for RFA\textsuperscript{16,17,22} patients were needed in each arm, accounting for a drop-out rate of 10\%, resulting in a total study population of 44 patients (\textit{x}=0.05, \textit{b}=0.10, two-sided testing). Data analysis was performed using SPSS statistical software package [SPSS version 16.0.2]. Mean (±SD) was used for normal distribution and median (IQR) was used for skewed distribution. The Fisher exact test and Mann-Whitney U test were used to compare groups when appropriate. Differences were considered statistically significant if \textit{p}<0.05. To calculate CIs the Confidence Interval Analysis package was used (Confidence Interval Analysis Version 2.2.0). For sample size calculation and random sequence generation, nQuery Adviser (Version 7) was used.

**RESULTS**

**Patients**

Between April 2006 and April 2008, 55 patients with HGD/EC in a BO segment <5 cm underwent endoscopic investigation and staging for eligibility in the Academic Medical Center (Amsterdam, The Netherlands), Sint Antonius Hospital (Nieuwegein, The Netherlands) or the University Medical Center Hamburg-Eppendorf (Hamburg, Germany) (Fig. 1). Staging ER was performed prior to randomisation in 30 of 55 patients. Eight patients were not eligible for study for the following reasons: non-lifting of the lesion (\textit{n}=2), no residual IM after ER (\textit{n}=2), lymphatic tumour invasion in the ER specimen (\textit{n}=1), residual carcinoma after two ERs (\textit{n}=1), tumour at the deep margin (\textit{n}=1) and acute ER-related oesophageal perforation (\textit{n}=1). In two patients, EUS-guided fine needle aspiration of local lymph nodes was performed, and malignancy was excluded. Therefore, 47 of 55 screened patients fulfilled all study criteria after investigation and staging and were randomised to SRER (\textit{n}=25) or RFA (\textit{n}=22) (Fig. 1). The baseline characteristics of patients in both groups were similar (table 1).

**Complete remission of neoplasia and IM**

CR-neoplasia was reached in all 25 patients (100\%) after SRER and in 21 of 22 patients (96\%) after ER/RFA (Fig. 1, table 2). CR-IM was achieved in 23 of 25 patients (92\%) after SRER and 21 of 22 patients (96\%) after ER/RFA. The single ER/RFA patient who failed to achieve CR-neoplasia and CR-IM underwent oesophagectomy to treat persistent HGD. The choice of surgery in preference to escape ER was due to the fact that previous ER made it impossible to perform additional ER. The surgical resection specimen showed residual HGD, while 20 lymph nodes were negative for malignancy. Two SRER patients failed to achieve CR-IM: one patient had a small rim of visible BO without dysplasia after two SRER sessions, but because of post-ER stricturing and poor healing after previous treatment no further treatment was performed, and one patient had persistent IM at the neo-Z-line without visible BO after two SRER sessions and RFA.
**Figure 1. Patient enrolment and outcomes**


<table>
<thead>
<tr>
<th>Screening phase</th>
<th>SRER (n=25)</th>
<th>ER + RFA (n=22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGD/EC in BO &lt;5cm n=55</td>
<td>23/25 (92%)</td>
<td>21/22 (96%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Work-up ER n=30</td>
<td>23/25 (92%)</td>
<td>21/22 (96%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Randomization n=47</td>
<td>23/25 (92%)</td>
<td>21/22 (96%)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment phase</th>
<th>SRER (n=25)</th>
<th>ER + RFA (n=22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR-N: 25/25 CR-IM: 23/25</td>
<td>25/25 (100%)</td>
<td>21/22 (96%)</td>
<td>0.47</td>
</tr>
<tr>
<td>- 2 patients did not reach CR-IM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFA +/- ER n=22 CR-N: 21/22 CR-IM: 21/22</td>
<td>21/22 (96%)</td>
<td>21/22 (96%)</td>
<td>1.00</td>
</tr>
<tr>
<td>- 1 patient had persistent HGD and underwent surgery</td>
<td></td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Follow-up phase</th>
<th>SRER (n=25)</th>
<th>ER + RFA (n=22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRER n=25 Lost to follow-up n=6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After a median of 25 months: CR-N: 25/25 CR-IM: 20/25</td>
<td>25/25 (100%)</td>
<td>21/22 (96%)</td>
<td>0.47</td>
</tr>
<tr>
<td>- 1 patient had recurrent carcinoma, treated successfully with ER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3 IM in the cardia during follow-up</td>
<td>21/22 (96%)</td>
<td>21/22 (96%)</td>
<td>1.00</td>
</tr>
<tr>
<td>RFA +/- ER n=22 Lost to follow-up n=1 After a median of 22 months: CR-N: 20/20 CR-IM: 18/20</td>
<td>21/22 (96%)</td>
<td>21/22 (96%)</td>
<td>0.47</td>
</tr>
<tr>
<td>- 2 IM in the cardia during follow-up</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Table 1. Baseline patient characteristics.</th>
<th>SRER (n=25)</th>
<th>ER + RFA (n=22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>21:4</td>
<td>19:3</td>
<td>1.00</td>
</tr>
<tr>
<td>Median age [years] [range]</td>
<td>68 [45-88]</td>
<td>69 [55-73]</td>
<td>0.97</td>
</tr>
<tr>
<td>Median BO [cm]</td>
<td>C2, M4 [IQR C1-3; M2-5]</td>
<td>C2, M4 [IQR C1-3; M2-5]</td>
<td>0.99</td>
</tr>
<tr>
<td>Visible lesion prior to treatment</td>
<td>17/25 (68%)</td>
<td>18/22 (82%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Staging ER prior to randomization</td>
<td>10</td>
<td>12*</td>
<td>0.39</td>
</tr>
<tr>
<td>ER after randomization</td>
<td>15</td>
<td>6*</td>
<td>0.06</td>
</tr>
<tr>
<td>Worst diagnosis histology of biopsies or ER specimens</td>
<td>13 EC/12 HGD</td>
<td>15 EC/7 HGD</td>
<td>0.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Outcome parameters and characteristics of endoscopic treatment.</th>
<th>CR-neoplasia</th>
<th>CR-IM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRER (n=25)</td>
<td>25/25 (100%) (95% CI 86% to 100%)</td>
<td>21/22 (96%) (95% CI 77% to 100%)</td>
<td>0.47</td>
</tr>
<tr>
<td>ER + RFA (n=22)</td>
<td>21/22 (96%) (95% CI 77% to 100%)</td>
<td>21/22 (96%) (95% CI 77% to 100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Severe complications</td>
<td>6 (1 acute perforation, 5 stenoses)</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Moderate complications</td>
<td>18 (1 early bleeding, 17 stenoses)</td>
<td>4 (1 late bleeding, 3 stenoses)</td>
<td>0.00</td>
</tr>
<tr>
<td>Mild complications</td>
<td>5 (5 acute bleedings)</td>
<td>3 (2 acute bleedings, 1 acute non-transmural laceration)</td>
<td>0.71</td>
</tr>
<tr>
<td>Sessions SRER/ER + RFA</td>
<td>2 (IQR 1-3)</td>
<td>3 (IQR 3-4)</td>
<td>0.07</td>
</tr>
<tr>
<td>Escape treatment</td>
<td>8/25 (32%)</td>
<td>4/21 (19%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Dilution sessions (median) [range]</td>
<td>4 (1-19)</td>
<td>3 (1-4)</td>
<td>0.39</td>
</tr>
<tr>
<td>Total no. of therapeutic sessions (median)</td>
<td>6 (range 1-20, IQR 3-9)</td>
<td>3 (range 1-8, IQR 3-4)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Oesophageal stenosis
Symptomatic oesophageal stenosis occurred in 22 of 25 (88%) SRER patients versus 3 of 21 (14%) RFA patients (RR=6.2 (95% CI 2 to 18; p<0.001) (table 2). All RFA patients who developed stenosis had undergone ER of relatively large lesions prior to RFA, with stenosis developing at the ER sites. In the SRER group, five stenoses were graded as severe complications; all other stenoses were graded as moderate complications. Most stenoses resolved upon balloon or bougie dilation, while one patient required incision therapy in addition to dilation. The median number of dilations was 4 (range 1-19, IQR 2-5) for post-SRER stenoses and 3 (range 1-4) for post-ER/RFA stenoses (p=0.39).

Acute complications
There was one severe acute complication in the SRER arm: a perforation occurred during ER, which was treated non-surgically with a covered stent. After stent removal and healing, SRER treatment was continued. Additionally, in the SRER arm, five mild acute bleedings occurred and were treated endoscopically by hot biopsy forceps, epinephrine injection and/or clip placement. Acute complications in the ER/RFA arm were mild and included two bleedings immediately after ER treated endoscopically with a clip and APC, and one superficial mucosal laceration during RFA that required no intervention, but prevented a second ablation pass (table 2).

Early complications
There was one bleeding in the SRER arm, graded as a moderate complication, in a patient presenting with haematemesis within 24 h after ER due to an arterial bleed from the resection wound that was treated by placement of a clip. After blood transfusion (Hb level drop from 7 to 5.2 mmol/l, 2 units of packed red blood cells transfused), the patient was discharged.

Late complications other than stenosis
There was one delayed bleed after RFA, graded as a moderate complication. This patient developed melaena after re-initiating oral anticoagulation therapy (acenocoumarol) for atrial fibrillation 2 weeks after focal RFA. Upper endoscopy showed a visible vessel in the treatment area, which was injected with epinephrine (1:10 000) and coagulated using bipolar electrocoagulation. The patient underwent blood transfusion (baseline Hb level unknown, Hb level after bleeding 5.7 mmol/l, 2 units of packed red blood cells transfused).

Number of treatment sessions

**SRER patients**
For SRER, the MBM technique (n=12), ER-cap technique (n=8) or MBM and ER-cap (n=5) were used. In seven patients, SRER was performed in a single session. A median of 5 (IQR 2-7) resections was performed per session. Per patient, a total median number of 10 (IQR 6-13) resection specimens were removed. In three SRER patients (3/25, 12%), APC was used to treat residual BO tissue bridges between resection wounds. Escape treatment to reach CR-neoplasia and CR-IM was required in eight patients (8/25, 32%): RFA for residual BO because post-SRER scarring and stenosis impeded further ER (n=5); APC for residual BO because stricturing did not allow for passage of an ER-cap or HALO\textsuperscript{90} catheter (n=1); or APC for ablation of tiny islands <5 mm (n=2).

**ER/RFA patients**
Prior to RFA 18/22 patients underwent ER of a visible lesion with the ER-cap technique (n=11), the MBM technique (n=6) or the simple snare technique (n=1). Escape treatment to reach CR-neoplasia and CR-IM was performed in four patients (4/21, 19%): hot biopsy forceps to remove a BO island (<2 mm, n=2); ER for residual visible BO (n=1) (histology showed no IM or dysplasia); and ER plus APC (n=1) for an elevated island of BO (histology showed a radically removed T1sm1 cancer). The median number of therapeutic sessions to achieve CR-neoplasia and CR-IM was not significantly different in both groups (SRER 2 (IQR 1-3) vs RFA 3 (IQR 3-4); p=0.07). However, due to stenosis requiring dilations, the total number of endoscopic interventions per patient was significantly higher in SRER (6 (IQR 3-9) vs 3 (IQR 3-4); p<0.001). The median duration of the treatment period was not significantly different between SRER and RFA (5 (IQR 5-13) vs 8 (IQR 5-10) months, respectively; p=0.26).

Follow-up: persistence of CR-neoplasia and CR-IM
Median follow-up from initial treatment to March 2010 was 24 months (IQR 18-29) overall, and median follow-up from the final treatment session to March 2010 was 18 months (IQR 11-23), with a median of 3 (IQR 3-4) follow-up endoscopies for both groups (table 3). Forty-five patients (96%) remained under endoscopic follow-up. Two of 47 patients were not available for endoscopic follow-up: one ER/RFA patient failed CR-neoplasia and underwent surgery; another ER/RFA patient died 4 months after reaching CR-neoplasia and CR-IM due to myocardial infarction (unrelated death). In the SRER group, one patient (4%) was diagnosed with cancer at the neo-Z-line 16 months after SRER and was treated with ER. The resection specimen showed T1m3 carcinoma. Follow-up endoscopy after 4 months revealed no dysplasia or cancer. In the ER/RFA group, no recurrence of neoplasia was observed during follow-up. None of the patients who reached CR-IM during the treatment phase showed endoscopic signs of recurrence of BO on any follow-up endoscopy. At the last follow-up endoscopy, histological signs of IM were found in three SRER and two RFA patients: all had repeated findings of IM at the neo-Z-line and were considered failures for CR-IM at the last follow-up endoscopy (p=1.00). Six other patients had a focal IM in a single biopsy during a single follow-up endoscopy without being reproduced at subsequent endoscopies, including four SRER patients (two focal IM at the neo-Z-line, two buried BO glands in neosquamous biopsies) and two RFA patients (focal IM at the neo-Z-line). These six patients were not considered as failures for CR-IM at the last follow-up endoscopy.
<table>
<thead>
<tr>
<th></th>
<th>SRER [n=25]</th>
<th>ER + RFA [n=22]</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost to FU</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unrelated death</td>
<td>0</td>
<td>1</td>
<td>0.47</td>
</tr>
<tr>
<td>FU (months)</td>
<td>25 (IQR 19-29)</td>
<td>22 (IQR 17-30)</td>
<td>0.52</td>
</tr>
<tr>
<td>FU from final treatment session (months)</td>
<td>20 (IQR 11-24)</td>
<td>15 (IQR 11-22)</td>
<td>0.27</td>
</tr>
<tr>
<td>Follow-up endoscopies</td>
<td>3 (IQR 3-4)</td>
<td>3 (IQR 3-4)</td>
<td>0.48</td>
</tr>
<tr>
<td>Biopsies from neosquamous (median)</td>
<td>14 (IQR 11-22)</td>
<td>23 (IQR 16-28)</td>
<td>0.03</td>
</tr>
<tr>
<td>Biopsies from neo-Z-line (median)</td>
<td>12 (IQR 10-15)</td>
<td>15 (IQR 11-18)</td>
<td>0.06</td>
</tr>
<tr>
<td>Recurrent neoplasia</td>
<td>1 (4%)</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Visible BO</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Repeated IM including at the last FU endoscopy</td>
<td>3</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>Single finding of focal IM at the neo-Z-line, not reproduced during FU</td>
<td>2</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>Single finding of buried Barrett’s glands, not reproduced during FU</td>
<td>2</td>
<td>0</td>
<td>0.49</td>
</tr>
<tr>
<td>IM at any time point during FU (total)</td>
<td>7</td>
<td>4</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table 3. Follow-up (FU) after endoscopic treatment.


