Optical coherence tomography for the staging of tumor infiltration in superficial esophageal squamous cell carcinoma

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**Background:** Optical coherence tomography (OCT) is a noninvasive technology that can produce high-resolution cross-sectional images in real-time without acoustic coupling, enabling precise assessment of tumor invasion in superficial esophageal squamous cell carcinomas (SESCCs).

**Objective:** To elucidate the usefulness of in vivo OCT for the staging of SESCCs.

**Design:** A single-center, prospective study in 2 phases: phase I to establish the OCT criteria classified into 3 categories (epithelium or lamina propria mucosa [EP/LPM], muscularis mucosa [MM], submucosa [SM]) and phase II to evaluate these criteria.

**Setting:** An academic medical center.

**Patients:** Sixty-two patients with a histological diagnosis of SESCC by routine endoscopy. In the phase I study, 35 images from 16 patients were used. In the phase II study, 109 images from 46 subsequent consecutive patients enrolled from January 2007 to May 2009 were used.

**Interventions:** We performed OCT for preoperative staging followed by endoscopic submucosal dissection or a surgical procedure and compared precisely the visualized OCT sites with the corresponding tissue sections.

**Main Outcome Measurements:** The accuracy of OCT for the staging.

**Results:** The overall accuracy rate was 92.7% (EP/LPM, 94.9%; MM, 85.0%; SM, 90.9%). The OCT signal penetration depth was sufficient to depict the boundary of the deepest region of cancer, the thickness of which was less than 1.5 mm.

**Limitations:** The small number of patients.

**Conclusions:** To our knowledge, this is the first study demonstrating that OCT might be useful for the preoperative staging of SESCCs with a high degree of accuracy. (Gastrointest Endosc 2010;71:899-906.)
As previously reported, EUS has been suggested as a useful method for the evaluation and staging of tumor infiltration in SESCCs,5 but the accuracy of EUS has not yet been satisfactory because of the poor resolution and the limited depiction despite an appropriate coupler design in clinical practice.5

Optical coherence tomography (OCT) is a noninvasive optical imaging technology that produces high-resolution and cross-sectional images of biological tissue in real time. Although the physical principle of OCT is analogous to that of B-mode US, it uses infrared light instead of acoustic energy. The axial resolution of OCT is 10 μm, which is much higher than that of EUS, the resolution of which is more than 100 μm.8 Both in vitro and in vivo OCT images can identify structures on a microscopic scale, such as mucosal layers, pit and gland morphology, and glandular structures;5 and recently OCT images have been called optical biopsy specimens. OCT might allow more accurate diagnostic assessment of the depth of tumor invasion in SESCCs, but no appropriate study has been performed.

We demonstrated that the 5-layered architecture imaged by OCT corresponded to the histologically normal esophageal wall components by using resected specimens of pig esophagus.6 The wall layers consisted of layer I, a small hyporeflective layer; layer II, a hyperreflective layer; layer III, a hyporeflective layer; layer IV, a hyperreflective layer; and layer V, a hyporeflective layer from the luminal surface to the outside, corresponding to squamous epithelium, lamina propria mucosa, muscularis mucosa, submucosa, and muscularis propria, respectively.

The goal of this study was to establish OCT image criteria for staging tumor infiltration in SESCCs and to evaluate prospectively these criteria to further improve the staging accuracy of SESCCs.

MATERIALS AND METHODS

Patients

Sixty-six patients with SESCC at our institution were enrolled. All patients had received a diagnosis of esophageal squamous cell carcinoma histologically by previous endoscopic biopsy. After the staging procedure by OCT, patients underwent endoscopic submucosal dissection or surgical resection.

Exclusion criteria for this study were to have received a previous histological diagnosis of esophageal dysplasia by endoscopic biopsy and to have had any previous treatment for esophageal diseases. Under the effect of radiation fibrosis or inflammation, the cellularity of the lamina propria is markedly increased along with the collagen content, creating increased optical turbidity that results in increased backscattering.7 Three patients treated with chemoradiotherapy and 1 patient treated with microwave ablation therapy were excluded. Two patients had 2 concomitant SESCC lesions and 1 patient had 3 such concomitant lesions.

A total of 144 OCT images from 62 patients were acquired in this study. Thirty-five OCT images from 16 patients were evaluated to establish the OCT image criteria in a phase I study, and 109 images from 46 subsequent consecutive patients were used for prospective evaluation in a phase II study from January 2007 to May 2009. The detailed characteristics of the materials are shown in Table 1. The macroscopic type of tumor was classified according to the Japanese classification of early carcinomas (0-I, polypoid; 0-IIa, elevated; 0-IIb, flat; 0-IIc, depressed) and a combination of these types (complex type).8 There were no significant differences in the characteristics of the materials among the phase I and II groups. Informed consent for both endoscopy and participation in this study was obtained from all patients. This study protocol was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of Tohoku University Graduate School of Medicine (2005-53, 2008-7), and all patients gave written informed consent before enrollment. No complications occurred in any patients during this study.

