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Frozen Section Analysis of Esophageal Endoscopic Mucosal Resection Specimens in the Real-Time Management of Barrett's Esophagus

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Abstract

Background & Aims—The aim of this study was to assess the validity of frozen section analysis of endoscopic mucosal resection (EMR) specimens from Barrett's esophagus as compared with permanent sections for the detection of neoplasia. Frozen sections help to give immediate feedback for surgical procedures. It has not been determined whether EMR can be adequately interpreted by using frozen sections to aid endoscopists in completely resecting neoplastic lesions.

Methods—EMR specimens from Barrett's esophagus with high-grade dysplasia (HGD) and/or carcinoma were tested by frozen section. Pathologists evaluated EMR specimens for the depth of invasion as well as the appearance of clear margins of resection. The κ statistic was calculated to assess the degree of agreement between the frozen section and permanent section diagnoses.

Results—Twenty-three consecutive patients underwent 30 EMRs with frozen section diagnosis. Frozen section revealed a carcinoma in 7 specimens (23%) and dysplasia in 20 (66%). Permanent sections found carcinoma in 8 specimens (26%), dysplasia in 19 specimens (63%), and normal or nondysplastic Barrett's esophagus in the remainder. The κ statistic for the depth of invasion of EMR specimens was 0.93 (near perfect agreement). The κ statistic for the margins of the EMR specimens was 0.80 (excellent agreement)

Conclusions—This study indicated that frozen section analysis of esophageal EMR specimens is valid as compared with permanent section. This technique might allow rapid evaluation about the degree and depth of involvement of cancers. This allows physicians to make decisions regarding further therapy if margins are involved or decrease the use of EMR for histologically benign-appearing lesions.

Esophageal adenocarcinoma is the most rapidly increasing cancer in white men in the United States.¹ Esophagectomy is the current recommended therapy for treatment of esophageal cancer. The high mortality and morbidity associated with esophagectomy have led to an interest in developing new endoscopic therapies that have potentially lower morbidity rates.² Endoscopic therapeutic options for esophageal cancer include endoscopic mucosal resection (EMR), thermal ablation, photodynamic therapy, and combined modality treatments.^{3,4} EMR is the only one of these treatments that allows precise staging of tumor invasion by using

histologic criteria. In addition, this therapy could be therapeutic if the tumor is confined to the mucosa and clear margins of resection are obtained. Mucosally confined lesions have been shown to have a very low rate of lymph nodal spread, making them suitable for endoscopic treatment.^{5,6} New endoscopic techniques and equipment are being developed in an attempt to achieve en bloc or complete resection of neoplastic lesions, as gastroenterologists develop new methods to determine completeness of resection. If this were known in a timely fashion, unnecessary mucosal resections could be avoided, and more aggressive resections could be done in situations in which peripheral margins are involved.

Methods

Twenty-three consecutive patients referred to the Barrett's Esophagus Unit at the Mayo Clinic, Rochester, Minnesota, with a diagnosis of high-grade dysplasia (HGD) and/or esophageal adenocarcinoma underwent upper endoscopy with endoscopic ultrasound. All endoscopically apparent abnormalities or regions that had been previously identified as containing HGD were targeted for EMR. Before EMR, to allow for a safer resection, an average of 10–15 mL of dilute epinephrine (1:100,000 dilution) solution was injected in the submucosa underneath the lesions that was considered amenable to EMR. EMR was performed by using a commercially available disposable EMR kit, Olympus EMR-001 (Olympus America Inc, Melville, NY). A forward resecting cap was placed at the end of the endoscope. The distal end of the cap has a small ledge where a crescent snare can be placed around the circumference of the cap. The mucosal abnormality is suctioned into the cap, resected with the snare, and removed within the cap (Figure 1).

