Confocal laser endomicroscopy for superficial esophageal squamous cell carcinoma

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Background and study aims: Confocal laser endomicroscopy (CLE) allows subsurface imaging of gastrointestinal mucosa in vivo. The goal of the present study was to compare the endomicroscopic characteristics of cells and intrapapillary capillary loops (IPCLs) in normal and superficial esophageal squamous cell carcinoma (SESC).
Patients and methods: We recruited consecutive patients with SESC diagnosed by conventional endoscopy and confirmed by histopathology between July 2006 and May 2008. The confocal endoscopic images of these patients were collected and compared with the corresponding histology. The characteristic patterns of cells and IPCLs was then analyzed from these images of malignant and normal mucosa. The quality of images and interobserver variations of two endoscopists were also evaluated.
Results: Overall, 64 samples from 57 subjects (27 SESCs, 30 controls) were examined by CLE. The confocal images corresponded to the hematoxylin and eosin staining from the same sites. The confocal images showed that there was a significantly higher proportion of squamous epithelial cells with irregular arrangement (79.4% vs. 10.0%, P < 0.001), increased diameter of IPCLs (26.0μm vs. 19.2μm, P < 0.001), and irregular shape IPCLs (82.4% vs. 36.7%, $P = 0.0002$) in the SESC group compared with the controls. Massive IPCLs with tortuous vessels (44.1% vs. 0%, $P < 0.0001$), and long branching IPCLs (23.5% vs. 3.3%, $P = 0.0204$) were frequently observed in the SESC group. In this study, about 35.5% of images were graded as good quality, and the interobserver agreement for the prediction of cancerous mucosa was graded as substantial.
Conclusions: CLE can be used to distinguish cancerous from normal epithelium, which gives it potential value for early detection of esophageal carcinoma. The difficulty in obtaining good images in the esophagus by CLE is a latent problem.

Introduction

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Esophageal squamous cell carcinoma is a common cause of digestive cancers all over the world and especially in northern China [1]. Unfortunately, the prognosis for this disease remains poor, and the 5-year survival rate is less than 15% [2]. To date, early detection is still regarded as the best option for improving the prognosis of this disease, and endoscopy is one of the most widely used techniques for early diagnosis.

In the past decades, advances in biomedical optics have led to the improvement of conventional endoscopy for the detection of dysplasia and early cancer in the esophagus. It has been reported that chromoendoscopy and magnifying endoscopy provide numerous details of the esophageal surface. Dyeing techniques, such as Lugol's solution, can further improve the early diagnosis of esophageal cancer [3–5]. Observation with magnifying endoscopy of the microvascular architecture, known as intrapapillary capillary loops (IPCLs), of superficial esophageal carcinoma has proved to be an effective method for the diagnosis of depth of invasion [6,7].

Recently, intensive research has been undertaken to develop new gastrointestinal endoscopy techniques for a precise and even "real-time" endoscopic diagnosis [8,9]. Endocytoscopy and confocal laser endomicroscopy (CLE) stand at the forefront of the novel endoscopic techniques. Endocytoscopy with × 400–1100 magnification has been integrated into regular endoscopes [10–12], and the accuracy of endocytoscopy in differentiating nonmalignant and malignant tissues can be improved to 82% [13].

CLE is a newly developed endoscopic technique. This technique allows not only observation of living cells and tissue but also of the vascular networks of the mucosal layer in the gastrointestinal

tract during ongoing endoscopy [14]. With the 1000-fold magnifying ability, this technique enables visualization of the cells of the esophageal squamous epithelium and IPCLs. Recently, a prospective study showed that squamous cell cancers were diagnosed by CLE with an accuracy of 95% [15]. In addition, the success of CLE in detecting mucosal and vascular patterns of cancerous and noncancerous mucosa in the colon, stomach, and in Barrett's esophagus has been reported [16 – 19].

The subject of this paper is the systematic investigation of the endomicroscopic characteristics of cells and IPCLs on the normal and malignant esophageal mucosa, and the assessment of the potential application of CLE to detect superficial esophageal squamous cell carcinoma (SESC).

Patients and methods
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Patient inclusion and exclusion criteria
The SESC group consisted of consecutive patients with SESC diagnosed by conventional endoscopy or magnifying endoscopy and confirmed by histopathology between July 2006 and May 2008 at Qilu Hospital. Individuals with no symptoms, and confirmed by upper endoscopy served as controls. Patients with lesions of the protruding type were excluded because the distal tip of the endoscope cannot make contact with the mucosa well, which makes it difficult to obtain images for analysis. Patients with acute gastrointestinal bleeding, impaired renal function, jaundice, those who were pregnant or breastfeeding, or who had allergy to fluorescein were also excluded. All of the patients with cancer had indications for endoscopic or surgical resection. The study was approved by the institutional ethical committee of Qilu hospital, and written informed consent was obtained prior to endomicroscopy.