Instrument specifications

We used a prototype OCT system developed by Light Lab Imaging (Boston, Mass) and HOYA (Tokyo, Japan). As described previously,6,9 the OCT images were obtained by using a superluminescent diode light source with a center wavelength of 1300 nm, a bandwidth of 70 nm, and power output of 10 mW, resulting in a 11-μm axial image resolution. The focal point was 3 mm from the catheter outer sheath, and the transverse resolution was 30 μm. By scanning the interrogating beam across the tissue surface, a series of tomograms was obtained and used to construct a 2-dimensional image. We used a 1.5-mm diameter OCT probe providing a 360-degree scan perpendicular to the probe. OCT images were automatically acquired with dimensions of 4-mm length and 1.5-mm depth at a rate of 4 frames per second and were typically displayed in gray
In our system, these images were configured to represent hyperreflective signals as white and hyporeflective signals as black.

**OCT procedure**

The enrolled patients underwent routine endoscopy (GIF-H260; Olympus Optical Co, Ltd, Tokyo, Japan) followed by the OCT procedure. The OCT probe was inserted through the accessory channel under direct endoscopic observation, and every part of the lesion that was detected as a key to the tumor staging under the endoscopic observation was scanned by the OCT probe. Based on the set criteria, we scanned every site that was flat and reddish, protruded (granular, nodular, polypoid), and depressed. The OCT examination added 10 to 25 seconds per site examined. The OCT examination was performed by 1 of 2 experienced endoscopists (K.U., S.Y.) who had more than 6 months of experience with OCT before the study started. As previously mentioned, the quality of the OCT imaging could be maintained by keeping the probe placed lightly or firmly on the wall of the esophagus to maintain mechanical stability and minimize motion artifacts. Under direct endoscopic observation, we also took care when placing the probe so that the OCT imaging plane was perpendicular to the esophageal wall for precise correlation of OCT images with microscopic tissue sections. All images of both the OCT examination and the synchronized endoscopic examination were recorded digitally and individually as real-time video sequences. In addition, the representative OCT images were recorded from each site as still images for future review at the clinical imaging.

**Histological evaluation**

After the staging procedure was completed, patients underwent endoscopic submucosal dissection or surgical procedure. The resected specimens were fixed in 10% buffered formalin for 24 hours and cut into tissue blocks at the position and direction corresponding to the OCT imaging. The position and direction were carefully confirmed by reviewing the simultaneously recorded videos of both the OCT examination and endoscopic examination. These blocks were processed for standard paraffin embedding and sectioned into 5-μm thick slices followed by hematoxylin and eosin staining. According to the guidelines, the histological staging of the infiltration was determined by an experienced pathologist (A.I.) who was blinded to the preoperative staging in the phase II study, which was as follows: confined to epithelium (EP), invading lamina propria mucosa (LPM), invading muscularis mucosa (MM), invading submucosa (SM).

**Establishment of OCT image criteria (phase I study)**

After the representative OCT images from the first 16 patients were evaluated by gastroenterologists (K.U., S.Y., W.H.) and a pathologist (A.I.), the in vivo OCT imaging of a normal esophagus wall was identified (Fig. 1) and the depths of the tumor infiltration in the SESCCs were classified into 3 categories based on the guidelines: clinical EP/LPM, clinical MM, and clinical SM. Then the criteria (Fig. 2) were defined as follows.

- **Clinical EP/LPM**: The thickness of layer I or normal layer I with regular interfacial signal of layer II (Fig. 3) or involvement of the tumor signal into layer II without involvement of layer III (Fig. 4)
- **Clinical MM**: Involvement of the tumor signal into layer III with regular interfacial signal of layer IV (Fig. 5)
- **Clinical SM**: Destruction of layers I to III and irregular interfacial signal of layer IV (Fig. 6) or loss of layer V architecture by high backscattering (Fig. 7)
Evaluation of OCT image criteria (phase II study)

A prospective study of the accuracy of the criteria in 46 subsequent consecutive patients was performed from January 2007 to May 2009. The OCT images were reviewed by one gastroenterologist (W.H.) who was blinded to the endoscopic evaluation and information of the patients, and his assessment of the staging was recorded by a standardized questionnaire. This investigator had assessed the OCT image of the esophageal wall with reference to the histological structure in our previous study6 and had accumulated sufficient experience with OCT from 2005. Then, the accuracy of the OCT criteria was investigated with a comparison with the histological evaluation of the resected specimens.