DISCUSSION

This multicentre randomised trial showed that in patients with BO <5 cm containing HGD or EC, both SRER and focal ER plus RFA are highly effective, with high rates of CR-neoplasia and CR-IM. After a median follow-up of 24 months after initial treatment, recurrences of neoplasia or BO were rare for both groups. Regarding safety, SRER and RFA resulted in comparably low rates of acute complications, but SRER carried a significantly higher risk for the late complication of oesophageal stenosis, resulting in more procedures per patient in the SRER group due to dilation sessions. In addition, significantly more complications were graded as ‘severe’ in the SRER arm (1 perforation and 5 stenoses) with no severe complications in the ER/RFA arm. The most important finding of the study is a significantly higher stenosis rate of 88% in SRER compared with 14% after ER/RFA. Although all SRER stenoses were effectively treated with dilation, 5 of 22 SRER stenoses were quite resistant to treatment (>5 dilatations and need for combination treatment). In addition, dilation of these stenoses may cause significant complications as illustrated by a recent multicentre SRER study that reported two perforations after dilation for an SRER induced stenosis.31 More importantly, treatment of SRER induced stenoses doubled the total number of endoscopic procedures in the SRER group compared with the ER/RFA group. In the ER/RFA group, the three patients with stenosis all had undergone widespread ER prior to RFA to remove all visible lesions. Studies of patients treated with RFA, not preceded by ER, have reported stenosis rates <6%.32,33 This suggests that ER, not RFA, was the primary cause of stenosis in these patients. Oesophageal stenosis is a recognised complication of SRER of BO, varying from 2% to 56%.10,11,13,14 Compared with other series, the 88% stenosis rate after SRER in our study is quite high. An explanation may be that the three study centres prospectively screened all patients with BO <5 cm with HGD/EC and offered participation to all eligible patients. In contrast, other retrospective series may have included less complicated cases, for example with a shorter BO segment or BO mainly consisting of tongues. It has been shown that the stenosis rate after SRER increases with the length of the BO.31,32 We assume that our patient population had a relatively long circumferential extent (median C2, M4, IQR C1-3, M2-5) which may account for the relatively high stenosis rate. Our rigorous follow-up in this prospective study may also have contributed to the observed stenosis rate, with all stenoses and dilations being fully recognised and transparently reported. Although no significant differences were found in eradication rates of neoplasia and IM, we cannot conclude that SRER and ER/RFA are equally effective; the current study was powered to evaluate the difference in symptomatic stenosis rate between both treatment modalities, based on previous experience of the study centres. Uncontrolled studies have reported success rates for CR-neoplasia of SRER and ER/RFA varying between 88-100%10,12,14,15,16 If a difference of >10% in CR-neoplasia for one of both treatment modalities would be clinically relevant and assuming a 90% success rate for both treatment modalities, 155 patients would have been necessary in each treatment arm to be able to prove equivalence or non-equivalence. Given the relative rareness of HGD/EC in BO this is an unrealistic number of patients for a randomised study in this field. In addition, it is debatable whether it would be ethical or clinically relevant to perform such a study, knowing that combined ER and RFA has an excellent success rate with a low risk for symptomatic stenosis. In our study there was one
underwent extensive biopsy sampling during follow-up (median 12 biopsies obtained from relevant finding, and may predict recurrence of neoplasia. On the other hand, our patients were located at the neo-Z-line. This suggests that IM in the Z-line after SRER may be a recent multicentre SRER study of 169 patients in which all recurrences of HGD/EC (2%) and recurrent carcinoma (4%) in the SRER arm, located at the neo-Z-line of a patient that had undergone SRER or ER/RFA. Since RFA does not result in significant scarring of the oesophagus, escape ER after RFA has been incorporated in the treatment algorithm in other studies. Although it can be argued that escape treatment may influence the results, we feel that comparing SRER and ER/RFA inclusive of escape treatment in both treatment arms makes our results better translatable to clinical practice. A limitation of this study concerns its external validity: treatment was carried out in centres with a tertiary referral function for endoscopic treatment of early Barrett’s neoplasia. Hence, patients were treated by endoscopists highly experienced in SRER and RFA treatment. The outcomes of our study may therefore not apply to general practice. However, because of the low incidence of early Barrett’s neoplasia in the general population, it is desirable to centralise care for these patients in well-trained expert centres. In summary, this randomised multicentre trial showed that SRER and focal ER plus RFA are highly effective in the treatment of patients with BO <5 cm containing early neoplasia. SRER, however, resulted in a significantly higher stenosis rate than ER/RFA, and consequently required a higher number of therapeutic sessions due to dilations. Therefore, for patients with BO containing early neoplasia, a combined approach of focal ER for visible lesions followed by RFA for complete eradication of the remaining BO may be preferred.

**REFERENCES**

15. Peters FP, Brakenhoff KP, Curvers WL et al. Endoscopic cap resection for Barrett’s oesophagus with high-grade dysplasia or early adenocarcinoma - CHAPTER 8 PART II - Radiofrequency Ablation

16. 10 cm.37


Radiofrequency ablation combined with endoscopic resection, for eradication of Barrett’s oesophagus containing early neoplasia in 132 patients: results of a European multicentre study (EURO-II)
ABSTRACT

Objectives:

— Barrett’s oesophagus (BO) containing high-grade intraepithelial neoplasia (HGIN) or early cancer (EC), can be treated by radiofrequency ablation (RFA) with prior endoscopic resection (ER) in case of focal lesions, as demonstrated by a number of relatively small-sized, single-centre studies.

— Aim and methods:

Aim of this prospective study was to evaluate efficacy of RFA, with or without prior ER, for BO with early neoplasia, in 13 European centres with expertise in BO neoplasia. Patients with BO ≤12 cm with HGIN/EC were included. ER was performed in case of focal lesions limited to ≤2 cm length and ≤50% circumference. RFA was performed at 0-3-6-9-12 months, with max. 2 circumferential and 3 focal RFA treatments. Escape-ER as part of protocol, was allowed for residual BO after RFA, or for suspicious lesions found during the treatment period. To ensure uniformity and compliance, investigators were trained at the coordinating site. A coordinating study team attended all treatments and first follow-up at each site. Central pathology review was performed at the coordinating site. Primary outcomes were eradication of intestinal metaplasia (IM) and neoplasia.

— Results:

132 patients, median BO length C3M6, underwent en-bloc (n=62), piecemeal ER (n=57) or no ER (n=13). Primary outcomes were eradication of intestinal metaplasia (IM) and neoplasia.

— Conclusion:

This is the largest prospective multicentre study on RFA combined with ER for treatment of BO containing HGIN/EC. These outcomes suggest that this treatment approach is very effective and safe, when performed by trained, expert endoscopists in carefully selected patients.

INTRODUCTION

Barrett’s oesophagus (BO), a complication of long-standing gastroesophageal reflux disease, is the most important risk factor for the development of oesophageal adenocarcinoma.1,2 Patients with high-grade intraepithelial neoplasia (HGIN) or early cancer (EC), may be treated endoscopically given a low risk of lymph node metastasis, but more advanced cancers are indications for surgery.3,4 The cornerstone of endoscopic treatment for early Barrett’s neoplasia is endoscopic resection (ER), which allows for removal of visible lesions and accurate histological assessment of infiltration depth, differentiation grade and lymph-vascular invasion.1,5,6 After focal ER, however, the residual Barrett’s mucosa remains at risk for malignant transformation and cancer recurrences are found in 30% of patients during follow-up.3,4 To prevent such metachronous lesions, the residual Barrett’s segment can be eradicated by radiofrequency ablation (RFA). This endoscopic ablation technique has been shown to be successful in eradicating non-dysplastic Barrett’s mucosa and BO containing low-grade intraepithelial neoplasia (LGIN) and HGIN.9,10 The combination of ER and RFA for mucosal abnormalities and early cancer (EC) has also been proven safe and effective in a number of relatively small sized studies.11-14

We performed a multicentre trial in 13 European centres, to evaluate the safety and efficacy of RFA, combined with ER for visible lesions, in patients with BO containing HGIN or EC.

MATERIALS AND METHODS

Study Design

This prospective cohort trial was conducted at thirteen tertiary-care medical centres in Europe, with expertise in detection and treatment of early Barrett’s neoplasia. To ensure standardization of the technique, the principal investigator of each center received hands-on training in RFA at the coordinating study centre (JBe). After focal ER, however, the residual Barrett’s mucosa remains at risk for malignant transformation and cancer recurrences are found in 30% of patients during follow-up.3,4,7 After focal ER, however, the residual Barrett’s mucosa remains at risk for malignant transformation and cancer recurrences are found in 30% of patients during follow-up.3,4 To prevent such metachronous lesions, the residual Barrett’s segment can be eradicated by radiofrequency ablation (RFA). This endoscopic ablation technique has been shown to be successful in eradicating non-dysplastic Barrett’s mucosa and BO containing low-grade intraepithelial neoplasia (LGIN) and HGIN.9,10 The combination of ER and RFA for mucosal abnormalities and early cancer (EC) has also been proven safe and effective in a number of relatively small sized studies.11-14

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We performed a multicentre trial in 13 European centres, to evaluate the safety and efficacy of RFA, combined with ER for visible lesions, in patients with BO containing HGIN or EC.
Treatment protocol
At baseline, all mucosal irregularities were removed by ER to allow for histological staging. ER was performed using the ER-cap technique (Olympus, Hamburg, Germany), multiband mucosectomy (Duette®, Cook Endoscopy, Limerick, Ireland) or the Euroligator (Mandel+Rupp, Erkrath, Germany). Based on prior experiences, the extent of ER allowed for inclusion in this study, was limited to 2 cm in length and 50% of the circumference to prevent complications at subsequent RFA treatment, due to post-ER strictureing. A minimum of 6 weeks after any ER, the first RFA treatment was performed using either the HALO360 system for circumferential ablation, or the HALO90 system for focal ablation, as described in detail previously.12-14 The gastro-oesophageal junction was treated circumferentially with HALO90 ablation at least once. RFA treatment was performed at 0-3-6-9-12 months, with a maximum of 2 circumferential and 3 focal RFA treatments. ‘Escape’ ER as part of the treatment protocol was performed for any visible lesions detected prior to any of the scheduled RFA treatments, or for residual neoplasia persisting after the maximum number of RFA sessions. Any residual non-dysplastic Barrett’s epithelium persisting after the maximum number of allowed RFA sessions was recorded, and at the discretion of the responsible endoscopist either removed by escape-ER, biopsied and treated with APC only in case of areas <5 mm, or kept under endoscopic surveillance. If endoscopic eradication of all visible Barrett’s mucosa was reached, biopsies for histological correlation were obtained immediately distal (<5 mm) to the neo-squamocolumnar junction, and from four-quadrants every 2 cm of the original extent of the BO segment. Additional follow-up was then performed 6 and 12 months after the last treatment session, and annually thereafter.
During the entire study period, all patients were prescribed high-dose proton pump inhibitor therapy, supplemented with an H2-receptor-antagonist and sucralfate suspension for two weeks after each therapeutic endoscopy.

Outcome parameters

**Primary outcome parameters:**
- Histological eradication of neoplasia, defined as all biopsies negative for neoplasia, 12 months after the first RFA treatment.
- Histological eradication of IM 12 months, defined as all biopsies negative for IM, 12 months after the first RFA treatment.

**Secondary outcome parameters:**
- Adverse events, defined as ‘acute’ (during procedure), ‘early’ [0-48hrs] and ‘late’ (>48hrs). Adverse events were only recorded if they were clinically significant and graded as ‘mild’ (unplanned hospital admission, hospitalization <3 days, hemo-globin drop <3g/dl, no transfusion), ‘moderate’ (4-10 days hospitalization, <4 units blood transfusion, need for repeat endoscopic intervention, radiologic intervention), ‘severe’ (hospitalization >10 days, ICU admission, need for surgery, > 4 units blood transfusion, in the case of stenosis: >5 dilatations, stent placement or incision therapy) or ‘fatal’ (death attributable to procedure <30 days or longer with continu-ous hospitalization).
- Durability of eradication of neoplasia during follow-up.
- Durability of eradication of IM during follow-up.

Histological analysis
At each centre histological evaluation was performed by a local expert pathologist, followed by central pathology review of all ER specimens, work-up biopsies, and biopsies from the first follow-up endoscopy, at the coordinating study site. In case of discrepancies, revision by a second expert pathologist was performed to reach consensus. ER specimens were evaluated for neoplasia according to the WHO classification,15 tumour infiltration depth, differentiation grade, presence of lymph-vascular invasion and radicality of resection at the vertical margin. Biopsies were evaluated for presence of IM and neoplasia, and for the presence of subsquamous areas of IM in biopsies from neosquamous mucosa.

Statistical analysis
Statistical analysis was performed with SPSS Statistics 17.0 Software for Macintosh. For descriptive statistics mean ± standard deviation (SD) was used in case of a normal distribution of variables, and median (interquartile range (IQR)) in case of a skewed distribution.

Ethical considerations
The ethical committee of each institution reviewed and approved the protocol and the patient informed consent form. All patients signed an informed written consent form prior to inclusion. The trial was registered at www.trialregister.nl (NTR1211).

RESULTS

**Patients**
A total of 132 patients was included, 107 men, mean age 65 ±14 years, with a median BO length of C3 (IQR 1-7) M6 (IQR 4-9). In 119 patients ER was performed, using the ER-cap technique with submucosal lifting (n=52) or a ligate-and-cut technique (n=67). En-bloc resection was performed in 63 patients (52%) and piecemeal resection in 57 patients (48 %) with a median of 2 pieces (IQR 2-4) per session. Worst pathology in the resected specimens was EC (n=78), HGIN (n=31), LGIn (n=7) or no-dysplasia (n=3). Prior to RFA, the worst histological grade in the residual Barrett’s segment was HGIN (n=36), LGIn (n=45) or no-dysplasia (n=51).

**Primary endpoints**
During the treatment period 6/132 patients (5%) dropped out from the study (withdrawal of consent, n=3; second primary cancer, n=3). By August 2011, eight patients had not yet reached the 12-month endpoint. According to intention-to-treat analysis, all 6 dropouts were considered as failures for eradication of neoplasia. After a median of 1 (IQR 1-2) HALO360 ablation and 2 (IQR 1-3) HALO90 ablations, eradication of neoplasia and IM at 12 months was reached in 115/124 (92%) and 105/124 (84%) patients, respectively. After additional escape ER, as part of the treatment protocol, to remove residual columnar mucosa after the maximum number of RFA sessions (no dysplasia, n=3; LGIn, n=1), and for visible lesions popping-up during the treatment period (HGIN, n=1; EC, n=1), eradication of neoplasia and IM was reached in 117/124 (94%) and 111/124 (90%) patients, respectively.
In a per-protocol analysis (i.e. censoring the 6 patients as unrelated drop outs instead of blindly labeling them as failures), eradication of neoplasia and IM at 12 months was reached in 117/118 (99%) and 111/118 (94%) patients, respectively.

Secondary endpoints

**Durability of response during follow-up**

After a median follow-up of 21 months [IQR 15-27], recurrence of neoplasia was found in 2 patients (1.5%). The first patient had a C1M2 BO, successfully treated by en-bloc resection for mucosal cancer, followed by two focal RFA sessions for residual BO with HG1N. After two follow-up endoscopies without any endoscopic or histological signs of recurrence of neoplasia or IM, a small visible lesion with HG1N was detected at the neosquamocolumnar junction, 24 months after the last RFA session. The lesion was removed en-bloc by MBM, and histological evaluation showed a small focus of radically removed HG1N and IM. The second case was a patient with a C5M7 BO treated by en-bloc resection for mucosal cancer, followed by RFA for residual BO with HG1N. He required two circumferential and three focal ablation sessions with poor healing in between the treatment sessions but finally achieved CR-neo and CR-IM. During the first follow-up endoscopy, a small island of columnar mucosa was detected and removed by biopsy, showing LG1N. At the second endoscopy no columnar mucosa was detected yet a grade B reflux oesophagitis hampered optimal inspection. At 12 months HG1N was detected in a biopsy from the same island that was thought to have been removed by biopsy before. This area was subsequently effectively treated with ER. In 4 patients (4%), a small endoscopically visible area of columnar epithelium with IM upon biopsy recurred. In 9 patients (8%), non-dysplastic IM was diagnosed in a single biopsy from a normal appearing neosquamocolumnar junction, during a single follow-up endoscopy. In 1 patient (1%) buried Barrett’s was detected. This patient was treated successfully for C4M5 BO with HG1N. During the first follow-up endoscopy, buried glands were diagnosed in a neosquamous biopsy from the middle part of the initial BO. Repeated detailed endoscopic inspection did not reveal any visible Barrett’s mucosa. However, a biopsy from the same area again confirmed the presence of subsquamous IM. The area with the buried Barrett’s was therefore treated anew with balloon-based circumferential RFA (2x 12 J/cm²). Two, six and 18 months after the repeat RFA treatment, a total of 16 biopsies and 3 ER specimens from the healed area did not show any signs of subsquamous glands.