Confocal laser endomicroscopy system
All procedures in this study were performed using a confocal laser endomicroscope (Pentax EC-3870K, Pentax Corp., Tokyo, Japan). The confocal laser endomicroscope consisted of the confocal laser microscope mounted on the distal tip of a conventional video endoscope. The diameter of the distal tip is 12.8 mm. The working channel is free to perform endomicroscopic-guided biopsies or immediate therapy. Control of imaging plane depth of the tissue is achieved using two buttons on the endoscope control body. During laser endoscopy a single line laser delivers an excitation wavelength of 488 nm. Light emanating from the focused point, which is normally produced by fluorescence, is recaptured back into the same optical fiber. The optical slice thickness is 7 μm with a lateral resolution of 0.7 μm. The field of view is 475 × 475 μm. The range of the z-axis was 0 – 250 μm below the surface layer. Confocal images and endoscopic images can be generated at the same time.

Endoscopic procedure
The procedure for the preparation of patients for endomicroscopic examination is similar to that for standard upper endoscopy. First, each patient was given 80 mg dimethylpolysiloxane orally to remove adherent esophageal mucus. Then, standard methods of conscious sedation (e.g. midazolam hydrochloride and meperidine citrate) were used. In addition, 20 mg scopolamine butylbromide was administered intramuscularly 10 minutes before the endoscopy to reduce gastric and esophageal motility. Finally, fluorescein sodium (5 mL of a 10% sterile solution)

was injected intravenously. The gastrointestinal mucosa turned yellow after about 20 seconds. The mucosal surface was washed using 10 mL saline, and three sites in each cancerous lesion were examined using endomicroscopy. Next, histopathologic examination of these three sites was carried out, and the most severe site was selected as representative of the lesion. For each subject in the control group, three normal squamous epithelial sites were also examined: 30 cm and 35 cm from the incisors in the mouth and 1 cm above the gastroesophageal junction. From these three sites, the site located at 35 cm from the incisors was selected as representative of a normal/control subject.

During the examination, the target site for imaging was placed at the lower left corner of the endoscopic view, the confocal lens was placed in contact with the mucosa using a blue laser guide, and gentle pressure was applied, if necessary, to maintain mucosal contact; suction was applied during the active phase of imaging. The position of the focal plane within the specimen was adjusted using the buttons on the control body of the endoscope. By pressing the right button intermittently, digital images from the upper to deeper parts of the epithelial layer were obtained. All images from the above-mentioned sites were stored digitally. A targeted biopsy of the examined areas was performed: the biopsy site was located 5 mm immediately to the left of the "polyp" created by suction. To avoid possible biases or mistakes, all of the lesions were carefully observed by two experienced endoscopists (YQL and TY), who were very familiar with confocal endomicroscopy.

Histopathologic analysis
A targeted biopsy of the examined areas was performed immediately after endomicroscopy. The specimens were fixed with 10% formalin, embedded in paraffin, and sectioned vertically and transversely to facilitate the comparison between histology and confocal images. The sections were then stained with hematoxylin and eosin (H&E) for histopathologic examination. During the study, endoscopic ultrasound (EUS) was routinely performed after endomicroscopy to obtain a rough estimation of the tumor invasion depth. Then, endoscopic mucosal resection (EMR) or surgery was performed; the final diagnosis in the study rested on the histopathologic examination of the resection specimen (either EMR or surgical). Distant metastatic spread was assessed by abdominal and thoracic computed tomography (CT) examination. In this study, the criteria proposed by the World Health Organization and revised Vienna Consensus were used to diagnose the intraepithelial neoplasia and cancer. High-grade dysplasia refers to the entire epithelium being made up of atypical immature cells but without invasion of the lamina propria. High-grade dysplasia is equivalent to carcinoma in situ. Invasive cancer means that the cancer had penetrated the lamina propria, and the muscularis mucosa and submucosa layers had been invaded. The histopathologic images were assessed by two pathologists (JPZ and TGZ), independently and blindly to the endoscopic results, and the diagnoses were confirmed by both.

