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ORIGINAL ARTICLE

Surface maturation scoring for oesophageal squamous intraepithelial neoplasia: a novel diagnostic approach inspired by first endomicroscopic 3-dimensional reconstruction

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ABSTRACT

Objective Loss of surface maturation and cytonuclear atypia have been regarded as the pathological 'gold standard' for the diagnosis of oesophageal squamous cell intraepithelial neoplasia. However, there has been no satisfactory endomicroscopic method similar to this pathological approach to detect surface maturation and screen for oesophageal squamous cell intraepithelial neoplasia. The aim of this study was to apply a 3-dimensional (3D) confocal endomicroscopic imaging technique to investigate the surface maturation of the oesophageal epithelium and develop new 2-dimensional confocal endomicroscopic criteria based on surface maturation.

Design In the 3D reconstruction phase, intrapapillary capillary loops were reconstructed to demonstrate the stereo configuration of the oesophageal epithelium, and a novel surface maturation scoring (SMS) method for plane confocal images was developed based on the interpretation of the 3D microstructure. In the SMS diagnostic phase, 1214 patients were screened and confocal images from 64 non-invasive oesophageal lesions were independently evaluated using SMS and previous methods.

Results We successfully obtained and interpreted 3D confocal images of the human oesophageal epithelium for the first time. The sensitivity (81.0%, 95% CI 58.1% to 94.6%) and specificity (90.7%, 95% CI 77.9% to 97.4%) of the newly established SMS were superior to previous confocal approaches in distinguishing squamous intraepithelial neoplasia from other non-invasive lesions.

Conclusions 3D confocal endomicroscopic imaging provides valuable insight into the stereo configuration of the human oesophageal epithelium. SMS is a novel and promising diagnostic method to distinguish neoplasia during ongoing endoscopy.

INTRODUCTION

Oesophageal cancer is one of the most common causes of cancer-related mortality worldwide,[1] and squamous cell carcinoma is still the predominant type of oesophageal cancer in the Eastern world.[1 2] Since carcinogenesis is a process relevant to angiogenesis,[3 4] most previous endoscopic screening methods, such as magnifying endoscopy, narrow-band imaging and confocal laser endomicroscopy (CLE), have focused on the diagnostic value of microvascular changes in predicting early oesophageal squamous cell carcinoma (ESCC) and oesophageal squamous intraepithelial neoplasia (ESIN).[5–11] Nevertheless, the diagnostic yield of ESIN by microvascular changes, limited by its indirect nature, has not been satisfactory. As a gold diagnostic standard, pathological evaluation of squamous intraepithelial neoplasia is based primarily on the presence of nuclear atypia and loss of normal surface maturation (polarity).[12] Surface maturation is the gradient transition of cellular morphology from the cuboidal basal layers to the flatter surface layers. It is one of the key features of non-neoplastic lesions which distinguishes them from neoplastic lesions. However, there was no endoscopic method similar to this pathological approach for detecting surface maturation and screening for ESIN.

Confocal endomicroscopy is a newly invented diagnostic tool that gives real time optical sectioning images of cellular resolution similar to histology. Conventionally, 2-dimensional (2D) images from optical cut planes are interpreted to predict the pathological results of evaluated lesions. Recently, bench-type confocal microscope, the predecessor of confocal endomicroscope, has been employed to visualise the 3-dimensional (3D) microstructure of the intestine in an animal model.[13 14] However, little is known about whether 3D volume rendering can be performed endoscopically in the human gastrointestinal mucosa.

In this study, we focused on finding superior methods similar to pathological approaches to recognise surface maturation and improve the screening accuracy of ESIN. We interpreted the microstructure of the oesophageal epithelium and intrapapillary capillary loop (IPCL) by 3D endomicroscopic reconstruction for the first time, and developed a 2D image based surface maturation scoring (SMS) method to evaluate surface maturation and explored its diagnostic value for ESIN.