Adverse events

No clinically relevant complications occurred during or immediately after any of the ER procedures. Acute complications during RFA consisted of mucosal laceration in 8/122 patients (7%) undergoing HALO™40 ablation, none of which required endoscopic intervention was necessary. One early complication (1%) was observed after RFA: fever resulting in prolonged hospital admission, graded as a mild complication. Late complications of treatment were seen in 9/132 patients (7%). Two late complications were graded as mild: non-objectified melena (n=1), and fainting after all three RFA sessions (n=1). Seven late complications were graded as moderately severe: hematemesis two weeks after HALO™ablation requiring repeat endoscopy (n=1), and oesophageal stenosis requiring a median of 1 (IQR 1-2) endoscopic dilatation (n=6). Of the patients who developed oesophageal stenosis, 3 underwent en-bloc ER prior to RFA, and 3 underwent piecemeal ER with 2 resections. In 7/132 patients (5%) a non-treatment related adverse event occurred during the treatment phase: pancreatitis (n=1), or coronary heart disease (n=6).

**DISCUSSION**

This multicentre trial of 132 patients in 13 European centres was initiated as a continuation on the EURO-I study, a pilot trial in 3 European centres in which 24 patients were enrolled. In this study, eradication of neoplasia and IM was reached in 92% and 84% of patients, respectively. In our analysis we considered all dropouts due to unrelated causes as failures (“intention-to-treat analysis”). After additional escape ER, eradication of neoplasia and IM was achieved in 94% and 90% of patients. According to per-protocol analysis (excluding drop-outs from the analysis), complete eradication of neoplasia and IM was reached in 99% and 94% of patients, respectively. These per-protocol results are almost identical to the eradication rates for neoplasia and IM of 100% and 96% in the EURO-I study. The results of this study add to the convincing evidence that ER for visible lesions combined with RFA for residual Barrett’s mucosa, should be preferred over surgery for BO with early neoplasia. In this study, ER had an indispensable role in this treatment approach: it allowed for removal and accurate histological staging of neoplastic lesions, which ensured optimal patient selection and rendered the mucosa flat for subsequent effective ablation with RFA. Furthermore, ER proved to be a safe and effective escape treatment in case neoplasia developed during the ablation phase or persisted after ablation. This is a unique feature of RFA since other ablation techniques generally result in significant esophageal scarring making additional ER often impossible. Based on experiences from the EURO-I trial, the extent of ER prior to RFA was limited to 2 cm in length and 50% of the circumference in order to prevent more complicated RFA procedures due to post-ER scarring. Mucosal laceration after HALO™ablation occurred in 7% of patients, which was much lower than the 21% in the EURO-I study, in which the limitation of ER extent was only introduced halfway through the study. Limiting ER therefore seems effective in preventing potential RFA complications after a prior ER. Due to these strict selection criteria, however, the results of this study may not be applicable for patients who require more widespread ER. In these patients complications such as mucosal laceration or oesophageal stricturing may occur more frequently than described in this study. In patients with widespread lesions, the ER should, however, still be performed for complete removal of all irregular mucosa. Post-ER scarring can then best be resolved by oesophageal dilatation up to at least 18 mm followed by circumferential RFA at a later stage. Mild complications related to treatment were observed in 2% of patients (fetor which prolonged hospital admission, anamnestic melena and fainting after RFA). Moderate complications were observed in 7% of patients, of which 5% were oesophageal stenoses. All these strictures could be dilated with a minimum of endoscopic dilatation sessions. None of the >500 treatment sessions in this study were associated with severe complications, and there were no treatment related deaths. The combination of limited ER and RFA was therefore very safe, and all complications could be managed either conservatively or endoscopically. Our study had a median follow-up of 21 months [IQR 15-27], during which recurrence
of neoplasia was observed in 1.5% of patients. Both recurrences were detected during endoscopic follow-up and could be removed by additional ER. In comparison to recurrences observed after ER monotherapy [25-33% within 5 years] the recurrence rate found in this study appears favorable and in line with other approaches that aim at completely eradicating the whole BO such as stepwise ER of the complete BO [2% recurrence during median follow-up of 32 months]. A recent randomized trial comparing stepwise ER with ER+RFA found that both approaches were highly effective in eradicating neoplasia (100% vs 96%) and preventing recurrences (4% vs. 0% during 22 months follow-up). Stepwise ER, however, was associated with a 88% oesophageal stenosis rate and required double the number of treatment sessions. Given the relative simplicity of ER+RFA and the fact that this is also effective for longer segments of BO, this approach should be preferred. The two recurrences of neoplasia in this study were both detected at the neosquamocolumnar junction. This is in concordance with two recent studies on SRER and RFA, in which recurrence of neoplasia also mainly occurred at this level. Other groups have also reported on the issue of neoplasia developing in the cardia months to years after complete removal of BO. To minimize the risk of recurrences, eradication of all IM at this level should therefore be optimized. Effective ablation of the gastro-oesophageal junction using the HALO device is difficult, given the often tortuous anatomy and presence of a hiatal hernia in most BO patients. In our opinion, the entire circumference of the gastro-oesophageal junction should be treated with the HALO device at least once during the treatment period. In this study, most patients even underwent multiple HALO ablations of this area since focal ablation of visible BO remnants was always combined with circumferential HALO treatment of the cardia. This is an important difference with RFA studies from the US in which circumferential HALO ablation of the cardia was not incorporated in treatment protocols. Since endoscopic differentiation between gastric mucosa and IM is almost impossible, it is difficult to assess if all Barrett’s mucosa has been eradicated. We have therefore used the histological eradication of IM in biopsies obtained immediately distal (<5 mm) to the neosquamocolumnar junction as an objective endpoint for effective treatment at this level. After eradication of BO, this area should be thoroughly inspected during follow-up endoscopies, as well as biopsied, to detect recurrence of neoplasia at a curable stage. The downside of this approach, however, is that this may lead to the detection of non-dysplastic IM in a normal appearing gastro-oesophageal junction, often as a single-biopsy-single endoscopy finding. One may argue that in patients with an initial diagnosis of early neoplasia in their BO, such a finding of IM reflects insufficient treatment or recurrence of their underlying disease. However, in patients with a normal appearing squamocolumnar junction, focal IM is found in 25% of patients, and this is not considered a premalignant condition in those cases. The presence of buried Barrett’s has been reported in about 53% of patients treated with argon plasma coagulation or photodynamic therapy. The 1% of subsquamous IM found in this study may therefore be considered very low, especially given the stringent biopsy protocol used during follow-up endoscopies. According to protocol, biopsies from neosquamous epithelium were only obtained after inspection with NBI, FICE or I-Scan, to prevent a histological finding of buried Barrett’s due to artifacts resulting from accidental biopsying residual columnar mucosa. Since buried Barrett’s in normal appearing neosquamous mucosa after RFA are very rare, one may question if extensive biopsies from the neosquamous mucosa will remain necessary after thorough inspection with white light endoscopy and NBI, FICE or I-Scan. Strengths of this study are the baseline training of participating centres, quality control, stringent endoscopic work-up, central pathology review, and the prospective, European multicenter set-up. Training at the start of the study was organized for all endoscopists participating in this study. They received hands-on training at the coordinating study site, and the first three RFA procedures at each center were supervised on-site by the principal investigator. All treatment sessions as well as the first follow-up endoscopy were attended on site by a coordinating study team who ensured prospective registration of data, and standardization of the technique throughout the study. All patients underwent thorough endoscopic work-up with at least 2 high-resolution endoscopies, and histological revision of all ER specimens and pre-treatment biopsies was performed at the coordinating study site. Lastly, the European multicenter setting enabled inclusion of a large number of patients with a different demographic background, which increased the generalizability of this study. Limitations of the study are that patients underwent endoscopic work-up and treatment at centres with extensive expertise in management of Barrett’s neoplasia. Results from this study should therefore be extrapolated to general practice with care. However, one may question if future Barrett’s management should not be centralized in such dedicated centres, to maintain the safety and efficacy results reported for endoscopic treatment. In addition, ER was not required to be performed as part of the study protocol, and ER procedures were therefore not attended by one of the study coordinators. Registration of ER procedures and extent of ER may therefore have been less accurate than registration of the RFA procedures. This is the largest European multicentre trial on the efficacy and safety of RFA, with or without ER for mucosal irregularities, for patients with early neoplasia in BO. Results show that if performed by trained endoscopists, in carefully selected patients, a combined treatment approach of RFA and ER is highly effective and safe.
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Properties of the neo-squamous epithelium after radiofrequency ablation of Barrett epithelium containing neoplasia

Roos E. Pouw, Joep J. Gondrie, Agnieszka M. Rygiel, Carine M. Sondermeijer, Fiebo J. W. ten Kate, Robert D. Odze, Michael Vieth, Kausilia K. Krishnadath, Jacques J. Bergman

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ABSTRACT

— Objectives: Endoscopic radiofrequency ablation (RFA) eradicates intestinal metaplasia and intraepithelial neoplasia associated with Barrett oesophagus (BO), restoring an endoscopically normal neosquamous epithelium (NSE). We evaluated the post-RFA NSE for genetic abnormalities and buried glandular mucosa (BGM).

— Methods: Eligible patients underwent RFA for BO containing early cancer and/or high-grade intraepithelial neoplasia (HGIN) with subsequent complete histological reversion to normal NSE. At baseline, the BO was sampled by brush cytology and biopsies. At least 2 months after RFA, the NSE was sampled by brush cytology, keyhole biopsies and endoscopic resection (ER). The untreated squamous epithelium was biopsied as control. The baseline BO and post-RFA NSE were evaluated for immunohistochemical expression of Ki-67 and p53; and genetic abnormalities (DNA-FISH: chromosome 1 and 9, p16 and p53). In addition, biopsy depth was compared for biopsies from the NSE and untreated squamous epithelium. Presence of BGM in NSE was assessed with primary and keyhole biopsy, and ER.

— Results: All pretreatment specimens from all 22 patients, showed abnormalities on immunohistochemical staining and FISH, while all post-RFA NSE specimens were normal. Post-RFA biopsies from the NSE all contained full epithelium whereas 37% contained lamina propria, a finding no different from biopsies from untreated squamous epithelium (36% lamina propria). Deeper keyhole biopsies contained lamina propria in 51%. All ER-specimens contained submucosa. No biopsy or ER-specimen contained BGM.

— Conclusions: Rigorous evaluation of the post-RFA NSE in patients who, at baseline, had BO containing early cancer/HGIN, demonstrated neither persistent genetic abnormalities nor BGM.

INTRODUCTION

Barrett oesophagus (BO) is a metaplastic and sometimes neoplastic alteration in the oesophageal epithelium associated with injury from gastro-oesophageal reflux. Malignant degeneration in BO, when it occurs, progresses through a series of phenotypic cellular changes detected and graded on microscopy; beginning with non-dysplastic intestinal metaplasia (IM), then low- (LGIN) and high-grade intraepithelial neoplasia (HGIN), and then invasive cancer. These phenotypic changes are preceded and precipitated by an accumulation of genetic mutations and other genetic insults, resulting in cellular proliferation, cellular autonomy, and ultimately neoplasia. Selected patients with BO may be treated with an endoscopic ablation technique intended to eradicate the abnormal cells and allow restoration of a histologically normal neosquamous epithelium (NSE). While there is some data from trials of photodynamic therapy (PDT) and radiofrequency ablation (RFA) to suggest that patients converted from neoplastic BO to NSE will have a reduction in cancer progression, we know less about the genetic status of the post-ablation NSE and whether it harbors occult buried glandular mucosa (BGM) that has the potential to develop further neoplasia. For example, incidental cases of cancer arising underneath NSE after PDT and argon plasma coagulation (APC) have been reported. The most recently developed endoscopic ablation technique is RFA, with a number of reports showing favorable safety and effectiveness when applied for LGIN, HGIN, and in combination with endoscopic resection (ER) for early cancer. Thus far, all reports have shown no BGM in a large number of biopsies obtained from the post-RFA NSE (1521). RFA may, however, result in scarring of the NSE leading to false negatives for BGM. Furthermore, it is conceivable that the post-RFA NSE may harbor the same genetic abnormalities as the baseline neoplastic BO and therefore be of no lower risk for malignant transformation. Our study population underwent RFA for BO containing HGIN and/or early cancer, and subsequently achieved complete restoration of a histologically normal NSE. We compared the genetic abnormalities of the baseline BO and post-RFA NSE. We also assessed the NSE and untreated squamous epithelium with various biopsy techniques to determine depth of each biopsy and presence of BGM.

METHODS

Design
This report is derived from 3 sequential prospective clinical trials conducted at the Academic Medical Centre (AMC), Amsterdam, the Netherlands. Each study was approved by the Ethics Committee of the AMC and all patients signed an informed consent form. The first 2 trials were single-centre pilot studies (AMC-I, AMC-II), while the third was the first European multi-centre study of RFA for BO (EURO-I). The trials assessed the safety and effectiveness of RFA for treating BO containing HGIN and/or early cancer (defined as cancer limited to the upper 1/3 rd of the submucosa, a/a sm1) after ER of visible lesions. Despite minor differences in methods the clinical trial protocols were generally comparable (table 1).
process was repeated every 6 months after initial complete response was demonstrated.

During the initial complete response, four-quadrant brush-cytology specimens were obtained from the baseline BO. Then, after RFA at least 2 months after a complete response was confirmed, four-quadrant biopsy specimens and brush-cytology specimens were obtained from the NSE.

**Evaluation of cell proliferation and p53 accumulation**

Biopsies were processed routinely and stained with hematoxylin and eosin (H&E), then reviewed to classify neoplasia according to the revised Vienna classification. To evaluate proliferative activity, Ki-67 staining was applied using a mouse monoclonal antibody (Dako, Glostrup, Denmark). To assess expression of p53 protein, the p53 ab-8 mouse monoclonal antibody (Neomarkers™, Stratech Scientific Ltd, Cambridgeshire, UK) was used. Immunohistochemical staining procedures are described in detail elsewhere. For Ki-67, positive staining of the luminal surface of the epithelium was considered abnormal (scored as positive). For p53, presence of intense nuclear staining in any part of the epithelium was considered abnormal (scored as positive). Ki-67 staining and weak p53 staining of nuclei in the basal layer of (neo-) squamous epithelium was considered normal.

**Evaluation of genetic abnormalities by FISH**

Brush-cytology specimens were immediately processed to cytospin slides and FISH was applied with directly labeled fluorescent chromosomal centromeric probes (CEP) for chromosome 1 and 9, and the locus specific probes (LSI) for regions of 9p21 (p16) and 17p13.1 (p53) (Wisyis, Downers Grove, IL). Dual color probes were used combining CEP9 (SpectrumGreen) and LSI p16 (9p21) (SpectrumOrange) as well as single probes for CEP1 (SpectrumOrange) and LSI p53 (17p13.1) (SpectrumOrange). For each FISH cytology slide, at least 100 interphase nuclei were evaluated under a fluorescent microscope (Olympus BX60, Hamburg, Germany). Damaged cells and cells with indistinct and blurry signals were ignored. To establish frequencies of artifacts resulting from background hybridization variation, probes were applied to normal squamous epithelium from 20 BO patients without neoplasia. From these counts cut-off values were calculated. A sample was considered abnormal when the number of cells with abnormal counts was equal or greater to the cut-off value.