Evaluation of cells and IPCLs pattern of confocal images
Prior to the start of the study, two known superficial esophageal carcinomas and two normal controls were evaluated in order to classify the endomicroscopic images. Based on correlation with histology, irregularly arranged cells and altered IPCL patterns (caliber, number, shape) were found to be distinguishing characteristics between cancerous and normal tissue. Therefore, the
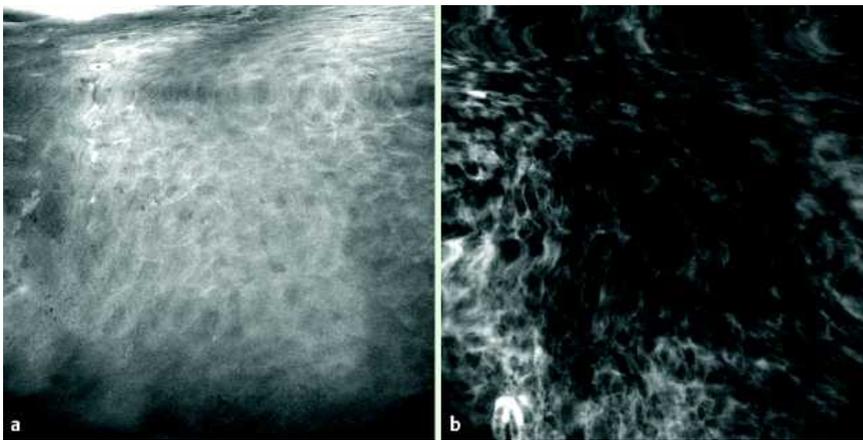
Fig. 1 Endomicroscopic characteristics of esophageal squamous epithelial cells. **a** Homogeneous squamous epithelial cells with regular arrangement. **b** Heterogeneous and darker squamous epithelial cells with irregular arrangement.
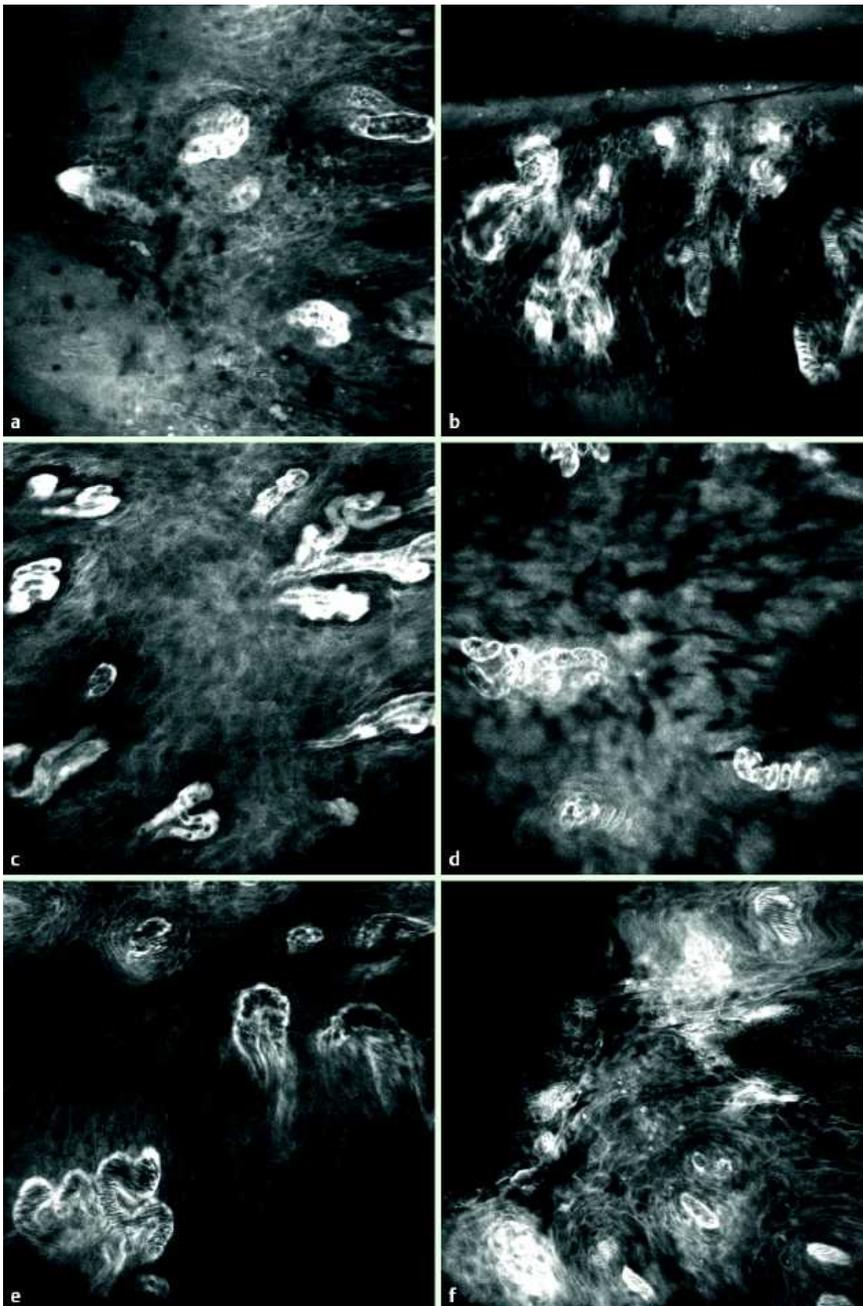
Fig. 2 Endomicroscopic characteristics of irregular intrapapillary capillary loops (IPCLs). **a** IPCLs with increased diameter. **b** Massive IPCLs with tortuous vessels. **c** Long branching IPCLs. **d** Spiral IPCLs. **e, f** IPCLs of different sizes, distributed unevenly.

