Endoscopic eradication of Barrett's oesophagus with early neoplasia
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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, with the lowest rates detected after radiofrequency ablation (RFA) (0-5.4%). 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. 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.

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.

RFA treatment protocol
As described in detail previously, 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
[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 lines 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).

Figure 1. Histological diagnosis of buried Barrett’s after an accidental biopsy of a small Barrett’s island. A, B: Antegrade view on a C9M10 BO, with early cancer, and a proximal stenosis. C: Follow-up 6 months after ER and 2 RFA sessions, showed an oesophagus covered with neosquamous epithelium. D: One biopsy, obtained at the upper end of the initial BO, showed buried glands. E, F: During repeat endoscopy with NBI, a 0.5x3 mm island of columnar epithelium was detected at the location of the biopsy with buried Barrett’s. G: The island was removed by focal endoscopic resection, which did not show any (subsquamous) glands. H: At following endoscopies with biopsies, no more buried Barrett’s were detected.

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).
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 48/69 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 biopsied, 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.9,10 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.11 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.18,19 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.”21 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 endoscopy does 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.22 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 (≤5mm).

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.7 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. Our experience with RFA for treatment of BO and associated neoplasia, and the buried glands, as demonstrated in one of our patients.

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