**Evaluation of post-RFA NSE tissue for biopsy depth and buried glandular mucosa**

After detailed endoscopic inspection of the oesophagus with high-resolution endoscopy and NBI, to ensure absence of residual columnar epithelium, patients were randomized to standard biopsies (FB-220U, Olympus, Tokyo, Japan) or to jumbo biopsies (FB-222U, Olympus, Tokyo, Japan). For jumbo biopsies, the diagnostic endoscope was switched for a therapeutic endoscope, to allow introduction of the jumbo biopsy forceps through the working channel. Four-quadrant “primary” biopsies were obtained from every 2 cm of the NSE over the entire length of baseline BO. Immediately after each primary NSE biopsy, a “keyhole” biopsy was taken from the same biopsy site. All primary and keyhole biopsies were collected in separate formalin containers for each level (i.e. 2 containers per level.

**Table 1. Overview of referenced trials, from which patients were derived for evaluation of genetic abnormalities and evaluation of biopsy depth and buried glandular mucosa.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Biopsy depth</th>
<th>BGM</th>
<th>Evaluation of cell proliferation</th>
<th>Evaluation of genetic abnormalities</th>
<th>Biopsies</th>
<th>Brush-cytology</th>
<th>Collection of tissue specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC-I</td>
<td>n=11, median age 60 yrs</td>
<td>100%</td>
<td>100%</td>
<td>Yes</td>
<td>Yes</td>
<td>1 1 2</td>
<td>1 1 2</td>
<td>1 1 2</td>
</tr>
<tr>
<td>AMC-II</td>
<td>n=12, median age 70 yrs</td>
<td>100%</td>
<td>100%</td>
<td>Yes</td>
<td>Yes</td>
<td>1 1 2</td>
<td>1 1 2</td>
<td>1 1 2</td>
</tr>
<tr>
<td>Euro-I</td>
<td>n=10, median age 64 yrs</td>
<td>100%</td>
<td>100%</td>
<td>Yes</td>
<td>Yes</td>
<td>1 1 2</td>
<td>1 1 2</td>
<td>1 1 2</td>
</tr>
</tbody>
</table>

Patients

Eligible patients were 18 to 85 years of age with BO length of 2-12 cm (table 1). All had HGIN and/or early cancer on at least two prior endoscopies, with diagnosis confirmed by an expert GI pathologist at AMC (FtK). Visible lesions were removed with ER at least 6 weeks prior to enrollment and RFA. Residual BO after ER was confirmed to contain LGIN or HGIN prior to RFA. Patients were excluded if: 1) ER showed cancer at a vertical margin, deep submucosal invasion (>T1sm1), poor or undifferentiated grade, lymphatic or vascular invasion, 2) biopsy of mucosa after RFA showed cancer, or 3) oesophageal stenosis. For comparison of baseline and post-RFA genetic abnormalities we included consecutive patients from the AMC-II and AMC-II study (n=23) (table 1). For the evaluation of post-RFA biopsy depth and presence of BGM in biopsies and ER specimens we considered consecutive patients from the AMC-I and AMC-II study (n=23) (table 1).

Endoscopic treatment

A detailed description of ER and RFA is beyond the scope of this paper. Briefly, after removal of visible lesions with ER and confirmation of eligibility, patients underwent a series of circumsferential and focal RFA procedures (HALO360 and HALO90 systems, BÂRRX Medical Inc., Sunnyvale, Ca, USA) at 2-month intervals until complete endoscopic and histological eradication of BO achieved, or, 5 RFA sessions performed. If BO persisted after 5 RFA sessions, focal ER was performed to achieve complete response in all patients. Initial complete response was defined as endoscopically normal NSE on high-resolution endoscopy with Lugol’s (2 %) chromoendoscopy or NBI, and histologically normal biopsy specimens (four-quadrant immediately distal to the neosquamocolumnar junction and every 1 cm of the NSE). This process was repeated every 6 months after initial complete response was demonstrated.

**Collection of biopsies and brush-cytology specimens**

Prior to RFA (after ER where applicable) four-quadrant biopsies (every 1 cm) and brush cytology specimens were obtained from the baseline BO. Then, after RFA at least 2 months after a complete response was confirmed, four-quadrant biopsy specimens and brush-cytology specimens were obtained from the NSE.

**Collection of tissue specimens from NSE**

After detailed endoscopic inspection of the oesophagus with high-resolution endoscopy and NBI, to ensure absence of residual columnar epithelium, patients were randomized to standard biopsies (FB-220U, Olympus, Tokyo, Japan) or to jumbo biopsies (FB-222U, Olympus, Tokyo, Japan). For jumbo biopsies, the diagnostic endoscope was switched for a therapeutic endoscope, to allow introduction of the jumbo biopsy forceps through the working channel. Four-quadrant “primary” biopsies were obtained from every 2 cm of the NSE over the entire length of baseline BO. Immediately after each primary NSE biopsy, a “keyhole” biopsy was taken from the same biopsy site. All primary and keyhole biopsies were collected in separate formalin containers for each level (i.e. 2 containers per level.
one with 4 primary NSE biopsies, one with corresponding keyhole biopsies). In addition, one set of four-quadrant “primary” biopsies was obtained from the untreated squamous epithelium of the proximal oesophagus. A NSE site that was separate from any baseline/escape ER was then selected, based on documented still images of preceding endoscopies, and marked with APC for subsequent ER. A multiband mucosectomy kit (Duette®, Cook, Limerick, Ireland) was assembled and the ER target area resected (Fig. 1). All primary and keyhole biopsies were routinely processed and stained with H&E. ER-specimens were sectioned in 2 mm slices, embedded in paraffin and a minimum of 4 serial cuts per slice were mounted on glass slides for standard H&E staining.

![Figure 1. Collection of biopsies, keyhole biopsies and ER-specimen from NSE after RFA.](image)

A: Antegrade view on an oesophagus covered with normal appearing NSE, after successful RFA of a C6M7 BO. B: Corresponding NBI image. C: Four-quadrant biopsies and keyhole biopsies are obtained every 1-2 cm of NSE. D: Endoscopic view on a biopsy site that was identified as an initial area of Barrett mucosa and that was marked with APC. E: The target area was removed using MBM. F: The ER resulted in a wound into the deep submucosal layer.

**Evaluation of depth of biopsy and presence of buried glandular mucosa**

Three international expert GI-pathologists from the Netherlands (FtK), Germany (MV) and the United States (RO) independently evaluated all biopsy and ER specimens in a blinded manner. They assessed the depth of each biopsy and ER specimen as either full epithelium including basal layer, lamina propria, muscularis mucosae and submucosa in each biopsy fragment. Further, each specimen was assessed for BGM.

**Study outcome variables**

**Primary outcome variables:**

- Immunohistochemical expression of Ki-67 and p53, baseline BO vs. post-RFA NSE;
- Genetic abnormalities on FISH, baseline BO vs. post-RFA NSE;
- Percentage of biopsies from post-RFA NSE containing lamina propria depth or deeper;
- BGM in primary and keyhole biopsies, and ER specimens from post-RFA NSE.

**Secondary outcome variables:**

- Comparison of sampling depth:
  - Primary biopsies obtained from post-RFA NSE vs. untreated squamous epithelium;
  - Primary biopsies vs. keyhole biopsies vs. ER from post-RFA NSE;
  - Standard vs. jumbo biopsy forceps from post-RFA NSE.

**Statistical analysis**

Statistical analysis was performed with SPSS 16.0.2 Software for Windows. For descriptive statistics mean (±SD) was used in case of a normal distribution of variables, and median (IQR) for variables with a skewed distribution. Where appropriate student t test and Mann-Whitney test were used. Pre- and post-treatment FISH score distributions were tested by x²-testing against cut-off values of controls.

**RESULTS**

**Evaluation of genetic abnormalities**

Of the 22 patients consented for genetic evaluation, 16 had undergone ER of visible lesions prior to RFA (Table 1). The worst pathological grade in the residual BO before RFA was LGIN (n=3) and HGIN (n=19). After treatment, complete eradication of endoscopic BO and histological clearance of neoplasia and columnar epithelium was achieved in all patients (all had histologically normal NSE). At baseline, 90% of patients showed abnormal immunohistochemical expression for Ki-67 and 100% showed abnormal p53 expression in the biopsies of their BO-neoplasia (Fig. 2). By comparison, post-RFA NSE for all patients showed a normal distribution of lightly stained nuclei at the basal layer of the squamous epithelium, but not in the superficial layers representing a normal staining pattern (Fig. 2). At baseline, all patients (n=22) showed an abnormal probe distribution of at least one of the FISH probes tested (Fig. 3). Numerical chromosomal abnormalities were found in 60% of patients: gain of chromosome 1 (40%), or gain of chromosome 1 and 9 (20%). Loss of tumour suppressor genes was found in 90 % of patients: loss of p16 (20 %), loss of p53 (40%), and loss of p16 and p53 (30%). By comparison, the post-RFA NSE FISH counts of the centromeric probes for chromosomes 1 and 9, and the locus specific probes for p16 and p53 showed normal diploid distributions in all patients (n=22) (Fig. 3).
Evaluation of depth of biopsy and presence of buried glandular mucosa

Of the 23 patients approached for the biopsy depth and BGM evaluation, 7 were excluded due to: baseline circumferential BO <2 cm (n=4), unrelated co-morbidity (n=2), unrelated death (n=1). The 16 eligible and consented patients had a median baseline endoscopic BO length of 7 (IQR 5-8) cm, with HGIN (n=14) or early cancer (n=2) as the worst baseline diagnosis. All patients had achieved durable eradication of neoplasia and IM after a median 26 (IQR 21-28) months follow-up. Two patients refused ER, but agreed to biopsy evaluation. For the primary study outcome variables, a depth of lamina propria or deeper was obtained in 37% of primary biopsy specimens obtained from the post-RFA NSE. There was no BGM detected in any of the primary or keyhole biopsies, or ER specimens, obtained from the post-RFA NSE (table 2). For the secondary outcome variables, there was no difference in sampling depth when comparing primary biopsies obtained from the post-RFA NSE (37% lamina proprial vs. those obtained from untreated squamous epithelium [36% lamina propria]). By comparison, keyhole biopsies from the post-RFA NSE, sampled more deeply than primary biopsies. ER specimens were deepest of all, containing submucosa in every sample. Furthermore, there was no difference between samples obtained from post-RFA NSE using standard (36% lamina propria) vs. jumbo biopsy (38% lamina propria) forceps.

<table>
<thead>
<tr>
<th></th>
<th>USE primary biopsies (n=60)</th>
<th>NSE primary biopsies (n=194)</th>
<th>USE vs. NSE depth</th>
<th>NSE keyhole biopsies (n=177)</th>
<th>NSE primary vs. keyhole biopsies</th>
<th>NSE ER (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full epithelium</td>
<td>100% (100-100)</td>
<td>100% (100-100)</td>
<td>p=ns</td>
<td>N/A</td>
<td>N/A</td>
<td>100% (100-100)</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>36% (26-46)</td>
<td>37% (30-45)</td>
<td>p=ns</td>
<td>51% (39-58)</td>
<td>p=0.002</td>
<td>100% (100-100)</td>
</tr>
<tr>
<td>Muscularis mucosae</td>
<td>10% (6-17)</td>
<td>10% (4-18)</td>
<td>p=ns</td>
<td>31% (17-38)</td>
<td>p=0.000</td>
<td>100% (100-100)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>0% (0-0)</td>
<td>0% (0-0)</td>
<td>p=ns</td>
<td>0% (2-13)</td>
<td>p=0.003</td>
<td>100% (100-100)</td>
</tr>
<tr>
<td>Buried glandular mucosa</td>
<td>0% (0-0)</td>
<td>0% (0-0)</td>
<td>N/A</td>
<td>0% (0-0)</td>
<td>N/A</td>
<td>0% (0-0)</td>
</tr>
</tbody>
</table>

Table 2. Overview of biopsy depth and presence of buried glandular mucosa.

ER: endoscopic resection specimens. NSE: neosquamous epithelium. USE: untreated squamous epithelium. Presented percentages are the mean (range) of the independent evaluations by three international, expert pathologists.
DISCUSSION

Data from clinical studies suggest that endoscopic RFA is a safe and effective modality for complete eradication of BO and associated neoplasia.\textsuperscript{15-21,23} Given that the endoscopic and histological appearance of the NSE after RFA is normal, we hypothesized that the genetic abnormalities commonly associated with neoplastic BO might be absent in the post-RFA NSE as well. Further, we sought to rigorously evaluate the post-RFA NSE with primary and keyhole biopsies as well as ER to categorically determine if occult BGM was present. We used immunohistochemical methods to compare proliferation activity (Ki-67) and accumulation of p53 protein in biopsies from the BO tissue before and after treatment. At baseline, 90% of patients had abnormal Ki-67 expression and all had abnormal p53 expression, whereas none showed any immunohistochemical abnormalities in biopsies obtained from the post-RFA NSE. We acknowledge the limitation of immunohistochemical evaluation of p53, since the results are method-dependent and do not correlate perfectly with presence of mutations. However, since increased proliferative activity and expression of p53 are known to be associated with neoplastic progression in BO, normal Ki-67 and p53 expression after RFA suggests that the NSE is more quiescent and has the potential for a reduction in risk for malignant transformation.\textsuperscript{6,7,24} We also compared numerical chromosomal changes and specific genetic abnormalities both before and after RFA by using FISH analysis of brush-cytology specimens. All patients at baseline showed an abnormal distribution of at least one of the FISH probes, whereas all samples from the post-RFA NSE showed a normal diploid signal count. One limitation of FISH is its restriction to the most frequently encountered alterations in neoplastic BO tissue, namely numerical chromosomal changes, p16 status and p53 status. We acknowledge that we did not evaluate the specific mutational status of p53/p16, the presence/absence of other genetic markers, hypermethylation, loss of heterozygosity, or alterations in the expression of other proteins. By comparison, Finkelstein et al. evaluated 21 patients with BO containing LGIN before and after RFA. Microdissection specimens from multiple targets for each patient were assessed (baseline and up to 2.5 years after RFA) for a panel of 16 allelic imbalance mutational markers affecting 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, and 22q using quantitative fluorescent PCR with capillary electrophoresis. At baseline, all patients had multiple mutational abnormalities. RFA achieved complete eradication of all BO tissue in 15/16 patients, and all of these 15 patients demonstrated absence of the previously detected mutations.\textsuperscript{25} The finding that the post-RFA NSE has normal Ki-67 expression and negative p53 expression, and absence of pre-existing genetic abnormalities, may suggest that the NSE after RFA regenerates from a different progenitor cell than that associated with the baseline BO. Different hypotheses regarding regeneration of NSE have developed recently, including migration of adjacent squamous stem cells, repopulation from submucosal duct pluripotent cells, and deposition of circulating stem cells.\textsuperscript{26-28} Further insight of this process of NSE repopulation after RFA would be valuable to understand if eradication of BO by RFA results in clearance of genetic abnormalities of the epithelium and, thus, a reduction of the risk for developing cancer.\textsuperscript{29} Another important issue with regard to BO ablation is the possibility of occult BGM that is not readily detected on endoscopy and standard biopsy techniques. The clinical relevance of BGM is largely unknown, but one hypothetical risk is the possibility of malignant progression.
of BGM not detectable by endoscopic inspection.14,15 The presence of BGM after ablation of BO has been reported in up to 53% of patients treated with APC or PDT.20-24 This is in contrast with an absence of reported BGM in over 6,000 biopsies from the post-RFA NSE obtained in a number of well-designed clinical trials on RFA for metaplastic and neoplastic BO.15-21 While the absence of reported BGM after RFA may be a true-negative finding related to complete ablation, it is also possible that there is occult BGM present after RFA and that standard biopsy techniques are incapable of sampling the subepithelial tissue (lamina propria or deeper) to detect the BGM. We addressed these questions in our study and found that there was no difference in primary biopsy depth when comparing specimens obtained from the post-RFA NSE (37% lamina propria) vs. specimens obtained from the untreated squamous epithelium (36% lamina propria). This suggests that the post-RFA NSE is not more resistant to biopsy as compared to untreated tissue, and our ability to sample the sub-epithelium of the post-RFA NSE is not impaired. We addressed the issue of occult BGM by adding keyhole biopsies and ER to more deeply evaluate the post-RFA NSE. We found that keyhole biopsies sample more deeply (51% lamina propria, 31% muscularis mucosae, 5% submucosa) than primary biopsies, and that ER samples deepest of all (100% submucosa). In our study, keyhole, or ER specimen did we identify BGM, using three independent blinded readings by three expert GI pathologists from the Netherlands, Germany and the United States. These findings make it highly unlikely that the reported absence of BGM after RFA is due to insufficient biopsy sampling depth. In a secondary outcome analysis, we compared the depth of biopsy between standard and jumbo biopsy forces. Jumbo forces are anecdotally believed to sample deeper aspects of the mucosa,36 however, their use requires a therapeutic keyhole, or ER specimen did we identify BGM, using three independent blinded readings by three expert GI pathologists from the Netherlands, Germany and the United States. These findings make it highly unlikely that the reported absence of BGM after RFA is due to insufficient biopsy sampling depth. In a secondary outcome analysis, we compared the depth of biopsy between standard and jumbo biopsy forces. Jumbo forces are anecdotally believed to sample deeper aspects of the mucosa,36 however, their use requires a therapeutic


CHAPTER 11

Pseudo-buried Barrett’s post radiofrequency ablation for Barrett’s oesophagus

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— Submitted
ABSTRACT

— Background:
In our experience, biopsies from small residual islands of non-buried Barrett’s mucosa after radiofrequency ablation (RFA) are occasionally reported by pathologists to contain “buried Barrett’s” upon histological evaluation, despite the fact that these islands of columnar mucosa were visible endoscopically.