following cell and IPCL patterns were identified and agreed upon by the investigators for the present study:

1. presence or absence of irregularly arranged squamous epithelial cells (○ **Fig. 1**)
2. caliber of IPCLs: measurement of caliber of IPCLs in the clearest image of each site examined. Three vessels with the largest caliber in every image were selected and the mean value was regarded as the diameter of IPCLs of the site. The vessel diameter was measured using image processing software (AutoCAD, Autodesk, San Rafael, California, USA)
3. number of IPCLs per image, manually counted
4. presence or absence of irregularly shaped IPCLs, such as tortuous, branching, spiral IPCLs (○ **Fig. 2**).

During the present study, all of the confocal endomicroscopic images from each site were stored in a specific folder. An image with cells and an image with IPCLs of each site were selected to be evaluated for cell pattern and IPCL pattern, respectively. The above four features of each area were recorded. All of the selected images were evaluated after the endoscopy by an investigator (YTG) who was blinded to the patient groups.

Evaluation of confocal image quality
The quality of all images was evaluated by an investigator (YTG) who was blinded to the endoscopic and histologic results. The quality of every image was scored as good (no moving artifacts, cells or IPCLs can be identified clearly), average (artifacts present but cells or IPCLs can be recognized), and poor (artifacts do not allow judgment of the image).
In addition, 50 endomicroscopic images of good or average quality were randomly selected. Two endoscopists (YAZ and JNZ), without any information of imaging sites, manually classified the 50 images independently, and predicted whether the tissue was normal or cancerous.

Statistical analysis
All statistical analyses were performed using the statistical software package SAS (9.10; SAS Institute Inc., Cary, North Carolina, USA). The Student's t-test was used for all continuous variables. If there was a significant difference, the receiver operating characteristic (ROC) curves were calculated, and the optimal cut-off value was determined to maximize the sum of sensitivity and specificity. The difference was considered significant when the P-value of two groups was less than 0.05. For the categorical variables, the chi-squared test or Fisher's exact test was used to determine whether there was a difference between the two groups. Sensitivity and specificity were calculated for the prediction of SESC with cellular and IPCL pattern in endomicroscopic images. To examine chance-adjusted agreement, the kappa value along with 95% confidence intervals (CIs) were calculated for interobserver agreement [20]. Strength of agreement was considered as follows: slight (kappa 0.01–0.2), fair (kappa 0.21–0.4), moderate (kappa 0.41–0.6), substantial (kappa 0.61–0.8), almost perfect (kappa 0.81–1.0).

Results
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A total of 27 SESC patients (21-male) and 30 controls were included in the study and no patient was excluded. The patients with SESC had a mean age of 61.1 years (range 45–72 years). The endomicroscopic examination lasted 15–35 minutes for the 57 subjects (mean 24 minutes). All subjects developed a slight yellow coloration of the skin and urine after examination, which disappeared within 24 hours. No severe side effects were observed.

In total, 34 lesions were found in the 27 patients with SESC (one lesion in 20 patients and two lesions in seven patients); all lesions together with 30 normal control sites were examined by confocal endomicroscopy. Among the 34 lesions, seven were located in the upper segment esophagus, 15 in the middle segment, and 12 in the lower segment esophagus. There were 13 lesions of the slightly elevated type, 12 of the flat type, and nine of the slightly depressed type. Lugol's dye spray was applied to the flat-type lesions to determine the extent of the lesion. The results showed that 11 lesions were less than 2 cm, 16 lesions were 2–5 cm, and seven lesions were larger than 5 cm. After examination of the endoscopic or surgical resection specimens, a histologic diagnosis was obtained. The histologic types of the 34 lesions were: 18 with high-grade dysplasia and 16 with invasive cancer. The 16 invasive cancer lesions included nine within the mucosa and seven that had invaded the submucosa layer. None of these 16 invasive cancer lesions showed distant metastatic spread. However, two of the seven lesions with cancer invading the submucosa had lymph node invasion ($T_1N_1M_0$). The remaining lesions had no lymph node invasion ($T_1N_0M_0$). The characteristics of lesions in the SESC group are shown in ○ **Table 1**.