METHODS

Phase I: 3D reconstruction

3D imaging

Eight healthy volunteers were recruited in the 3D imaging trial after informed consent was obtained. The inclusion criterion was healthy adults willing to participate in the 3D imaging experiment. The exclusion criteria included the following:

volunteers allergic to fluorescein and those unable to give informed consent. Confocal endomicroscopic imaging was performed using a Pentax EC-3870CIK confocal laser endomicroscope (Pentax, Tokyo, Japan). Preparation of the recipient for 3D-CLE was the same as in our previous study.[10] Each patient was given 80 mg dimethylpolysiloxane orally to remove adherent oesophageal mucus, and standard methods of conscious sedation (eg, midazolam hydrochloride and meperidine citrate) were used. Fluorescein sodium solution (5 ml) was injected intravenously as a contrast dye before withdrawal of the endoscope from the stomach. At a fixed point of the oesophageal epithelium, confocal images were recorded at continuous depths, with X/Y resolution of 1024×1024 pixels and a z-axis step increment of 7 μm.

Postimage processing

At each endomicroscopically scanned site, about 20–30 continuous depth images were digitally gathered in JPG format, and then converted by Acdsee (V.5.0, ACD Systems International, Inc., Victoria, Canada) to BMP format to prepare for the 3D reconstruction. These converted images were subsequently imported by Mimics (V.10.0.1, Leuven, Belgium), with a pixel size of 0.464 μm and a slice distance of 7 μm. The IPCLs were then semiautomatically masked by the thresholding and mask-editing tools. The 3D configuration of the IPCLs was calculated from the plane mask, and smoothened by the Remesh tool. To facilitate the interpretation of the 3D microstructure, the reconstructed tissue was partially made transparent to expose the reconstructed IPCLs together with the neighbouring tissue (figure 1A). The exposed worm-bitten appearance of reconstructed IPCLs demonstrated many tiny filling defects with diameters ranging from 6 to 12 μm.

Interpretation of 3D images

According to the well-established pathological experience, the epithelial maturation gradient is present in non-neoplastic lesions and absent from neoplastic lesions. The morphological transition of the epithelial cellular gradient from the basement membrane to the superficial layers can be observed in the schematic diagram of the 3D microstructure of the oesophageal epithelium (figure 1B). IPCLs rise obliquely upward in the reconstructed 3D image (figure 1A and supplementary video 1), slightly different from the schematic diagram. This 3D image demonstrates that the focal planes of confocal endomicroscope were oblique to the IPCL axis. Learning the oblique spatial relationship between the IPCL axis and confocal imaging plane, we resliced the oesophageal epithelium with a similar oblique spatial relationship to facilitate comparison between histological and confocal images.

The original plain endomicroscopic images, corresponding histological sections and 3D reconstructed images of the oesophageal epithelium were openly viewed and discussed by three endoscopists (ML, X-LZ and Y-QL) and a pathologist (C-JZ). Four major characteristics of surface maturation could be found in both the histological and confocal images.

First, from the basal layers around the IPCL to the outer layers, cellular thickness became thinner (figure 1C,D), which corresponds to the surface maturation gradient in the schematic diagram (figure 1A).

Second, the thinner and outer cells around the IPCL had a halo-like oval structure of cellular interspaces around the IPCL (figure 1D). Meanwhile, in the corresponding confocal image, an oval halo of distinct cellular interspaces could be visualised around the IPCL, which gradually abates outwardly (figure 1C).

Third, the 3D reconstructed and coexhibited image of the IPCL and the epithelial tissue were also evaluated from a different aspect (figure 1E and supplementary videos 2), which gave a major projection of one plane image together with reconstructed IPCLs. Each halo extends further in the same direction as its corresponding IPCL rises. Such polarity is the third characteristic of surface maturation. Similar polarity can also be found in the corresponding plane confocal image (figure 1C) and histological section (figure 1D).

Fourth, in the same field of confocal view, different halos around the respective IPCLs point towards a common direction, which is in the same direction as the IPCL rise (figure 1E). This behaviour of polarised halos mimics that of the different compasses at the same geographical location, pointing to the same direction. This is the fourth characteristic of surface maturation and we have called it the 'compass effect'.

All of these four features, beyond microvascular morphology or individual cell morphology, gave a major depiction of epithelial surface maturation in the confocal images.