— Aim:
Aim was to evaluate the frequency of buried Barrett’s in biopsies obtained from small residual Barrett’s islands (<5 mm) sampled post RFA, compared to biopsies from normal neosquamous epithelium.

— Methods:
Biopsies obtained from normal appearing neosquamous epithelium, and from small Barrett’s islands (<5mm) in 69 consecutive Barrett’s patients treated with RFA, were evaluated for the presence of buried columnar mucosa.

— Results:
2,515 biopsies were obtained from neosquamous epithelium during follow-up post RFA. We found a 0.1% rate of buried glands in biopsies from endoscopically normal neosquamous epithelium. However, when small islands of columnar mucosa were biopsied, buried glands were detected in 21% of biopsies.

— Conclusion:
To avoid accidental sampling of small islands resulting in a false positive histological diagnosis of buried Barrett’s, thorough inspection should be performed before obtaining biopsies during post-RFA follow-up.

INTRODUCTION

A potential limitation of ablation for Barrett’s oesophagus (BO) is the development of residual areas of columnar mucosa that reside underneath newly formed squamous epithelium (“buried Barrett’s”). Buried Barrett’s has been reported in up to 53% of patients after endoscopic ablation,1–5 with the lowest rates detected after radiofrequency ablation (RFA) [0–5.4%].6–12 The clinical relevance of buried Barrett’s is uncertain. One major concern is the possibility that buried Barrett’s remains undetected and progresses to malignancy.13–15 Others believe that the malignant potential of buried Barrett’s is negligible, since the buried glands are protected from the trophic influence of the luminal gastro-oesophageal refluxate.16

In our experience, biopsies from small residual islands of non-buried Barrett’s mucosa after RFA are occasionally reported by pathologists to contain “buried Barrett’s” upon histological evaluation, despite the fact that these islands of columnar mucosa were visible endoscopically (i.e. appeared as columnar mucosa, not as squamous mucosa). As a result, we hypothesized that a false-positive finding of buried Barrett’s may be explained by sampling error and by potential processing artifacts. Thus, in this study, our aim was to evaluate the frequency of buried Barrett’s in biopsies of small residual areas of columnar mucosa (<5 mm) sampled post RFA, and to compare findings in biopsies from endoscopically apparent neosquamous mucosa.

METHODS

Patients
This study included 69 consecutive patients who were treated with RFA for BO with early neoplasia at the Academic Medical Center, Amsterdam, the Netherlands.9–12

RFA treatment protocol
As described in detail previously,9–12 the BO segment was thoroughly inspected and any visible lesions were removed by endoscopic resection for histological staging. RFA treatment was performed with the HALO System (BARRX Medical Inc., Sunnyvale, CA, USA) every 8–12 weeks, until all endoscopically visible Barrett’s mucosa was eradicated.

Biopsy protocol and pathologic analysis
Biopsies of visible columnar mucosa were obtained prior to RFA and at intervals prior to complete eradication of all visible Barrett’s mucosa. All biopsies from endoscopically visible columnar mucosa were collected separately. The site of origin of the biopsies was recorded for all study patients as part of our standard study protocol of RFA for Barrett’s. After complete endoscopic eradication of all Barrett’s mucosa was achieved by RFA, follow-up was scheduled at 2, 6 and 12 months, and then annually. Follow-up endoscopies were performed with a high-resolution endoscope, with narrow-band imaging (NBI). Four-quadrant mucosal biopsies were obtained immediately distal (<5 mm) to the neo-squamocolumnar junction, and from every 2 cm of the entire length of the original Barrett’s segment.

Biopsies were embedded in paraffin, cut into 5-µm thick sections and stained with hematoxylin & eosin. The slides were assessed by two pathologists (FK, MV), who scored tissue type
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PART II - Radiofrequency Ablation

[squamous, columnar] and presence of buried columnar mucosa. Buried Barrett’s was diagnosed by the pathologists if columnar lined glands were located underneath an intact layer of squamous epithelium, regardless of the presence or absence of goblet cells, since all patients had proven goblet cells in their columnar lined oesophagus prior to RFA. Additional deeper cuts into the tissue block were made to evaluate for the presence of communication of the buried glands to the oesophageal lumen.

RESULTS

Biopsies from neosquamous epithelium

A total of 2,515 biopsies from 69 patients was obtained from neosquamous epithelium during follow-up post RFA. Histological evaluation showed buried glands without surface communication in three patients, in a single biopsy each (0.1%). Below, these three cases are described in more detail:

Case 1:
The first case was a patient with early cancer in a C9M10 BO, who was successfully treated by endoscopic resection followed by 2 RFA sessions. Two months after the last RFA session, no residual Barrett’s was seen during endoscopy and all biopsies from neosquamous mucosa were negative for columnar mucosa. Follow-up endoscopy, at 6 months, showed no columnar mucosa, however, one neosquamous biopsy from the upper end of the original Barrett’s segment contained a focus of non-dysplastic buried glands. During repeat endoscopy with NBI, a 0.5x3 mm island of columnar epithelium was detected at the location where the biopsy with buried glands had been obtained. The island was located immediately distal to a reflux stenosis at the upper end of the original BO, and was only observed after inspection with NBI. The island was removed by focal endoscopic resection, 9 biopsies were obtained at the same level, all without signs of subsquamous glands. At two following endoscopies a second endoscopic resection and 12 biopsies were taken at this level, none of which showed buried glands (Fig. 1).

Case 2:
The patient had early cancer in a C3M4 BO who was successfully treated by endoscopic resection and 2 RFA sessions. Two months after the second RFA treatment, no residual Barrett’s was seen and biopsies were obtained distal to the neo-squamocolumnar junction, and at two levels from the neosquamous epithelium. One biopsy, obtained just above the gastro-oesophageal junction, showed buried glands. At the next two follow-up endoscopies, 12 and 24 months after the last ablation, no (subsquamous) columnar epithelium was found in a total of 8 biopsies obtained immediately distal to the neo-squamocolumnar junction, and in 9 biopsies from neosquamous epithelium.

Case 3:
This patient was treated successfully by endoscopic resection and 2 RFA sessions for C4M5 BO with high-grade intraepithelial neoplasia (HGIN). During the first follow-up endoscopy, buried glands were diagnosed in a neosquamous biopsy from the middle part of the initial BO. Repeated detailed endoscopic inspection with NBI was performed at this level, but no visible Barrett’s mucosa was detected. However, a biopsy from the same area again confirmed the presence of subsquamous glands with goblet cells. The area with the buried Barrett’s was therefore treated anew with balloon-based circumferential RFA (2x 12 J/cm²). Two, six and 18 months after the repeat RFA treatment, a total of 16 biopsies and 3 endoscopic resection specimens from the healed area did not show any signs of subsquamous glands (Fig. 2).
Buried Barrett’s treated by repeat RFA.

Biopsies from endoscopically visible islands of residual columnar mucosa

In 18 of 69 patients, 52 small islands (median 1 mm (IQR 1-2)) of columnar mucosa were detected at an interim endoscopy in between the first and last RFA treatments. Targeted biopsies from these 52 islands showed buried glands in 11/52 samples (21%). Surface communication of the glandular structures was visualized in 9/11 biopsies (82%). All patients received additional RFA. Complete eradication of all neoplasia was reached in 68/69 patients (98.5%) and complete eradication of all Barrett’s in 67/69 patients (97.0%). The patient with residual HGIN and columnar mucosa was referred for surgical oesophagectomy, whereas the patient with residual non-dysplastic Barrett’s was kept under endoscopic surveillance.9-12

DISCUSSION

We found a 0.1% rate of buried glands in biopsies from endoscopically normal neosquamous epithelium. However, when small islands of columnar mucosa were neosquamous, buried glands were detected in 21% of biopsies. The low rate of buried Barrett’s in biopsies from neosquamous mucosa comports with the 0-5.4% rate found in over 700 patients from other studies on RFA for BO.4,14 In a previous study by our group, we found that biopsies from neosquamous epithelium post RFA revealed subepithelial lamina propria or submucosa in 37%, which was similar to biopsies from untreated squamous epithelium (36%) in the same patients. In addition, no buried glands were found in biopsies, deeper keyhole biopsies, and endoscopic resection specimens from neosquamous epithelium.15 Furthermore, two studies from the USA demonstrated that post-RFA neosquamous biopsies contain lamina propria or submucosa in 78 and 91% of biopsies, which did not significantly differ from biopsies from ablation-naive squamous epithelium.16,17 Biopsies after RFA therefore appear to be of adequate depth to evaluate the presence of buried glands.20

Buried Barrett’s is generally defined as “columnar epithelium covered by a layer of squamous epithelium, without communication to the luminal surface”.20 In our opinion another requisite for a diagnosis ‘buried Barrett’s’ is that the biopsy should be obtained from endoscopically normal neosquamous mucosa, without signs of columnar epithelium. This is difficult to guarantee when random biopsies are obtained in patients with visible Barrett’s mucosa after RFA, since there is a risk that residual Barrett’s islands or tongues are partially sampled, since post biopsy bleeding may obscure the endoscopist’s view.

There are several reasons that may explain why biopsies from endoscopically visible small columnar islands may result in a histological finding of buried glands. First, undermining of the Barrett’s mucosa underneath the adjacent squamous epithelium (Fig. 3a). Second, the oesophagus is a tubular organ and the biopsy forceps is typically aimed at a steep angle during biopsies. Columnar mucosa may be sampled unintentionally and may, consequently, appear to be situated underneath squamous mucosa. Third, if biopsies obtained from small columnar islands consist partially of columnar mucosa, and partially of squamous mucosa, and they are not orientated prior to fixation, embedding and sectioning may result in columnar mucosa that appears to be situated underneath squamous mucosa (Fig. 3b). In the first case described in this paper, buried Barrett’s was found in a single neosquamous biopsy 6 months post-RFA. After repeat inspection with NBI, a small columnar island was identified at the upper end of the original BO segment. We hypothesized that this small island was overlooked during preceding endoscopies, and was accidentally biopsied under the presumption of biopsying normal neosquamous epithelium. Due to the artifacts described above, this may have led to a histological finding of buried Barrett’s.

To avoid accidental sampling of small islands, thorough inspection should be performed before obtaining biopsies during post-RFA follow-up. In our experience, high-resolution endoscopy with NBI proved very valuable in detecting small residual islands of Barrett’s mucosa post RFA (Fig. 1; Chapter 5 Fig. 5). Although high-resolution endoscopes do not allow the use of a jumbo biopsy forceps, we prefer to optimize endoscopic inspection and obtain standard biopsies, instead of obtaining jumbo biopsies using a therapeutic endoscope that provides lower quality imaging.

In the second case, we detected buried glands in a biopsy obtained just proximal to the neo-squamocolumnar junction. As demonstrated by autopsy studies, there is a 4 to 8 mm overlap of squamous and gastric mucosa at the gastro-oesophageal junction.21 Although we do not know how this overlap is composed after RFA, cardia mucosa may undermine the neosquamous epithelium. Biopsies obtained from neosquamous mucosa close to the junction may, therefore, histologically result in buried glands due to the same artifacts that may occur when biopsying small islands. To avoid this, biopsies from neosquamous mucosa should be obtained at least 10 mm above the neo-squamocolumnar junction, and biopsies to assess for residual intestinal metaplasia at the cardia should be obtained just distal to the junction (≤5 mm).

Buried Barrett’s post RFA was reported in the AIM-dysplasia trial by Shaheen et al.7 In this study, 25.2% of patients had buried Barrett’s detected prior to RFA treatment and thus in
biopsies obtained from an oesophagus with visible Barrett’s mucosa. This rate was reduced to 5.4% of patients post treatment. However, since complete eradication of all IM was reached in 77.4% of patients, it may be expected that part of the buried Barrett’s post-RFA were found in patients who also had residual visible Barrett’s mucosa. When buried Barrett’s is diagnosed in neosquamous biopsies, it is advisable to perform detailed endoscopic inspection to detect any residual Barrett’s mucosa that can then be subsequently treated. If columnar mucosa is not observed, biopsies should be repeated. If buried glands are confirmed, repeat ablation of the affected area is effective to eradicate buried glands, as demonstrated in one of our patients.

Based on our experience with RFA for treatment of BO and associated neoplasia, and the buried glands, as demonstrated in one of our patients. Buried glands are confirmed, repeat ablation of the affected area is effective to eradicate subsequently treated. If columnar mucosa is not observed, biopsies should be repeated. If buried glands are confirmed, repeat ablation of the affected area is effective to eradicate buried glands, as demonstrated in one of our patients. Patients with experience with RFA for treatment of BO and associated neoplasia, and the results from this study, we recommend follow-up with high-resolution endoscopes and NBI, or comparable techniques, to carefully inspect the neo-squamo-columnar junction in the antegrade and retroflexed position, and to rule out the presence of small islands of Barrett’s mucosa. Biopsies should be obtained immediately distal (<5 mm) to the neosqua-mocolumnar junction to evaluate for residual columnar mucosa, and from four-quadrants of the neosquamous epithelium every 2 cm of the original extent of the Barrett’s segment, beginning 10 mm above the neo-squamo-columnar junction.