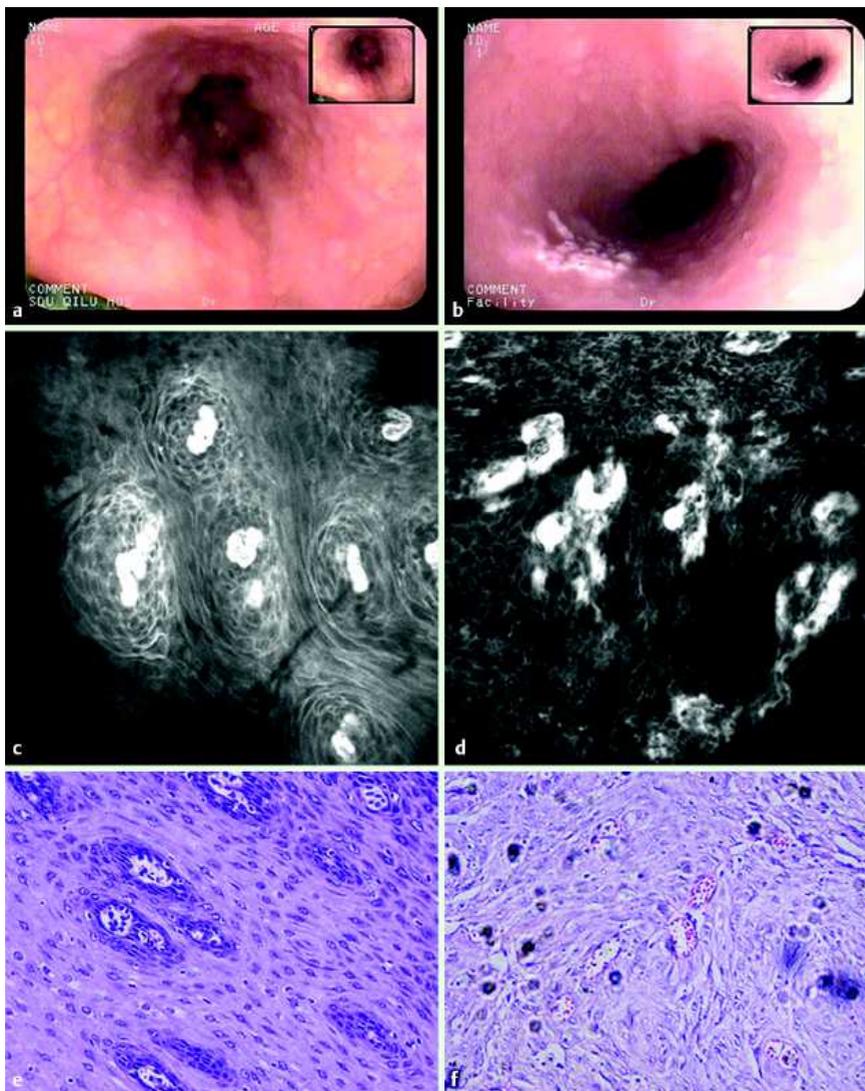### Comparison of endomicroscopic images with histopathology
Confocal images and endoscopic images of normal esophageal mucosa (○ **Fig. 3a**) were generated simultaneously.
In confocal images the squamous epithelial cells have a rhombus appearance with clear borders, and are arranged regularly and homogeneously. IPCLs are directed toward the luminal surface (○ **Fig. 3c**). This observation corresponds well with the H&E (○ **Fig. 3e**) staining of transverse sections of the biopsy specimens from the same site.
A superficial esophageal malignant flat-type lesion was identified by conventional endoscopy (○ **Fig. 3b**). The confocal images from the lesion showed darker esophageal squamous cells with irregular and heterogeneous arrangement. The number, caliber, and shape of the IPCLs were changed in cancerous epithelium (○ **Fig. 3d**). We observed similar phenomena in H&E staining of the same site (○ **Fig. 3f**).

Table 1 Characteristics of lesions in the superficial esophageal squamous cell carcinoma group.