The EMR specimens were then sent to the frozen section laboratory for processing. On arrival, the deep and lateral mucosal aspects of the specimen were inked to assist in margin evaluation. Then the specimen was serially sectioned along the short axis at approximately 2-mm intervals. This typically resulted in 4–5 separate cross-sections of tissue (Figure 2). A freezing microtome was used for preparing frozen sections. In this technique, the lower half of a tissue section is frozen in water and stained with the monochromatic dye toluidine blue. The upper half remains unfrozen and is thus free of any “freezing artifact.” Both halves are then formalin-fixed and paraffin-embedded for hematoxylin-eosin staining. The frozen sections are moderately thicker than typically prepared paraffin-embedded hematoxylin-eosin-stained sections, measuring 9–10 μm in thickness. Permanent sections were created that were 5 μm thick.

After freezing and staining, the slides were read immediately, and a preliminary report was conveyed to the clinician. The interpretation included specific mention of intestinal metaplasia. If this were found, the pathologist would then assess for the presence or absence of dysplasia or invasive cancer in the specimen and at the cauterized and inked margins (deep and lateral). The remainder of the specimen was then processed for routine histopathology. The histopathology was then interpreted on the permanent sections. All of the final histopathology was verified by an independent gastrointestinal pathologist by using predefined criteria (Figure 3 and Figure 4).

Results from frozen section and permanent section analyses were then correlated by using the κ statistic. All permanent sections were also blindly reviewed by a single reference pathologist. The degree of agreement between the reference pathologist and our clinical pathologists regarding the permanent sections' interpretation was also assessed by the κ statistic. The κ statistic is a chance corrected measure of the degree of agreement between 2 observations and ranges between -1.0 (perfect disagreement) and 1.0 (perfect agreement). Hence if the κ statistic is positive, the observed level of agreement is greater than by chance alone, indicating agreement between the 2 tests. A κ statistic value of zero indicates agreement completely accounted for by chance. A κ statistic value of 0.0 – 0.2 indicates slight agreement, 0.2 – 0.4

indicates fair agreement, 0.4–0.6 indicates moderate agreement, 0.6–0.8 indicates substantial agreement, and 0.8–1.0 indicates near perfect agreement.⁷ Statistical analysis was completed by using the JMP statistical analysis package (JMP version 5.1.2; SAS Institute, Cary, NC).

Results

Twenty-three consecutive patients underwent EMR; 7 patients (30%) had 2 EMRs done. Twenty-one patients (91%) were men, with the mean age of 71 ± 2 years. The referral diagnoses before EMR included HGD in 15 (65%), carcinoma in 7 (30%), and nondysplastic Barrett's esophagus in 1 patient. On upper endoscopy, 18 patients (78%) had presence of a nodule, which was targeted for endoscopic EMR. The remaining patients had either mucosal irregularity (endoscopically apparent areas of mucosal elevation larger than 1 mm) or normal-appearing mucosa.

Endoscopic ultrasound was performed by using the Olympus GF-UM130 (Olympus America Inc) at 7.5 and 12 MHz before EMR in 21 of 23 patients. Endoscopic ultrasound did not identify a lesion (uT0) in 18 patients (67%). Six lesions were staged as uT1b (submucosal invasion), 2 as T2 (invading the muscularis propria), and 1 as T1a (confined to the mucosa). EMR was performed on lesions rated as uT1b and T2 because the lesions appeared to lift with submucosal saline injection.

The dimensions of the specimens were mean length 0.9 cm (± 0.04), mean width 0.7 cm (± 0.03), and mean depth 0.5 cm (± 0.03). There was excellent agreement between frozen section and permanent section diagnoses of carcinoma or dysplasia in the body of the EMR specimens, with a κ statistic of 0.93 (Table 1). A similarly near perfect agreement was seen in terms of permanent section and frozen section margin assessment (Table 2).

The κ statistic for the agreement between frozen section and permanent section in terms of confirming the presence or absence of carcinoma was also high at 0.91 (± 0.10). The κ statistic for the agreement between the reference pathologist (after blinded assessment of the permanent sections) was 0.8, indicating excellent agreement. This would indicate a lack of bias between the interpretation of the frozen and permanent sections. Additional time required for the frozen section analysis ranged from 8–12.5 minutes. No complications were noted in any of the 23 patients after EMR.