Figure 3. Artifacts that may result in a histological finding of buried Barrett’s.
A: Histological image of a biopsy from a visible Barrett’s island, showing a patch of Barrett’s mucosa with communication to the luminal surface. However, if the biopsy would have been obtained just next to the island, sampling of only the glandular structures undermining the surrounding squamous epithelium [arrow], could have resulted in a histological finding of buried Barrett’s. B: Histological image of a biopsy that appears to contain buried glands. However, this biopsy shows the transition of squamous mucosa to glandular mucosa at the luminal surface [arrow], which may be missed because the biopsy was not stretched and oriented prior to fixation.

REFERENCES

FUTURE PROSPECTS
As described in this thesis, endoscopic treatment has steadily gained its place as the therapy of choice for patients with early Barrett’s neoplasia. From endoscopic resection (ER) monotherapy, which was abandoned due to a high recurrence rate of neoplasia in residual Barrett’s mucosa, we moved into the field of complete Barrett’s eradication to prevent recurrences. After disappointing results of photodynamic therapy for this purpose, we used stepwise radical endoscopic resection (SRER) to remove the entire Barrett’s segment during subsequent resection sessions. Although SRER proved highly effective, a European multicentre study showed that SRER is technically demanding and associated with a high rate of oesophageal stricturing especially in patients with longer Barrett’s segments. We therefore evaluated the use of a relatively new endoscopic ablation technique, radiofrequency ablation (RFA). The initial promising results of RFA to eradicate all Barrett’s mucosa with early neoplasia were reproduced in a number of large-scale European multicentre studies. Given its high efficacy, safety and relatively easy application, RFA with or without prior ER for visible lesions, has been implemented as the treatment of choice in a number of national and international guidelines. The studies described in this thesis have made an indispensable contribution to the evidence on which current management of early Barrett’s neoplasia is based. However, we should always strive for improvement. In the following paragraphs we will therefore make recommendations to improve Barrett’s management in the future, and we will speculate on studies that may help to reach these improvements.

1. Indications for endoscopic treatment in patients with Barrett’s oesophagus

High-grade intraepithelial neoplasia (HGIN) and mucosal cancer

Based on the currently available literature from different centers around the world, and the studies presented in this thesis, there is enough evidence that endoscopic treatment, with ER as its cornerstone, is the treatment of choice for BO patients with HGIN and mucosal cancer, with 5-yr disease free survival in up to 95% of patients.

Low-risk submucosal cancer

Barrett’s cancer infiltrating the submucosa is still regarded as an indication for surgery, given the risk of lymph node metastasis (N+) ranging from 0-22% for sm1, and 36-54% for sm2/3 cancer. These risk estimates are, however, based on retrospective cohorts with submucosal cancer diagnosed in surgical resection specimens. At the time these studies were performed, oesophagectomy was the treatment of choice for any grade of neoplasia, ranging from a single biopsy diagnosis of HGIN, to stage T3 cancer. Accurate histological differentiation between different depths of submucosal invasion did therefore not bear much clinical relevance. Surgical specimens were routinely cut in 5-10 mm slices and the area of deepest infiltration could have been missed easily. This may have resulted in underestimation of invasion depth, and thus a wrong interpretation of the N+ risk corresponding with a certain depth of invasion. In contrast, when infiltration depth is assessed in ER-specimens, which are routinely cut in 2-mm slices with additional cuts in case of submucosal invasion, the N+ risk for certain infiltration depths can be reported more accurately. This has already been demonstrated by the fact that the 4-12% risk of N+ for mucosal cancer reported in surgical
series, is in fact much lower (≤0.5%) when the diagnosis is based on ER-specimens. As reported by Manner et al.,14 and Alvarez Herrero et al.,15 the risk of N+ in ‘low-risk’ submucosal cancer diagnosed in ER-specimens, may also be lower than reported thus far. Low-risk criteria are defined as well to moderately differentiated cancer, with submucosal invasion of ≤500 μm, without signs of lymph-vascular invasion. Taking into account that the N+ risk may be lower than reported thus far, and that most patients with low-risk submucosal cancer are elderly with co-morbidities, endoscopic treatment should be considered as a valid alternative to surgery in these cases. Until more evidence is available, we think that an approach of endoscopic treatment with 3-monthly follow-up with endoscopy and EUS is an acceptable in patients with low-risk submucosal cancer, who are not optimal candidates for surgery. Studies comparing endoscopic treatment for low-risk submucosal cancer to surgery in a randomized setting will be difficult, since patients are rare, often have contraindications for surgery and may not always agree to participate in such a study. A study with ER for staging of the disease, followed by surgery to assess for lymph node metastasis also has its drawbacks, since micro-metastases and lymph node metastases can be missed. Therefore, multicentre prospective registration of endoscopically treated low-risk submucosal cancer, with endoscopic and EUS follow-up, will be important to evaluate if endoscopic treatment is indeed a valid alternative to surgery for selected patients.

Low-grade intraepithelial neoplasia (LGIN)
Recent data have demonstrated that after expert histological revision, the diagnosis of LGIN is down-staged in the majority of patients.18 If LGIN is confirmed by expert histological revision, however, it is a serious disease with a cumulative risk for developing HGIN or cancer of 83% within 97 months.19 Recent AGA-guidelines have indicated that RFA may be a therapeutic option for treatment of confirmed LGIN in BO to prevent neoplastic progression, despite the controversies surrounding the definition of LGIN.19 For LGIN in BO, Shaheen et al. and Sharma et al. demonstrated that RFA achieved eradication of all LGIN and intestinal metaplasia (IM) in 90-100% and 81-90% of patients, respectively.18,19 Another randomized trial performed in a multicentre European setting, is currently underway comparing RFA versus surveillance for patients with confirmed LGIN (SURF-study). Given these recent developments, an approach using RFA to eradicate BO in patients with confirmed LGIN, will most likely be adapted in the near future. In that case, there will be an increased need for expert histological revision of LGIN, or preferably for more objective markers of LGIN, such as biomarkers. 

Non-dysplastic Barrett’s oesophagus (NDBO)
Currently an approach of endoscopic surveillance with biopsies is used to detect malignant progression in these patients. However, surveillance is limited by the difficulty to detect early neoplasia endoscopically, by biopsy sampling error, by inter-observer variability between pathologists, and by questionable cost-effectiveness.20 The annual progression rates to HGIN (0.9%) or cancer (0.5%) are relatively low.21,22 Biomarkers predicting malignant progression may therefore help to identify those patients with an increased risk to develop neoplasia. These patients could then undergo more frequent endoscopic surveillance or prophylactic ablation of their NDBO. Currently, ablation of NDBO is still a subject of much debate given the low progression rates and lack of objective predictors for progression. However, if the annual progression risks are accumulative, as suggested by a recent Dutch nation-wide cohort study,23 then patients with a longer life expectancy will have a serious risk to develop cancer at some point in their life. This knowledge, as well as the promising preliminary results in the field of prognostic biomarkers24-28 and the demonstrated efficacy and safety of RFA, may be used to improve current management of NDBO. Clearly, endoscopic surveillance with its previously mentioned shortcomings is not efficient enough to halt the rising incidence of oesophageal adenocarcinoma.24 Therefore, alternatives such as prophylactic ablation of NDBO using RFA deserve to be evaluated.

The use of RFA to prevent neoplastic progression in NDBO compared to standard endoscopic surveillance should ideally be studied in a randomized setting. Given the relatively low annual progression rate, a large cohort of patients would be needed, warranting a multicentre setting. Preferably, only patients recently diagnosed with NDBO should be included in such a study, since these are the patients that best reflect day-to-day practice. These patients have the highest chance of under-diagnosis due to sampling error, and thus a higher risk of progression compared to patients who have already undergone multiple surveillance endoscopies. Furthermore, anticipating on the implications of such a study, its results will be used to make a decision between RFA versus surveillance in these recently diagnosed patients. The primary outcome parameter for such a randomized study should be progression to HGIN or early cancer, both currently indications for treatment. However, with the recent developments in the field of RFA for LGIN, one may question if patients diagnosed with LGIN at some point, are still willing to remain under endoscopic surveillance instead of undergoing RFA. In the future, the decision to use prophylactic RFA in patients with NDBO will likely be guided by the use of biomarkers to identify those patients with an increased risk of malignant progression. For now, we think that RFA for NDBO may be considered for individual patients who are expected to at increased risk for neoplastic progression, for example patients with a long BO segment, patients with a strong family history of oesophageal adenocarcinoma, and young patients with a significant life-expectancy. Quality of life may also play an important role in the decision to treat patients diagnosed with NDBO, since fear of developing cancer may negatively influence quality of life, especially in patients with a family history of Barrett’s cancer.20 Although surveillance after RFA will still be necessary since no long-term follow-up data after RFA for NDBO are available yet, the risk of malignant progression and patients’ fear to develop cancer, may be significantly decreased.

2. Endoscopic work-up of patients with early neoplasia in Barrett’s oesophagus
Work-up prior to endoscopic treatment usually consists of thorough endoscopic inspection, often using advanced imaging techniques, to detect mucosal irregularities. Targeted biopsies are then obtained from any abnormalities, as well as randomly throughout the BO. Often, a second endoscopy is scheduled to remove abnormalities by ER, followed by one or two additional endoscopies to re-stage the residual BO with biopsies prior to RFA. Chapter 4 showed that in all patients undergoing 5RER, the histological findings of the first procedure corresponded with the overall worst histopathology of the patient: all T1sm1 cancers were identified as a suspicious lesion and removed during the first procedure and...
no G3/G4 cancers or lymph-vascular invasion were diagnosed at subsequent ER sessions. This suggests that after thorough endoscopic work-up and ER of the most involved area with histological correlation, the remaining BO can be safely ablated without significant risk of leaving submucosal lesions undiagnosed and under-treated. The chance of finding early cancer in random biopsies from normal appearing flat-type mucosa is extremely low. Although not officially reported, no patients were excluded from any of the studies in this thesis based on this exclusion criterion.

In the future, endoscopic work-up may be performed more efficiently. Thorous endoscopic inspection can be performed to detect any mucosal irregularities that may be immediately removed by ER for histological staging. If the ER specimen does not show risk factors for lymph node metastasis, endoscopy can be performed after 6 weeks to inspect the ER-wound and to evaluate if there are any other mucosal abnormalities. If no other lesions are observed, the residual Barrett’s mucosa can be treated with RFA, without the need to obtain additional random biopsies. Also, in between RFA sessions, there will be no need to obtain biopsies from normal appearing flat-type mucosa. During work-up for endoscopic treatment, high-definition endoscopy allowing for wide-angle inspection of the oesophagus may therefore be much more useful than advanced imaging techniques allowing for detailed, focal inspection of the mucosa (e.g. confocal endomicroscopy and spectroscopy).

3. Developments in endoscopic resection

As demonstrated in chapter 3, multiband mucosectomy (MBM) was equally effective and safe as ER-cap for piecemeal resection of early BO neoplasia, however, significantly faster and cheaper. In the future, the use of the ER-cap technique will in our opinion only be indicated for patients with large lesions or lesions suspicious for submucosal invasion who are unfit for surgery and in whom the first ER will be their best shot for curative treatment. In those cases, the large flexible cap should be used to increase the chances of radical resection, in case the lesion actually extends into the submucosa.

MBM did result in significantly smaller resection specimens than ER-cap, which may imply that more adjacent resections should be performed to remove a lesion. Piecemeal ER does not allow for histological assessment of radicality at the lateral resection margins. However, as long as the visible lesion is completely removed and histological evaluation shows radically removed neoplasia at the deep resection margins, the lateral margins may be less relevant, since residual Barrett’s mucosa will be treated additionally by RFA.

Endoscopic submucosal dissection (ESD), does allow for en-bloc resection of neoplastic lesions. However, the advantage of using ESD compared to ER in BO may be limited. First, ESD may be effective and safe for radical removal of neoplastic lesions in the stomach or squamous oesophagus, but radical resection rates for Barrett’s oesophagus from Western series (22-64%) are quite disappointing. Endoscopic submucosal dissection (ESD), does allow for en-bloc resection of neoplastic lesions. However, the advantage of using ESD compared to ER in BO may be limited. First, ESD may be effective and safe for radical removal of neoplastic lesions in the stomach or squamous oesophagus, but radical resection rates for Barrett’s oesophagus from Western series (22-64%) are quite disappointing. Second, in contrast to piecemeal ER, ESD allows for en-bloc resection. However, as described above, if neoplasia is removed radically in the deep resection margins, histological evaluation of the lateral margins may be less relevant since the residual BO mucosa needs to be treated anyway. ESD will not be applicable for complete BO removal, given the high stricture rates associated with widespread ESD. Third, although ESD results in less local recurrences, the local recurrence rates after ER followed by RFA are so low that the potential benefit from applying ESD may be limited. Fourth, just as after ER, follow-up is still indicated after ESD to detect recurrences in the oesophagus and cardia at a curable stage. All in all, ESD in its current form will have a limited role in the management of early BO neoplasia.

4. Developments in endoscopic ablation

Although RFA is relatively easy to apply, and operator dependency is limited by standardization of RF energy delivery, the technique may be improved at some points. First, a flexible ablation balloon should be developed, which fits through the working channel of an endoscope, and which adjusts itself to the oesophageal diameter. This would make the sizing step, placement of a guide-wire, and repeated introductions with the endoscope redundant. Second, the energy settings for ablation may need to be re-evaluated. Currently the HALO360 catheter is used at 2x 12 J/cm², and the HALO90 at 2x2 15 J/cm² in Europe and 2x2 12 J/cm² in the US. In our first studies we demonstrated that thorough cleaning of the ablation zone in between ablation passes increased efficacy. However, this cleaning step requires additional procedure time and patience from the endoscopist. Although a second ablation pass will remain necessary to treat any areas that were skipped during a first ablation pass, increasing the energy settings might make the cleaning step unnecessary, while maintaining the same depth of ablation. Two randomized studies comparing efficacy of ablation in patients undergoing HALO360 or HALO90 ablation with extensive cleaning in between ablations vs. no cleaning, are currently being performed at our centre.

Up to now, RFA is the only technique that has been proven to be safe and highly effective for eradication of BO. Photodynamic therapy should be abandoned for Barrett’s ablation, given its poor efficacy and significant complication risk. APC is an effective, easily accessible and cheap ablation tool, but for ablation of an entire BO segment it is just not very practical. For ablation of small islands, however, APC is faster and cheaper compared to HALO90 ablation. In all patients not treated within an RFA-study protocol, we use APC for residual islands with a maximum diameter of 5 mm, and a maximum of 3 residual islands. Another ablation tool undergoing interesting developments is cryoablation. Initially cryospray-ablation was used for Barrett’s ablation. However, since the liquid nitrogen was sprayed on the mucosa from a certain distance, for a certain amount of time, results were very operator dependent. Recently, a balloon-based device has been developed for cryoablation. This flexible balloon adjusts itself to the oesophageal wall and is then filled with liquid nitrogen, freezing all mucosa in contact with the balloon. However, to surpass the current efficacy and safety profile of RFA, this technique should be able to reach the same constant depth of ablation, it should be well tolerated by patients, easy to use, and affordable.

Lastly, we should define when additional HALO90 treatment for residual Barrett’s islands should be ceased. After HALO360 ablation, at least one HALO90 procedure should be performed to circumferentially ablate the entire gastro-oesophageal junction, and any residual BO islands. If large islands persist, a second HALO90 procedure can be performed. However, if there are still residual islands of BO after the second HALO90 procedure, this may indicate that contact between the electrode and mucosa in these areas is suboptimal. Removing such
residual areas by MBM may be much faster, cheaper and more effective. As demonstrated in chapter 10, MBM can be applied without problems after prior RFA. As mentioned above, small islands <5 mm can also be touched up with APC.