Total number of lesions	34
Location of lesions, n	
Upper segment esophagus	7
Middle segment esophagus	15
Lower segment esophagus	12
Macroendoscopic type, n	
Slightly elevated	13
Flat	12
Slightly depressed	9
Size of lesions, cm	
< 2	11
2–5	16
≥ 5	7
Histological type	
High-grade dysplasia	18
Invasive cancer	16

Fig. 3 Comparison of endoscopic images and endomicroscopic images with histopathology. **a** Normal esophageal mucosa in endoscopic image. **b** Endoscopic image of superficial esophageal squamous cell carcinoma. **c** Confocal endomicroscopic image of normal esophageal squamous epithelium showed regular cells and IPCLs. **d** Confocal endomicroscopic image of superficial esophageal squamous cell carcinoma (SESC) showed irregular and heterogeneous cells and irregular IPCLs. **e** Hematoxylin and eosin staining of transverse section of normal esophageal squamous epithelium. **f** Corresponding histologic specimen with esophageal carcinoma.
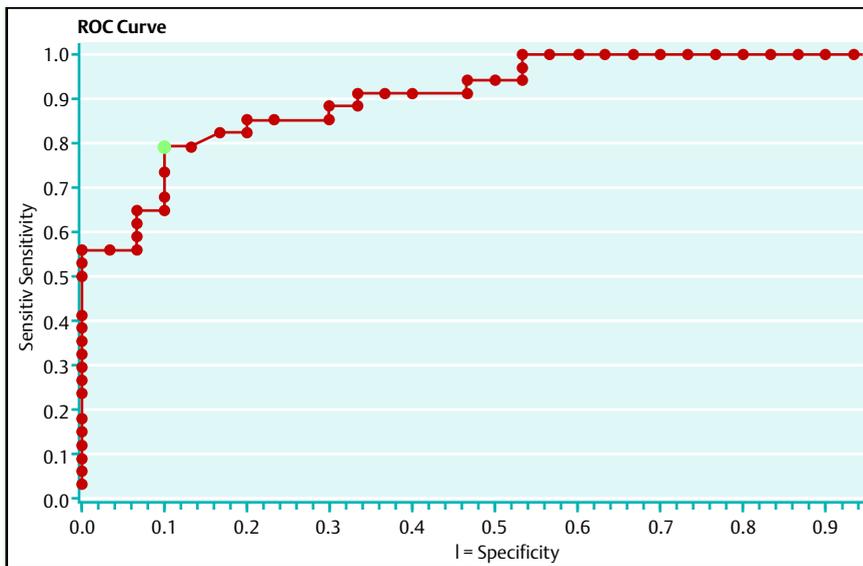
Comparison of confocal endomicroscopic findings in SESC group and controls

We observed that a significantly higher proportion of SESC patients (79.4%) had an irregular arrangement of squamous epithelial cells compared with controls (10.0%, P < 0.001). The sensitivity and specificity of an irregular cellular pattern were 79.4% and 90.0%, respectively. As to the caliber of IPCLs, the mean diameter in the SESC group was 26.0 µm, whereas that of the controls was 19.2 µm. There was a significant difference between the two groups (P < 0.001). Subsequently, the ROC curve was plotted for the mean diameter in SESC patients and controls to determine the cut-off value with the highest sensitivity and specificity (● **Fig. 4**).

The optimal cut-off value between the two groups was evaluated as 21.2 µm. Twenty-seven of the 34 lesions (79.4%) in the SESC group had an IPCL caliber of more than 21.2 µm compared with three of the 30 control samples (10.0%) (P < 0.001). With regard to mean number of IPCLs per field of view, though it seems that there was a decreased number in the SESC group, there was no statistical difference between the two groups (P = 0.073). IPCLs with irregular shapes appear more frequently in SESC patients (28/34, 82.4%) than in controls (11/30, 36.7%; P = 0.0002). These findings and their sensitivity, specificity, positive predic-

tive value, and negative predictive value have been highlighted in ● **Table 2**.

Of the 28 lesions with irregularly shaped IPCLs in the SESC group, 15 showed massive IPCLs with tortuous vessels, eight lesions had long branching IPCLs, four had spiral IPCLs, and one had IPCLs of different sizes that were distributed unevenly. In the 11 samples with irregularly shaped IPCLs in the control group, nine had spiral IPCLs, one had long branching IPCLs, and one had IPCLs distributed unevenly. These observations demonstrate that patients with SESC were more likely to show the presence of massive IPCLs with tortuous vessels (44.1% vs. 0%; P < 0.0001), a finding that was not seen in any of the controls. Similarly, SESC patients were more likely to demonstrate long branching IPCLs (23.5% vs. 3.3%; P = 0.0204). Conversely, a lower rate of SESC patients (11.8%) had spiral IPCLs compared with controls (30.0%; P = 0.0684); however, this difference was not statistically significant. Neither was there a significant difference with regard to IPCLs distributed unevenly between the two groups (P = 0.9283). These findings are shown in ● **Table 3**. Therefore, the irregular arrangement of squamous epithelial cells, increased diameter of IPCLs, massive IPCLs with tortuous vessels, and long branching IPCLs were distinctive features of patients with SESC under confocal endomicroscopy.