OCT signal penetration depth in SESCCs

In the phase II study, to investigate the OCT signal penetration depth, we evaluated the distance from the surface to the deepest region of the tumor in the histological section that corresponded to the OCT imaging and compared the signal penetration depth between the 2 groups, the visible group and the invisible group, from the point of view of whether the obvious 5-layered architecture and the identification of the deepest region of the tumor were demonstrated.

Statistical analyses

Continuous variables were expressed as the mean (range), and nominal variables were expressed as the frequency. Continuous variables were compared between the phase I and II study groups by using the Mann-Whitney U test. To compare proportions, we used a χ² test or, if appropriate, the Fisher exact test. A P value < .05 was considered statistically significant for each test.

RESULTS

Correlation of OCT image and histology

The in vivo OCT imaging of normal esophageal wall was similar to the corresponding histological findings (Fig. 1). OCT images of SESCCs were depicted as a small hyporeflective component like the normal layer I, so it was difficult to distinguish between the EP cancer and the normal esophageal tissue by OCT. However, OCT could visualize SESCCs that invaded deeper than LPM (Figs. 3-7) and defined the criteria of OCT imaging (Fig. 2). In particular, OCT depicted the hyperreflective microstructure in the muscularis mucosa (Fig. 5) or the slight invasion of the submucosal layer (Fig. 6), which enabled the exact assessment of tumor staging.

Prospective study

Correlations between OCT image criteria and histological results are shown in Table 2. The overall accuracy of the diagnosis of the depth of the cancer invasion was 92.7% (101/109), and the accuracy of EP/LPM, MM, and SM cancer was 94.7% (74/78), 85.0% (17/20), and 90.9% (10/11), respectively. We also investigated the accuracy in relation to the tumor locations. As a result, the accuracy in the proximal, middle, and distal part of the esophagus was 100% (9/9), 89.8% (53/59), and 95.1% (39/41), respectively (Table 3). The accuracy among the anatomical locations was not significantly different (P = .79).

OCT signal penetration depth in SESCC

The thickness of the lesion in the visible group (0.46 mm [range 0.10-1.5 mm], n = 100) was significantly thinner than that of the invisible group (2.5 mm [range 1.2-5.0 mm], n = 9) (Fig. 8) (P < .001). Although the thickness of all lesions in the visible group was less than 1.5 mm, that in the invisible group was more than 1.5 mm. However, 1
lesion less than 1.5 mm was invisible because of the backscattering from hyperkeratosis.

**DISCUSSION**

In this study, we defined the criteria for OCT imaging for staging tumor invasion based on the esophageal cancer treatment guidelines and found that OCT could be a novel technology with a high degree of accuracy for the preoperative staging of SESCCs.

The advantages of the OCT are the high-resolution image, and less need for acoustic coupling.

Regarding the first advantage, in view of the high-resolution images whose axial resolution allowed 10 μm, being approximately 10 times more precise than that of EUS, previous studies demonstrated the usefulness and efficacy of OCT in general clinical use. However, little was known about the usefulness of OCT for staging esophageal squamous cell carcinoma. In this study, most OCT images were depicted as precise wall components that were analogous to the histological findings of a loupe, and the overall accuracy rate was 92.7%, mainly because of the high-resolution imaging technology. In detail, the accuracy rate of the preoperative staging for EP/LPM, MM, and SM cancers was 94.9%, 85.0%, and 90.9%, respectively. Although the accuracy for MM cancers was less than that for EP/LPM and SM cancers, the accuracy rate was much higher than that of the study with high-resolution EUS (60%). According to the study of Murata et al using catheter probe EUS, the overall accuracy rate was 87% and the accuracy rate for EP/LPM and SM cancers was 81% and 87%, respectively, each of which was lower than the accuracy of OCT in this study. In other words, although EUS provided relatively low resolution and, in particular, the...
suboptimal delineation of the mucosa and submucosa, OCT could depict useful images close to microscopic resolution, which might provide more useful information for the management of SESCCs.

The second advantage of OCT is that there is less need for acoustic coupling with tissue as in cases of EUS. Although the previous study found that the application of a biocompatible chemical agent might enhance the OCT signal penetration depth, we could depict the exact structure regardless the tumor location without any acoustic coupling. Indeed, the accuracy of EUS was lower in the distal part of esophagus, where it was difficult to achieve adequate water preparation. Moreover, EUS with a water-filled balloon, which might be developed to resolve this problem, had another problem in that the interposition or the compression by the balloon might interfere with the EUS images. However, we showed that the accuracy of the OCT images was not significantly different among the tumor locations, so OCT might be a clinically useful advance in endoscopic tissue imaging.