5. Management of patients with widespread neoplastic lesions
Scarring after ER makes subsequent RFA more challenging, due to a more difficult sizing procedure, suboptimal contact between mucosa and electrode, and an increased risk of mucosal laceration and stenosis. As described in chapter 7, we found fewer complications when the extent of ER was limited to 2 cm in length, and 50% of the oesophageal circumference. In most patients, visible lesions can be removed within this restriction; however, in some patients more extensive resection is necessary. The most effective treatment approach to reach eradication of neoplasia and all Barrett’s mucosa will be SRER if the BO is <5 cm in length. However, for patients with a BO >5 cm, another solution should be found to combine widespread ER and RFA, while minimizing the risk of suboptimal treatment, mucosal laceration or stenosis. We already evaluated the use of circumferential RFA, followed by ER of irregular mucosa in the same session, in a small series of 24 patients. Although circumferential RFA could be performed uncomplicated in all patients, and histological specimens allowed for assessment of neoplastic invasion depth, this approach required some skill from the endoscopist and pathologist. Preferably, we would like to perform ER of visible lesions first, followed by RFA in the same session, before scarring of the ER-wound occurs. In pig studies, however, this approach was associated with severe strictureting and even perforation (unpublished data). The optimal approach to combine ER and RFA in the same session should therefore be studied better. Until then, a sequential approach of widespread ER followed by dilatations until 18 mm to allow for RFA using an 18-mm catheter, may be the best option to treat widespread mucosal irregularities in BO >5 cm.

6. Neosquamous regeneration after RFA
The process of regeneration and factors that contribute to conversion of BO into neosquamous epithelium after RFA are still poorly understood. Some patients undergoing RFA require only a single treatment session for complete reversion of their BO into neosquamous epithelium whereas others show poor healing without significant regression. There are several hypotheses concerning the origin of the neosquamous epithelium after ablation therapy: outgrowth from existing pools of squamous cell progenitors, repopulation from adjacent areas with squamous epithelium, or regeneration from multipotent progenitor cells from the esophageal glands or bone marrow. Further insight of this process of squamous repopulation after RFA would be valuable for two reasons. Firstly, it might help to identify patients with an anticipated poor response to RFA and/or to intervene in the regeneration process. Secondly, it may answer the question if eradication of BO by RFA results in permanent clearance of genetic abnormalities of the epithelium and thus in a reduced risk to develop cancer. Studies to increase our insight into neosquamous regeneration after RFA have been initiated, however, to get to the bottom of this intriguing matter expertise from different centers should be combined.

7. Predictors of response to RFA
Sporadically, we encounter patients with poor response after the first RFA treatment. These patients show minimal or slow regression of the Barrett’s epithelium after RFA, and some of these patients show delayed healing of the ablated areas. As described above, factors responsible for wound healing and response to RFA treatment are still unknown. By prospective registration of a variety of factors for all patients treated with RFA (e.g. BO length, grade of dysplasia at baseline, smoking, healing after ER, etc), we hope to be able to identify factors that may predict who will respond well to RFA treatment, and in whom treatment will be difficult and will require more time.

8. Management of reflux after endoscopic treatment of BO
BO is a complication of longstanding gastro-oesophageal reflux. Although no one has ever seen a BO ’develop’, one may question if PPI treatment after successful eradication or BO is enough to prevent recurrence. Currently, 5-year follow-up data have shown minimal recurrence of Barrett’s mucosa, which suggests that PPI treatment is sufficient. However, in patients with therapy resistant reflux, or patients showing very poor healing after RFA due to acidic irritation of the ablated mucosa, fundoplication may be necessary. The best timing for fundoplication in relation to RFA treatment still needs to be studied.

9. Follow-up after endoscopic treatment of Barrett’s oesophagus
As described in chapter 4, 7 and 8, the majority of recurrences of IM and neoplasia occur at the gastro-oesophageal junction (GO-junction). Eradication of all IM at this level should therefore be optimized. For SRER this implies that resections should extent deep enough into the cardia. Also, the entire circumference of the GO-junction should be resected, even in patients who only display tongues of Barrett’s mucosa. For RFA this implies that the entire circumference of the GO-junction should be treated at least once with the focal HALO90 device, since HALO360 ablation at this level is often insufficient due to poor contact between the electrode and the mucosa. Endoscopic differentiation between gastric mucosa and IM is almost impossible, making it difficult to judge if all Barrett’s mucosa has been eradicated. An endoscopically based endpoint for eradication of BO is therefore quite subjective. We have therefore used the criterion of histologically proven IM before treating patients with RFA, which enables us to use the objective endpoint of histological eradication of IM post-RFA. However, by using this endpoint, we do occasionally encounter a finding of focal IM in random biopsies obtained from the cardia during follow-up in patients who had already reached eradication of all Barrett’s mucosa. One may argue that residual IM in the cardia reflects incomplete cure of the underlying disease. However, IM of the cardia can be detected in up to 25% of patients with a normal appearing squamocolumnar junction and is not considered premalignant in those cases. We therefore repeat endoscopic inspection and biopsies within one year in the case of non-dysplastic IM. In the case of LGIN, HGIN or cancer, however, additional treatment is necessary. The behavior of the neosquamous columnar junction after endoscopic therapy is unclear. In accordance with our findings, other groups have also reported on the issue of neoplasia developing in the cardia months to years after complete removal of BO. During follow-up, this area should therefore be inspected thoroughly and biopsies should be obtained to assess for IM.
As described above, a histological parameter is preferable over an endoscopic parameter during follow-up of the GO-junction post-RFA. However, for the neosquamous epithelium the opposite is the case: thorough endoscopic evaluation of the neosquamous epithelium for columnar mucosa is in our opinion more important than taking random neosquamous biopsies. Currently, a lot of attention during follow-up after RFA goes out to the presence of “buried Barrett’s”. However, as demonstrated in chapters 10 and 11, buried glands are very rare if the neosquamous mucosa looks normal upon endoscopic inspection. As described in chapter 11, we found a 0.1% rate of buried glands in over 2,500 biopsies from endoscopically normal neosquamous epithelium. However, when small islands of columnar mucosa were biopsied, buried glands were detected in 21% of biopsies. In addition, the cases of subsquamous neoplasia we diagnosed in over 10 years time (all occurring after complex treatment with PDT or APC) were all detected as endoscopically visible abnormalities. All these subsquamous lesions could be treated endoscopically, referral for surgery was never needed and no patients died from this diagnosis. Therefore, after thorough endoscopic inspection of the neosquamous epithelium with high-definition endoscopy and narrow-band imaging (NBI) to detect areas of columnar mucosa, random ‘blind’ biopsies of the neosquamous epithelium have little additional value. In the future, endoscopic follow-up may consist of thorough endoscopic inspection of the neosquamous mucosa and GO-junction. Biopsies will only be obtained immediately distal to the GO-junction, and if necessary from any visible mucosal irregularities or columnar mucosa in the tubular oesophagus. If patients do not show recurrence of IM or neoplasia within the first year, endoscopic follow-up can be performed at longer intervals, or even omitted. EUS during follow-up may be reserved for those patients with risk factors for lymph node metastasis, such as submucosal invasion, poorly differentiated cancer, or lymph-vascular invasion in the diagnostic ER-specimen.

10. Training and centralization of Barrett’s management

Although new developments have made endoscopic treatment for Barrett’s neoplasia easier and accessible, it should be born into mind that ER and ablation are only technical parts of overall Barrett’s management. An endoscopist should also be able to recognize neoplastic lesions, and identify patients who are eligible for curative endoscopic treatment. Furthermore, adequate histological evaluation should be available, as well as surgical assistance in the case of failed endoscopic treatment or complications that cannot be managed endoscopically. Structured training aimed at improvement of endoscopic detection, endoscopic treatment and histological evaluation of ER specimens is therefore necessary. The last couple of years, such training programs have successfully been set up in Europe (www.endosurgery.eu, www.rfa-academia.eu). By using a teaching-the-teachers model, these training programs have managed to spread structured training to other countries where endoscopic therapy was still little practiced. Since Barrett’s neoplasia is not a very prevalent disease, it is difficult to get enough exposure to keep trained techniques at an adequate level. Patients will only benefit from all new developments in the field of endoscopic resection and ablation, if they are treated in centers with multidisciplinary expertise and if endoscopic treatment is performed by a well-trained endoscopist. In the future, treatment of BO should therefore be centralized in dedicated centers.

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THESIS SUMMARY
NEDERLANDSE SAMENVATTING
Endoscopic treatment has evolved as a valid and less invasive alternative to surgery in patients with early neoplasia in Barrett’s oesophagus (BO). Treatment protocols are constantly being improved based on results from ongoing research in this field, and technical developments in endoscopic imaging, resection and ablation. Endoscopic resection (ER) is the cornerstone of endoscopic therapy, allowing for curative removal of neoplasia and accurate histological staging. To prevent recurrences in the remainder of the Barrett’s segment after focal ER of neoplasia, it is advocated to eradicate all intestinal metaplasia. To achieve this, different approaches, such as stepwise radical ER and radiofrequency ablation (RFA), have been studied. This thesis contains a number of studies that have added greatly to the current management of patients with early Barrett’s neoplasia. Below, we have summarized the most important findings from these studies.

The first part of this thesis is focused on ER. Chapter 1 contains a review on different techniques that have been developed and modified for ER of early neoplasia in the upper gastro-intestinal tract. Furthermore, indications for ER in the oesophagus and stomach are discussed.

During work-up prior to endoscopic treatment, endoscopic ultrasound (EUS) is often used for locoregional staging of early oesophageal neoplasia. However, its value next to endoscopic inspection and diagnostic ER may be questioned, since diagnostic ER allows for histological assessment of submucosal invasion, and other risk factors for lymph node metastasis, e.g. poor differentiation and lymph-vascular invasion. Chapter 2 describes a retrospective cohort study on the additional value of EUS during work-up including ER. In this large study in 131 patients with early oesophageal and cardia neoplasia, we found that the additional value of EUS alone changed the treatment policy. Furthermore, the results of this study strengthened the role of diagnostic ER as a final step in the work-up for endoscopic treatment.

The most widely used technique for ER of early Barrett’s neoplasia is the ER-cap technique. ER-cap requires submucosal lifting and positioning of a snare in the cap, making it technically demanding and laborious. Multi-band mucosectomy (MBM) is a relatively new ER technique, which uses a modified variceal band ligator and requires no submucosal lifting or positioning of a snare. Chapter 3 describes a multicenter, randomized controlled trial comparing ER-cap and MBM for piecemeal ER of early Barrett’s neoplasia in 84 patients. The study concludes that piecemeal ER with MBM is significantly faster and cheaper than with ER-cap. In addition, MBM appears not to be associated with more perforations despite the lack of submucosal lifting. MBM may thus be preferred for piecemeal ER of early Barrett’s neoplasia.

After focal ER of neoplasia, there is a risk of 30% that patients will develop metachronous lesions in the remainder of their BO. To prevent these recurrences during follow-up, remaining Barrett’s mucosa can be removed by stepwise radical endoscopic resection (SRER). By combining the experience from four European centers to eradicate BO <5 cm
As described previously, our multicenter study on SRER showed that this approach is effective and safe treatment modality for this indication. After the promising results of RFA at the Academic Medical Center, we wanted to evaluate if these results could be reproduced in a larger European multicenter setting. We therefore initiated a pilot study in three European centers, including 24 patients [EURO-I study]. As described in Chapter 7 RFA, with or without prior ER for visible lesions, achieved eradication of neoplasia and intestinal metaplasia in 95% and 88% of patients, and after additional escape ER in 2 patients in 100% and 96%, respectively. Complications after RFA included melena (n=1) and dysphagia (n=1). Furthermore, during the first half of this study, 5 mucosal lacerations were observed within the ER-scar area during circumferential RFA. We related these lacerations to extensive baseline ER and scarring. None of the lacerations required intervention or caused complaints and, therefore, they were regarded as mild complications. However, since lacerations may potentially provoke severe bleeding or oesophageal perforation, the investigator group added a restriction to the baseline extent of ER (max 50% of circumference, 2 cm length), after which no further lacerations were observed. After an additional median follow-up of 22 months no neoplasia recurred. This pilot study, performed at the coordinating study site for all ER-specimens, pre-RFA biopsies, and continuous quality control throughout the study period, suggests that RFA plus ER is very effective and safe for treatment of BO containing early neoplasia, when performed by trained, expert endoscopists in carefully selected patients.

As described previously, our multicenter study on SRER showed that this approach is highly effective in eradication all neoplasia and intestinal metaplasia, however, technically demanding and associated with a high stricture rate. The promising results of RFA, its high safety profile, and relatively easy application, raised the question which approach is superior for treatment of BO ≤5 cm with early neoplasia. We therefore initiated a randomized controlled trial in three European centers with experience in both SRER and RFA, to compare the safety and efficacy of both techniques. As described in Chapter 8, complete eradication of all neoplasia was achieved in 100% of SRER patients, and in 96% of ER+RFA patients. Complete eradication of all intestinal metaplasia was achieved in 92% of SRER patients, and 96% of ER+RFA patients. Although SRER and ER+RFA achieved comparable high rates of eradication of neoplasia and intestinal metaplasia, SRER was associated with a higher number of complications and therapeutic sessions. For patients with early neoplasia in BO ≤5 cm, the combined use of focal ER followed by RFA may thus be preferred over SRER.

After the promising results from the European pilot study described in chapter 7, a large-scale European multicenter study was set up in 13 centers with expertise in management of early Barrett’s neoplasia [EURO-II study]. For this study, patients with BO measuring up to 12 cm in length were included. These patients were treated by a combination of ER for visible lesions, followed by RFA to eradicate the remainder of the Barrett’s mucosa. Based on the experiences from the first European trial, ER was limited to 2 cm in length and 50% of the circumference. A unique feature of this trial was that all endoscopists participating in this study were trained in RFA at the coordinating site to standardize technique. Furthermore, all RFA procedures as well as the first follow-up endoscopy for each patient were attended on-site by a coordinating study team to ensure protocol compliance. Histological revision was performed at the coordinating study site for all ER-specimens, pre-RFA biopsies, and biopsies from the first follow-up visit. As described in Chapter 9, eradication of intestinal metaplasia and neoplasia was reached in 91% and 96% of patients, respectively. Adverse events during RFA occurred in 10% of patients, all superficial mucosal lacerations at an ER-scar or proximal reflux-stenosis, none requiring additional intervention and therefore all graded as “mild”. This largest prospective multicenter study, with a unique training set-up, and continuous quality control throughout the study period, suggests that RFA plus ER is very effective and safe for treatment of BO containing early neoplasia, when performed by trained, expert endoscopists in carefully selected patients.

After RFA, the neosquamous mucosa appears completely normal upon endoscopic inspection. To evaluate if the neosquamous mucosa is indeed comparable to normal squamous epithelium in the oesophagus, we were the first to study the histological and immunohistochemical properties of neosquamous epithelium that regenerates after RFA, as described in Chapter 10. First, we assessed if RFA is able to eradicate pre-existing genetic abnormalities in neoplastic BO. For this, we obtained biopsies and brush cytology specimens from the baseline Barrett’s segment and from the post-RFA neosquamous mucosa in 22 patients. These tissue specimens were used for fluorescent in-situ hybridization (FISH) and immunohistochemical staining. We found that all specimens obtained from the pre-treatment BO showed genetic abnormalities, while all post-RFA specimens were normal. Second, we evaluated sampling depth for biopsies obtained from untreated squamous mucosa, and for biopsies from post-RFA neosquamous mucosa. Lamina propria was sampled in 37% of
biopsies obtained post-RFA, which was comparable to the 36% of biopsies from untreated squamous mucosa containing lamina propria. Third, we wanted to assess the presence of buried Barrett’s in post-RFA neosquamous mucosa. For this, we obtained four-quadrant biopsies every 2 cm of neosquamous epithelium. To sample the mucosa even deeper, keyhole biopsies were obtained from each biopsy site. Furthermore, we performed ER of an area of neosquamous mucosa. No buried Barrett’s glands were found in any of the biopsies or keyhole biopsies. Also none of the ER-specimens, all containing submucosal tissue, showed buried glandular mucosa. In summary, the results of our study showed that the genetic abnormalities present at baseline in patients with neoplastic BO are completely absent after RFA. Further, we found that there is no difference in our ability to sample the lamina propria between post-RFA and untreated squamous epithelium, so scarring due to RFA is unlikely. Lastly, we did not detect buried Barrett’s in any post-RFA biopsy using a rigorous assessment with keyhole biopsies and ER. These data therefore suggest that the neosquamous epithelium in patients treated with RFA for BO with neoplasia, may have a reduced risk for malignant transformation and that we are not missing occult buried Barrett’s with our standard post-RFA follow-up biopsy regimen.