Fig. 4 Receiver operating characteristic curve analysis for IPCLs diameter in SESC patients and controls. The green point shows the cut-off value.

Table 2 Comparison of endomicroscopic findings in patients with superficial esophageal squamous cell carcinoma and controls.

Confocal findings	SESC group n = 34	Controls n = 30	P-value	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Irregular arrangement of cells, n (%)	27 (79.4)	3 (10.0)	< 0.001	79.4	90.0	90.0	79.4
IPCLs diameter, μm (mean±SD)	26.0 ± 4.3	19.2 ± 3.9	< 0.001	–	–	–	–
IPCL diameter ≥22.9 μm, n (%)	27 (79.4)	3 (10.0)	< 0.001	79.4	90.0	90.0	79.4
IPCL number (per FOV), (mean ± SD)	4.5 ± 1.0	5.6 ± 1.2	0.073	–	–	–	–
IPCL irregular shape, n (%)	28 (82.4)	11 (36.7)	0.0002	82.4	63.3	71.8	76.0

FOV, field of view; IPCLs, intrapapillary capillary loops; NPV, negative predictive value; PPV, positive predictive value; SESC, superficial esophageal squamous-cell carcinoma.

Sensitivity and specificity of combinations of cellular and IPCL patterns on confocal images

Both cellular pattern and microvascular pattern are visible in most confocal images. Therefore, the combinations of cellular pattern with IPCLs are more significant and practical for the prediction of malignant lesions than single characteristics. We investigated the sensitivity and specificity values for various combinations of endomicroscopic findings for the diagnosis of SESC (⊙ **Table 4**).

⊙ **Table 4** illustrates that abnormal cells or increased diameter of IPCLs had a high sensitivity (94.1%) for predicting SESC. The combinations of abnormal cells and increased diameter of IPCLs, and abnormal cells and tortuous IPCLs both had a specificity of 100% in SESC patients.

Evaluation of confocal images

For the total 64 samples (34 lesions and 30 control sites), 3100 confocal images were obtained, including 1820 images from lesions and 1280 images from controls sites. Of all the images, 35.5% (1100/3100 images) were graded as good quality and 1250 images (40.3%) were average. There were 630 good-quality images (34.6%, 630/1820) of cancerous mucosa and in 470 good-quality images of control mucosa (36.7%, 470/1280). In addition, 25.1% of SESC group images and 15.6% of control images were of poor quality.

The mean kappa value for interobserver agreement for the prediction of cancerous mucosa was 0.670 (95%CI 0.445, 0.895). Interobserver agreement was graded as substantial.

Discussion
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Early diagnosis of esophageal squamous cell carcinoma can improve the prognosis, and several studies have reported on this. For example, capsulated brushing cytology has been applied to screen an asymptomatic population in whom the tumor invasion depth could not be determined; standard endoscopy may detect most superficial esophageal carcinomas but misses the flat-type lesions; Lugol's dye spray chromoendoscopy can detect 100% of high-grade intraepithelial neoplasia [21], but its use has several disadvantages, such as iodine allergy, the absorption of iodine [22], the sensation of esophageal burning, and esophageal spasm [23], even bronchospasm [24].

The most recent innovation in endoscopic imaging can help to visualize tissue at the cellular level. In fact, CLE may provide images of individual epithelial cells and microvascular architecture of the gastrointestinal tract. The present study showed the distinct endomicroscopic characteristics of cells and IPCLs between normal and cancerous esophageal tissue, and investigated the ability of CLE to predict malignant lesions. In the present study, irregularly arranged squamous epithelial cells, increased diameter of IPCLs, massive IPCLs with tortuous vessels, and long branching IPCLs were effective features in distinguishing SESC patients. At the start of the study, we assumed that spiral IPCLs were an irregular pattern representing SESC. However, as the study progressed we found more spiral IPCLs in normal controls than in patients with SESC. We concluded that this microvascular pattern might not be specific for cancerous lesions. Abnormal

Table 3 Comparison of IPCLs with irregular shapes in patients with superficial esophageal squamous cell carcinoma and controls.