Discussion

Endoscopic therapy has become a viable alternative to esophagectomy for early stage cancers or HGD in the setting of Barrett's esophagus. Compared with biopsy specimens, EMR specimens are significantly larger and allow for more precise assessment of depth of tumor invasion into the mucosa and submucosa. However, currently this technique is limited by its inability to define the involvement of the margins of resection by neoplasia at the time of resection. In addition, there are no current techniques that allow this degree of accuracy in rapidly assessing depth of neoplastic involvement. Endoscopic ultrasound does have an advantage of being able to inspect the regional lymph nodes. However, it is not very accurate in determining depth of invasion.^{8,9} Because the concept of EMR is very similar to a surgical resection, it is very important to develop methods of rapid assessment of tissue margins during the procedure. If the lesions are not fully resected during the initial resection, subsequent resection is often associated with increased difficulties because fibrosis and inflammatory change have usually occurred, and the mucosa is much less likely to be removed by a second EMR because of these changes.

Esophagectomy is commonly recommended for therapy of HGD or carcinoma arising in the background of Barrett's esophagus. However, esophagectomy is often not indicated because

the patient population is generally older, and the procedure is associated with a 3%–10% mortality rate and a 40% morbidity rate.¹⁰ EMR was initially popularized in Japan for the treatment of early upper gastric and esophageal cancers. It has become the treatment of choice for early esophageal and gastric cancers so long as the lesions are confined to mucosa (above the muscularis mucosa). Lesions that penetrate below the muscularis mucosa should be treated with esophagectomy, but endoscopic treatment with EMR is not usually recommended for more invasive lesions because of potential metastases in more than 30% of patients.^{11–14}

During the past decade, EMR has gained acceptance in Europe and the United States as an alternative modality for the treatment of neoplastic lesions arising in the background of Barrett's esophagus.^{3,4,15} Currently EMR and other endoluminal treatments for upper gastrointestinal neoplasia are accepted as alternatives for patients who are less than ideal candidates for esophagectomy.¹⁶ The primary advantage of EMR is that it not only treats the lesion, but it also removes large portions of mucosa and submucosa, which allows histologic staging and diagnosis of lesions in Barrett's esophagus.¹⁷ EMR is a relatively safe procedure, but it is still associated with a 15% complication rate. The procedure should not be used in patients for whom it cannot provide obvious clinical benefit.

Even though newer techniques such as chromoendoscopy, magnification endoscopy, narrow band imaging, and autofluorescence imaging have attempted to improve the identification of neoplasia and dysplasia in Barrett's esophagus, none of these techniques is as accurate as histology in finding the presence of dysplasia and the depth of invasion.^{18–21} Initial enthusiasm for these imaging techniques has been followed by less than optimal results in larger series.²² It appears to be very difficult to find smaller areas of dysplasia. In addition, the depth of involvement by tumor is very difficult to assess, even with techniques such as optical coherence tomography.²³

This study establishes the potential role of frozen section analysis of EMR specimens. The ability to rapidly report on results of EMR specimens will enable the application of EMR to more neoplastic lesions and will ensure a greater degree of complete resections. At the current time, margins are only estimated by their endoscopic appearance, which is known to be unreliable.²⁴ A high degree of agreement was seen between results from the body and margins of the EMR specimens obtained from frozen sections and permanent sections. However, permanent section evaluation was not performed in a blinded fashion, so that the high agreement between the frozen and permanent sections could be partially explained by the bias induced by using a single pathologist for both modalities. However, a blinded re-review by our reference pathologist of the permanent sections had excellent agreement with the initial diagnosis, indicating that it would be unlikely that the pathologists altered their diagnosis because of prior knowledge of the frozen section results. The few discrepancies seen appear to be related more to interpretation differences than sampling. The tissue evaluated on frozen section is processed in water (no fixative used), so that it dries within a few days and is unavailable for review. New sections are cut for permanent section evaluation. Although the tissue evaluated on frozen and permanent sections is not the same, we believe the artifact introduced by sampling is minimal because there are only a few micrometers between the levels.

The ability to accurately assess the presence or absence of invasive cancer is especially helpful to provide quick feedback on the need to persist with wider resection at a suspicious site. This proved very helpful in a patient with known intramucosal carcinoma and bland-appearing mucosa on endoscopy. Prompt feedback from frozen sections allowed targeting of other involved sites. The correlation between frozen sections and permanent sections was somewhat lower for assessing the margins. Thicker sections made for frozen sections (10 μ m) compared with permanent section (5 μ m) might have contributed to this difference in interpretation. The

presence of cautery artifact might also have decreased the accuracy of assessment of the margins.