All studies in this thesis show that buried Barrett’s after RFA are extremely rare. In our experience, however, biopsies from small residual islands of non-buried Barrett’s mucosa after RFA are occasionally reported to contain “buried Barrett’s” upon histological evaluation, despite the fact that these islands of columnar mucosa were visible endoscopically. In Chapter 11 the frequency of buried Barrett’s in biopsies obtained from small residual Barrett’s islands (<5 mm) sampled post RFA, was compared to biopsies from endoscopically normal neosquamous epithelium. In 2,515 biopsies from endoscopically normal neosquamous epithelium, buried glands were found in 0.1% of biopsies. However, when small islands of columnar mucosa were biopsied, buried glands were detected in 21% of biopsies. This study therefore concludes that to avoid accidental sampling of small islands resulting in a false positive histological diagnosis of buried Barrett’s, thorough inspection should be performed before obtaining biopsies during post-RFA follow-up.
Endoscopische therapie heeft zich bewezen als een effectief en minder invasief alternatief voor chirurgie, in de behandeling van patiënten met vroege neoplasie in Barrett oesophagus (BO). Endoscopische resectie (ER) is de hoeksteen van endoscopische therapie. Met ER kunnen vroege neoplastische afwijkingen worden verwijderd en de patholoog kan met het verwijderde stukje weefsel vervolgens een nauwkeurige histologische diagnose te stellen. Om recidieven na focale ER van Barrett neoplasie te voorkomen, wordt tegenwoordig steeds vaker het gehele Barrett segment behandeld. Hiervoor zijn verschillende benaderingen mogelijk, zoals stapsgewijze radicale endoscopische resectie en radiofrequente ablatie (RFA). Dit proefschrift bevat een aantal studies die een belangrijke bijdrage hebben geleverd aan de huidige behandeling van patiënten met vroege neoplasie in BO. Hieronder zijn de belangrijkste bevindingen van deze studies samengevat.

Het eerste deel van dit proefschrift draait om ER. Hoofdstuk 1 bevat een review over verschillende technieken die zijn ontwikkeld voor ER van vroege neoplasie in de bovenste tractus digestivus. Ook worden indicaties voor ER in de slokdarm en maag besproken. Tijdens de voorbereiding voor endoscopische behandeling wordt endoscopische echografie (EUS) vaak gebruikt ter stadiering van infiltratie diepte en aanwezigheid van lymfeklier metastasen. Men kan zich echter afvragen wat de toegevoegde waarde is van EUS naast endoscopische inspectie en diagnostische ER, met name omdat diagnostische ER een histologische beoordeling van infiltratie diepte en andere risico factoren zoals differentiatie en lymfvascularne invasie mogelijk maakt. Hoofdstuk 2 beschrijft een retrospectieve cohort studie naar de toegevoegde waarde van EUS tijdens de voorbereiding voor endoscopische therapie. In deze grote studie met 131 patiënten, vonden wij dat de toegevoegde waarde van EUS zeer beperkt was. In geen van de 131 patiënten veranderden de bevindingen tijdens EUS het beleid. Bovendien bevestigden de resultaten van deze studie de waarde van diagnostische ER tijdens de voorbereiding voor endoscopische behandeling.

De meest gebruikte techniek voor ER van vroege Barrett neoplasie is de ER-cap techniek. Voor ER-cap is submucosale lifting nodig, evenals positionering van een resectiesnaar in de cap, waardoor deze techniek moeilijk en tijdrovend is. Multi-band mucosectomie (MBM) is een relatief nieuwe ER techniek, die gebruik maakt van een gomodificeerde rubber band ligator. Voor MBM is geen submucosale lifting of positionering van een snaar in de cap nodig. Hoofdstuk 3 beschrijft een multicenter, gerandomiseerde studie die ER-cap en MBM vergelijkt voor piecemeal ER van vroege Barrett neoplasie in 84 patiënten. De studie concludeert dat piecemeal ER met MBM significant sneller en goedkoper is dan met ER-cap. Bovendien was MBM niet geassocieerd met meer complicaties dan de ER-cap, ondanks dat er geen submucosale lifting wordt gebruikt. MBM verdient dus de voorkeur voor piecemeal ER van vroege Barrett neoplasie.

Na focale ER van neoplasie, is er een risico van 30% dat patiënten metachrone laesies ontwikkelen in het restant van hun BO. Om deze recidieven te voorkomen tijdens follow-up,
kan het resterende Barrett segment worden verwijderd middels stapsgewijze radicale endoscopische resectie (SRER). Door de ervaring met deze techniek in vier Europese centra te bundelen, konden wij 169 patiënten met vroege neoplasie in een BO <5 cm includeren, wat dit de grootste studie met de langste follow-up maakt die tot nu toe over deze methode is geschreven. Zoals beschreven in hoofdstuk 4 kon met SRER complete eradication van neoplasie en intestinale metaplasie worden bereikt in 97.6% en 85.2% van de patiënten, resp. Na een mediane follow-up van 32 maanden, bleef complete eradication van neoplasie en intestinale metaplasie behouden in 95.3% en 80.5% van de patiënten, resp. Echter, SRER is technisch uitdagend en was geassocieerd met slokdarm stenosering in 50% van de patiënten. Het risico op stenosering was gecorreleerd met de lengte van het verwijderde Barrett segment. Wij denken daarom dat SRER beperkt moet blijven tot behandeling van patiënten met een BO van maximaal 5 cm lang.

Het tweede deel van dit proefschrift draait om radiografisch onderzoek (RFA). Hoofdstuk 5 is een review over stapsgewijze circumferentiële en focale RFA met het HALO systeem. Dit review beschrijft de technische achtergrond van RFA en geeft een samenvatting van de verschillende indicaties voor RFA in BO.

Het Academisch Medisch Centrum in Amsterdam was het eerste centrum wereldwijd waar patiënten met hooggradige dysplasie in BO werden behandeld met RFA en waar RFA werd toegepast na ER van focale neoplasie. Doel van hoofdstuk 6 was om de resultaten te rapporteren van de eerste 44 patiënten met vroege Barrett neoplasie die in ons centrum werden behandeld met RFA. In 31 patiënten werd ER van zichtbare lesies verricht voor RFA. Na RFA werd complete eradication van alle neoplasie en intestinale metaplasie bereikt in 43 patiënten (98%). Complicaties na RFA waren oppervlakkige mucosale laceratie ter plaatse van een ER litteken (n=3) en voorbijgaande dysphagie (n=4). Deze eerste ervaringen met RFA voor behandeling van BO met vroege neoplasie, laten zien dat RFA, met of zonder voorafgaande ER, een effectieve en veilige behandel methode is voor deze indicatie.

Na de veelbelovende resultaten van RFA in het Academisch Medisch Centrum, wilden wij evalueren of deze resultaten konden worden gereproduceerd in een grotere Europees multicenter setting. Hiervoor initiërden wij een pilot studie in drie Europese centra, waarin 24 patiënten werden geïncludeerd (EURO-I studie). Zoals beschreven in hoofdstuk 7 werd met RFA, met of zonder voorafgaande ER van zichtbare afwijkingen, complete eradication van neoplasie en intestinale metaplasie bereikt in 95% en 88% van de patiënten. Na aanvullende ‘escape’ ER in 2 patiënten werd eradication van neoplasie en intestinale metaplasie zelfs bereikt in 100% en 96%, resp. Complicaties na RFA waren melena (n=1) en dysphagie (n=1). Bovendien werden tijdens de eerste helft van de studie vijf oppervlakkige mucosale laceraties gezien ter hoogte van een ER litteken. Wij relatieden deze laceraties aan uitgebreide ER bij inclusie. Geen van deze laceraties behoefde behandeling, maar omdat laceraties potentieel kunnen leiden tot een bloeding of zelfs een perforatie, werd het studie protocol aangepast met een beperking voor de uitgebreidheid van een ER tot 2 cm in lengte en 50% van de circumferentie. Na deze aanpassing werden er geen laceraties meer gezien. Na een follow-up van 22 maanden werden er geen recidieven van neoplasie gezien. Deze

pilot studie in Amsterdam, Düsseldorf en Brussel, toonde aan dat vroege neoplasie in BO effectief en veilig behandeld kan worden met RFA, al dan niet in combinatie met ER voor zichtbare afwijkingen.

Zoals eerder beschreven, toonde onze multicenter studie naar SRER dat deze benadering erg effectief is voor eradication van neoplasie en intestinale metaplasie. SRER is echter technisch moeilijk en geassocieerd met een groot aantal stenosens. De veelbelovende resultaten van RFA, de veiligheid en de relatief makkelijke applicatie van de techniek, deden de vraag rijzen welke van beide technieken beter is voor de behandeling van BO <5 cm met vroege neoplasie. Daarom verrichtten wij een gerandomiseerde studie in 3 Europese centra met ervaring in SRER en RFA, om de veiligheid en effectiviteit van beide technieken te vergelijken. Zoals beschreven in hoofdstuk 8 werd volledige eradication van alle neoplasie bereikt in 100% van de SRER patiënten en in 96% van de ER+RFA patiënten. Volledige eradication van alle intestinale metaplasie werd bereikt in 92% van de SRER patiënten en in 96% van de ER+RFA patiënten. Hoewel met SRER en ER+RFA een vergelijkbaar hoog percentage complete eradication van neoplasie en intestinale metaplasie werd bereikt, was SRER geassocieerd met een significant hoger aantal stenosens en behandeldsessies. Voor patiënten met vroege neoplasie in een BO <5 cm, lijkt een combinatie van focale ER gevolgd door RFA daarom de voorkeur te verdienen boven SRER.

Na de veelbelovende resultaten van de Europese pilot studie die beschreven is in hoofdstuk 7, werd er een grote Europese multicenter studie opgezet in 13 centra met expertise in de behandeling van vroege Barrett neoplasie (EURO-II studie). Voor deze studie werden patiënten met een BO tot 12 cm geïncludeerd. Deze patiënten werden behandeld met een combinatie van ER voor zichtbare afwijkingen, gevolgd door RFA van het resterende Barrett weefsel. Gebaseerd op de ervaringen van de eerste Europese studie, werd ER beperkt tot 2 cm in lengte en 50% van de circumferentie. Een unieke eigenschap van deze studie was dat alle endoscopisten in deze studie getraind werden in RFA in het coördinerende centrum, waardoor de techniek kon worden gestandaardiseerd. Bovendien werden alle RFA procedures en de eerste follow-up scopie van elke patiënt bijgewoond door een coördinerend studie team, om naleving van het studie protocol te garanderen. Histologische revisie werd verricht in het coördinerende studie centrum voor alle ER-preparaten, pre-RFA biopten en biopten van de eerste follow-up. Zoals beschreven in hoofdstuk 9, werd eradication van intestinale metaplasie en neoplasie bereikt in 91% en 96% van de patiënten, resp. Complicaties tijdens RFA traden op in 10% van de patiënten, alle oppervlakkige mucosale laceraties, waarvan geen aanvullende behandeling vereiste. Deze grootste prospectieve multicenter studie, met een unieke training set-up en continue kwaliteitscontrole gedurende de gehele studie periode, suggereert dat RFA in combinatie met ER erg effectief en veilig is voor de behandeling van BO met vroege neoplasie, indien verricht door getrainde endoscopisten in goed geselecteerde patiënten.

Na RFA oogt het neosquameuze epitheel endoscopisch volledig normaal. Om te evalueren of het neosquameuze epitheel endoscopisch volledig normaal is, werden 169 patiënten met vroege neoplasie in BO effectief en veilig behandeld kan worden met RFA, al dan niet in combinatie met ER voor zichtbare afwijkingen.
wordt beschreven in hoofdstuk 10. Allereerst hebben we beoordeeld of RFA preëxistente genetische afwijkingen in het neoplastische Barrett segment eradiceert. Hiervoor hebben wij bipten en borstelcytologie verkregen van de BO voor RFA en van het neosquameuze epitheel post-RFA, in 22 patiënten. Deze samples werden gebruikt voor fluorescente in-situ hybridisatie (FISH) en immunohistochemische kleuring. Hierbij vonden wij dat alle pre-RFA samples genetische afwijkingen bevatten, terwijl alle post-RFA samples normaal waren. Ten tweede hebben wij geanalyseerd of de biopsie diepe van bipten van neosquameuze epitheel post-RFA even diep gaan als bipten van onbehandeld plaveiselepithel in de slokdarm. Lamina propria werd verkregen in 37% van de bipten post-RFA, wat vergelijkbaar was met de 36% in bipten van onbehandeld plaveiselepithel in de slokdarm. Ten derde wilden wij de aanwezigheid van ‘buried Barrett’s’ in het neosquameuze epithel analyseren. Hiervoor werden vierkadrants bipten verkregen elke 2cm van het neosquameuze epithel. Om een nog dieper weefselmonster te verkrijgen werden er ook ‘sleutelgat’ bipten genomen uit elke biopsie opening. Bovendien werd er een ER verricht van neosquameuze epithel. In geen enkel biop, sleutelgatbiop of ER-preparaat werd ‘buried Barrett’s’ gevonden. Samenvattend toont deze studie dat genetische afwijkingen die worden gevonden in de BO voor RFA, compleet afwezig zijn in het post-RFA neosquameuze epithel. Bovendien vonden wij geen verschil in biopsie diepte tussen post-RFA en preëxistent plaveiselepithel in de slokdarm. Als laatste werden er geen ‘buried Barrett’s’ gevonden in geen enkel weefsel preparaat.

Alle studies in dit proefschrift laten zien dat ‘buried Barrett’s’ na RFA zeer zelden zijn. In onze ervaring wordt bij histologische beoordeling van bipten van kleine eilandjes Barrett epithel echter af en toe de aanwezigheid van ‘buried Barrett’s’ verslagen, ondanks dat deze eilandjes endoscopisch zichtbaar waren. In hoofdstuk 11 wordt de aanwezigheid van ‘buried Barrett’s’ in bipten van zichtbare eilandjes Barrett epithel vergeleken met bipten van endoscopisch normaal neosquameuze epithel. In 2,515 bipten van endoscopisch normaal neosquameuze epithel werd ‘buried Barrett’s’ gevonden in 0.1% van de bipten. In bipten van kleine eilandjes met cilindrisch epithel werd echter ‘buried Barrett’s’ beschreven in 21% van de bipten. Deze studie concludeert dan ook dat bipten van kleine eilandjes resterend Barrett epithel moeten worden voorkomen door goede endoscopische inspectie voor het nemen van bipten tijdens follow-up na RFA, om een fout positieve histologische diagnose van ‘buried Barrett’s’ te voorkomen.
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— Sir Isaac Newton
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