Surface maturation scoring

For clinical application of endomicroscopic images, the above features of surface maturation were further summarised and briefly described as follows:

- Existence: halos present around the IPCLs and outwardly abate.
- Gradient: thickness of each layer of cells becoming outwardly thinner.
- Polarity: a single halo extending further in a certain direction.
- Compass effect: different halos within the same confocal image have a polarity in a common direction.

Schematic diagrams and examples of the above four features were listed (figure 2). Each image was scored according to the four major features of surface maturation: 1 for presence and 0 for absence of each feature. The sum of these scores, ranging from 0 to 4, was calculated as the SMS. Complete absence of all the four characteristics of surface maturation (SMS=0) was considered to be ESIN.

Phase II: SMS diagnosis

Patient enrolment

From May 2008 to April 2010, consecutive outpatients with oesophageal symptoms or due to undergo screening endoscopy, or who were under surveillance for known intraepithelial neoplasia at the Qilu Hospital were informed and encouraged to participate in this trial. The exclusion criteria included the following: patients without suspected lesions; known allergy to fluorescein; pregnancy; lactation; food retention; oesophageal stenosis; postoperative cases; acute bleeding; severe organ dysfunction; oesophageal carcinoma; Barrett's oesophagus; leiomyoma; and indefinite histological results. Patients with invasive cancer and other coexisting lesions were also excluded before further analysis. The nature of these lesions was confirmed by histology based on biopsy specimens or resected specimens (for advanced carcinoma and leiomyoma). Apparently normal oesophageal epithelium under white-light mode and histological invasive lesions were not included in this study. Informed written consent was obtained from all the participants. This trial was approved by the Institutional Ethics Committee of Qilu Hospital and was conducted in accordance with the revised Declaration of Helsinki (1989).