The ability of frozen section analysis to detect deep margins by carcinoma allowed the termination of EMR in patients with invasive carcinoma. It is widely accepted that submucosal invasion is associated with high rates of lymph nodal invasion. Quick detection of submucosal invasion on frozen section can avoid multiple EMRs in a patient that required surgical resection. This can potentially reduce complications from multiple EMRs, which are generally performed in the case of larger lesions, which have a greater potential to have submucosal invasion.

Frozen section examination of EMR specimens can be widely performed because of the presence of frozen section laboratories in a large majority of centers performing surgery. This should be feasible with close collaboration and cooperation between pathologists and endoscopists. It is very important for endoscopists to discuss with their pathologists the nature of the endoscopic resection to establish the need to have interpretations to comment on the margins and the depth of invasion as well as the histologic diagnosis. Accuracy of readings will increase with greater experience. Hence frozen section analysis of EMR specimens can allow more precise targeting of EMR in Barrett's esophagus and avoid multiple EMRs in patients in whom surgery is the recommended treatment. This capability will also be very helpful when en block resections of large neoplastic lesions are performed. In this situation, it would be imperative to be certain that the margins are free from neoplastic involvement. In these larger lesions, the application of this technique could be seen in initially performing a diagnostic mucosal resection in the area with the greatest depth of penetration as identified by endoscopic ultrasound. The confirmation of depth of resection by EMR would establish whether the lesion is potentially resectable. The remainder of the lesion could then be removed in a piecemeal fashion, with the margins and further depth assessment done by frozen section. Application of markers by using electrocautery "markers" at the periphery of the EMR specimen will help to better orient the pathologist for margin assessment, allowing additional resections along positive margins.

In conclusion, frozen section analysis of esophageal EMR specimens appears to be feasible and accurate, when compared with final permanent section histopathology. It has the potential to help achieve complete resection of neoplastic lesions arising in Barrett's esophagus and avoid resections in lesions not amenable to curative endoscopic therapy.

Abbreviations used in this paper

EMR, endoscopic mucosal resection; HGD, high-grade dysplasia.

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Figure 1.
EMR procedure: visible nodule in a background of Barrett's esophagus, which is lifted with a submucosal saline-epinephrine injection. The lesion is then resected with a pre-loaded snare by using suction and electrocautery.

Figure 2.
EMR specimen serially sectioned after the margins have been inked.

Figure 3.

(A) Survey view of a frozen section EMR. Stratified squamous epithelium is seen at top. Arrow highlights an esophageal submucosal mucus gland within the frozen section (original magnification 10×; toluidine blue). (B) Infiltrating malignant glands of invasive adenocarcinoma as seen in a frozen EMR (original magnification 100×; toluidine blue). (C) Frozen section EMR showing submucosal extension of the carcinoma, which is also present at the deep margin (original magnification 40×; toluidine blue).

Figure 4. High-power views of a frozen section (A) and corresponding permanent section (B) from an EMR with low-grade dysplasia. The surface epithelium and underlying glands from both show nuclear hyperchromasia and stratification. Normal polarity with respect to the basement membrane is preserved. (A) Toluidine blue; original magnification 200 $\times$. (B) Hematoxylin-eosin; original magnification 200 $\times$.

Table 1

Results of Frozen Sections/Permanent Sections for Body of EMR Specimens

	Carcinoma	Dysplasia	Non dysplastic Barrett's esophagus/ squamous
Frozen section	7	20	3
Permanent section	8	19	3

Kappa statistic, 0.93 (standard error, 0.06).

Table 2

Results of Frozen Sections/Permanent Sections for Margins of EMR Specimens

	Carcinoma	Dysplasia	Non dysplastic Barrett's esophagus/ squamous
Frozen section	4	11	14
Permanent section	4	10	16

Kappa statistic, 0.8 (standard error, 0.09).

Information on margins was not available on one specimen on frozen section.