Conversely, there are 3 limitations of this modality: (1) the depth of the penetration, (2) the inability to distinguish between cancer cell invasion and inflammatory cell infiltration, and (3) the inability to distinguish between the EP cancer and the normal esophageal tissue. Five of 32 clinical MM or clinical SM sites might be misinterpreted only by the OCT image because of these limitations. In 1 case of EP/LPM cancer, in which the thickness was more than 1.5 mm, the OCT image showed the loss of the 5-layered architecture, resulting in the misinterpretation as clinical SM. In another case of MM cancer misinterpreted as clinical SM, OCT imaging also showed the loss of the 5-layered architecture because of hyperkeratosis. In the other case of EP/LPM cancer, the muscularis mucosa appeared to be split into 2 layers, which caused misinterpretation as clinical MM. In 2 cases, we could not distinguish between inflammatory cells and cancer cells, which caused misinterpretations.

In all cases, we placed the OCT probe lightly or firmly on the target tissue, resulting in better quality of the OCT imaging and minimizing poor-quality images caused by
motion artifact. This method was supported by a previous study showing that, as the pressure increased, the tissue stretched around the probe and the layer thickness decreased, with the result that OCT had a certain fixed penetration depth and a deeper region became visible. However, we could not always visualize the mucosal and submucosal architecture by OCT, mainly because of the insufficient light penetration through thick lesions or the scattering from hypercellular tissues. These invisible lesions might have increased backscattering at the surface or cytological features such as an increased nucleus-to-cytoplasm ratio, which might alter light scattering. As previously mentioned, the OCT image penetration depth has been as great as 2 mm, depending on the tissue structure, depth of focus of the probe, and pressure applied to the tissue surface. For example, the maximal signal penetration in normal and pathological tissue of the larynx was 0.81 mm and 0.65 mm, respectively. In this study, the maximal signal penetration in SESCCs was 1.5 mm (Fig. 8), deeper than in previous investigations. However, the ability of OCT to precisely identify the layers of mucosa and submucosa could have important clinical implications in the management of SESCCs because SESCCs generally originate in the epithelium.

**Table 2.** The accuracy of the preoperative staging by OCT imaging compared with the corresponding histological findings

<table>
<thead>
<tr>
<th>Histological results</th>
<th>OCT results</th>
<th>Clinical EP/LPM</th>
<th>Clinical MM</th>
<th>Clinical SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP/LPM</td>
<td>74</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>2</td>
<td>17</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

OCT, Optical coherence tomography; EP/LPM, confined to epithelium or lamina propria mucosa; MM, invading muscularis mucosa; SM, invading submucosa.

**Table 3.** The differences in the rate of accuracy for the OCT imaging according to the locations of superficial esophageal squamous cell carcinomas

<table>
<thead>
<tr>
<th>Location</th>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td>Distal</td>
<td>39</td>
<td>2</td>
</tr>
</tbody>
</table>

OCT, Optical coherence tomography.

**Figure 7.** Another typical case of tumor invasion in SM cancer. A, OCT image of the loss of a 5-layered architecture (bar = 1000 μm). B, Corresponding histological finding (H&E, orig. mag. ×80).

**Figure 8.** The penetration depth of the OCT signal in SESCCs. The OCT signal could penetrate the cancer tissue enough to visualize the boundary between the deepest region of tumor invasion and normal tissue, when the thickness of the lesion was less than 1.5 mm.
Second, we could not identify any differences in the subcellular details such as the presence of nuclear abnormalities, which are important for distinguishing between inflammatory cells and cancer cells. In 2 EP/LPM cases, these OCT images showed the loss of layers I to III without change in the underlying layer IV, which was misdiagnosed as clinical MM, but the histological examination demonstrated the component of the inflammatory hypercellularity in the lamina propria.

Third, it was difficult to distinguish between EP cancer and the normal esophageal tissue because we could not detect any obvious differences among normal, inflammatory, and cancer tissue in the squamous epithelium layer. However, the recent study demonstrated that OCT could be useful for the differentiation of neoplastic and non-neoplastic tissue, especially in the pancreaticobiliary duct system.

Therefore, further development of OCT technology in terms of the signal penetration depth, the resolution such as ultrahigh-resolution OCT, and the functional imaging such as Doppler OCT will overcome these problems, and we should assess synthetically not only by OCT but by other endoscopic modalities. Then, OCT might be a more useful method as the diagnostic assessment for the standard guideline of ER in SESCCs.

In conclusion, to our knowledge, this study is the first to show that OCT might allow a precise assessment of the depth of tumor infiltration in SESCCs, providing a high degree of accuracy for staging, suggesting that it could be a powerful diagnostic modality for the management of SESCCs. Although future trials with larger numbers of subjects might be warranted, OCT could be a novel technology for the diagnostic assessment of the adequate treatment of SESCCs.

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REFERENCES