	SESC group n = 34	Control n = 30	P-value
Massive IPCLs with tortuous vessels, n (%)	15 (44.1)	0 (0.0)	< 0.0001
Long branching IPCLs, n (%)	8 (23.5)	1 (3.3)	0.0204
Spiral IPCLs, n (%)	4 (11.8)	9 (30.0)	0.0684
IPCLs distributed unevenly, n (%)	1 (2.9)	1 (3.3)	0.9283
Total, n (%)	28 (82.4)	11 (36.7)	0.0002

IPCLs, intrapapillary capillary loops; SESC: superficial esophageal squamous-cell carcinoma.

Table 4 Sensitivity and specificity of various combinations for diagnosing superficial esophageal squamous cell carcinoma.

Confocal findings	Sensitivity, %	Specificity, %
Abnormal cells or increased IPCLs diameter	94.1	73.3
Abnormal cells or tortuous IPCLs	79.4	86.7
Abnormal cells or long branching IPCLs	91.2	83.3
Abnormal cells and increased IPCLs diameter	44.1	100
Abnormal cells and tortuous IPCLs	38.2	100

IPCLs, intrapapillary capillary loops.

cells or increased diameter of IPCLs had a high sensitivity (94.1 %) for predicting SESC. Combinations of abnormal cells and increased diameter of IPCLs, and abnormal cells and tortuous IPCLs had a specificity of 100 % in SESC patients.

It has been reported that cancerous, inflammatory, and normal mucosa could be predicted on the basis of nucleus, cytoplasm, shape, and arrangement of cells by endocytoscopy [12,13,25]. In confocal endomicroscopic images, the shape and arrangement of squamous cells were clearly visible, whereas the nucleus could not easily be identified due to the use of fluorescein sodium as the contrast agent. However, malignant lesions can be predicted with a high sensitivity and specificity by using shape and arrangement of cells only.

IPCLs are the distinct microvascular characteristic of esophageal epithelium. In the normal esophageal mucosa, submucosal vessels that pierce the muscle layer are connected to the arborescent vascular network. Intrapapillary capillaries arise from the fourth branch of the arborescent vessels into the epithelial papillae and form single loops called IPCLs [26]. In this study, IPCLs could be clearly visualized with CLE. The capillaries were much brighter than epithelial cells due to the flow of fluorescein in the vessels. The subtle architecture of microvascular caliber, vessel wall, and even the blood cells can be identified.

In previous studies, IPCLs could be observed using magnifying endoscopy or the narrow-band imaging (NBI) system to predict inflammatory or malignant lesions [27]. Kumagai et al. [28] observed characteristic changes, such as dilatation and elongation, in the IPCLs according to the depth of esophageal tumor invasion. Another study of early esophageal squamous cell cancer revealed different types, including elongated, dilated IPCLs and abnormal tumor vessels [29]. Pech et al. [15] reported that twisted, irregular, and elongated tumor vessels with a larger diameter were characteristic of IPCLs in neoplastic lesions detected by CLE. Other investigators reported that the IPCL diameter ranged from 12.9 µm of the m1 cancer lesions ex vivo [28] to 30 – 42 µm in vivo [29]. In our study, the mean diameter in the SESC group was 26.0 µm in vivo.

Compared with endocytoscopy and magnifying endoscopy, the endomicroscopy technique has great advantages. For example, it can provide detailed inspection for not only squamous epithelial cells but also microvascular patterns. Furthermore, it provides up to 1000-fold magnified imaging immediately in vivo. The alterations of the shape and size of IPCLs can be observed clearly and easily. It avoids the minor error in ex vivo evaluation caused by treating the specimen for microscopic observation.

The advantages of endomicroscopy make it more suitable for the diagnosis of esophageal tumor in the early stage.

We propose the use of endomicroscopy because endomicroscopy emphasizes features of both cells and capillary patterns, which are important for the diagnosis of esophageal tumors in the early stage. However, there are limitations of the present study and the newly developed endoscopy. In our study, we could not compare the endomicroscopic features between intramucosal and submucosal carcinoma due to the small number of cases. This is a significant limitation because the rates of lymph-node metastasis have been shown to be 0 % for mucosal carcinomas and 45 % for submucosal carcinoma of the esophagus [30], and submucosal infiltration is a contraindication for endoscopic mucosectomy. In addition, endomicroscopic image quality cannot be assured because of the presence of moving artifacts caused by heart beat and breath. In this study, only 35.5 % of images were graded as good, which was lower than confocal colonscopy examination [16].

In conclusion, the patterns of squamous cells and IPCL in normal and malignant esophageal lesions could be identified clearly under CLE, which gives the technique potential value for early detection of esophageal carcinoma. However, the difficulty in obtaining good images in the esophagus by CLE is a latent problem.

Acknowledgment
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Competing interests: None

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