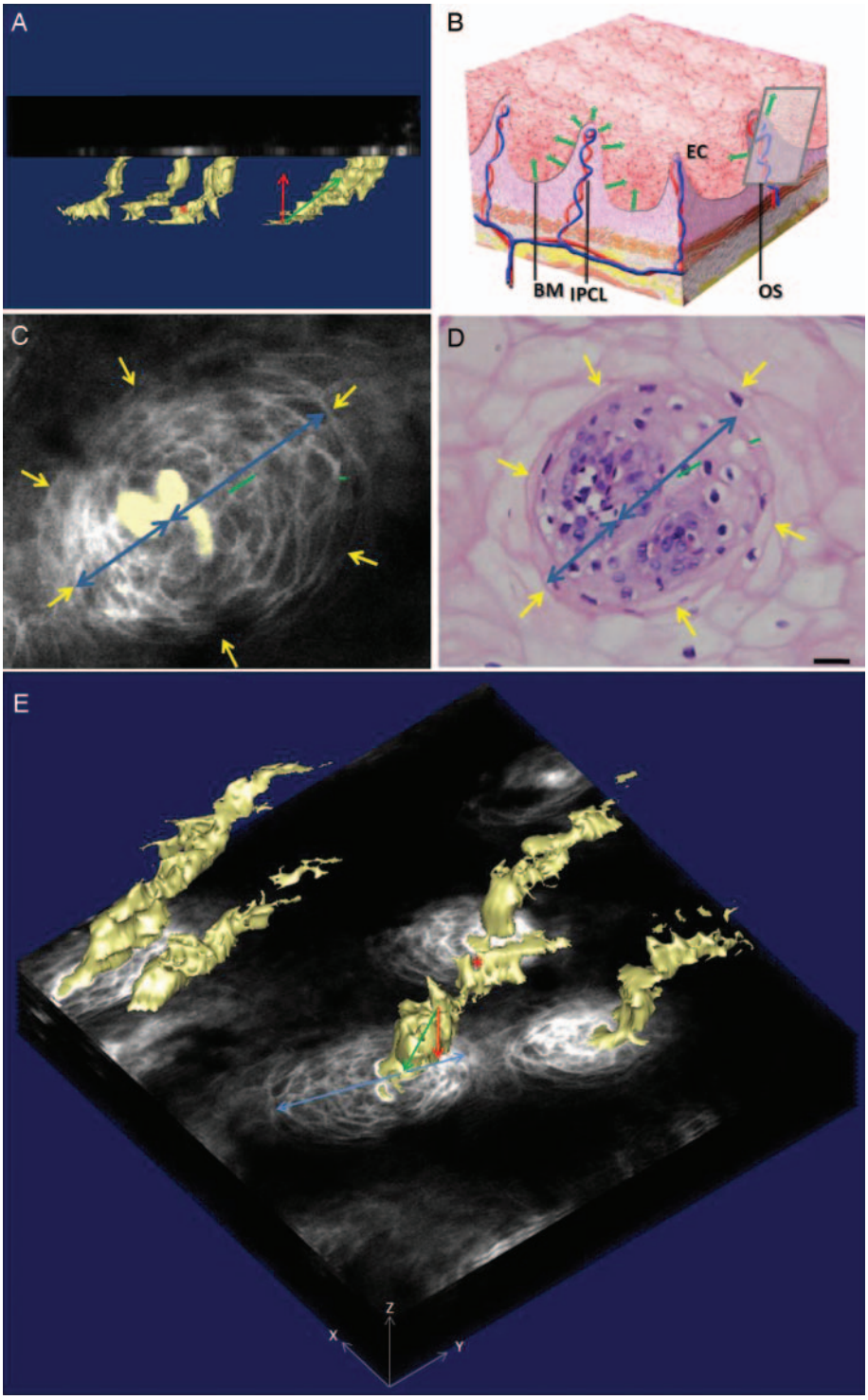

Figure 1 Microstructure of the oesophageal epithelium. (A) 3-Dimensional (3D) reconstructed and coexhibited intrapapillary capillary loops (IPCLs) and the epithelium are observed from a lateral angle, showing the oblique spatial relationship between the focal plane and the IPCL axis. The deeper half of the reconstructed epithelium was made transparent to expose the reconstructed IPCLs (red asterisk). The IPCL axes (green arrow) were oblique to the confocal scanning z-axis (red arrow). (B) Schematic 3D microstructure of the oesophageal epithelium. The surface maturation gradient (green arrows) can be observed from the basement membrane to the superficial layers. At an optical section oblique to the IPCL, the maturation gradient has a larger scale of projection in the direction of the IPCL rise. BM, basement membrane; EC, epithelial cells; OS, optical section. (C) Confocal images of sections oblique to the IPCL axis. During reconstruction, the IPCL was masked (yellow region) and further 3D configuration was calculated from the stacks of these masks. (D) Histological images of sections oblique to the IPCL axis (H&E stain). Scale bar: 10 μm. The cellular thickness becomes thinner from the basal layers around the IPCL to the outer layers (long green line vs short green line) in both (C) and (D). The halo-like oval structure of cellular interspace around the IPCL was obvious in both (C) and (D) (yellow arrows). (E) 3D reconstructed and coexhibited image of IPCLs and epithelial tissue. Reconstructed IPCLs have many tiny filling defects (red asterisk) caused by blood cells. The IPCL axes (green arrow) were oblique to the confocal scanning z-axis (red arrow). Polarity of the single halo can be seen on (C), (D) and (E): halos extend further in the same direction as the IPCLs rise (long blue bilateral arrow vs short blue arrow).

Image collection

Preparation of the patient for CLE was the same as in our previous study. First, each patient was given 80 mg dimethylpolysiloxane orally to remove adherent oesophageal mucus. Then, standard methods of conscious sedation (eg, midazolam hydrochloride and meperidine citrate) were used. Each patient was first screened under the standard definition white-light mode using EC-3870CIK.[10] All oesophageal lesions suspected under white-light mode were subsequently examined by CLE. Three endoscopists (X-MG, TY and X-LZ, each had >10 years' endoscopic experience, performing >10 000 oesophagogastroduodenoscopies and 300 CLEs) performed these endoscopic procedures, while a subordinate endoscopist (Y-YD) collected the images per lesion. None of the four endoscopists participated in the further evaluation proceedings after the data sets were given to the coordinator (ML).

Image distribution and blinding

The clinical information, white-light mode endoscopy results and corresponding endomicroscopic images were organised by the coordinator (ML) and kept from the histopathology reviewer (C-JZ) and the CLE image evaluators (RJ, ZL and HL). The CLE image selector (C-QL) was unaware of the purpose of this study, and one of the evaluators (HL), the proposer of confocal

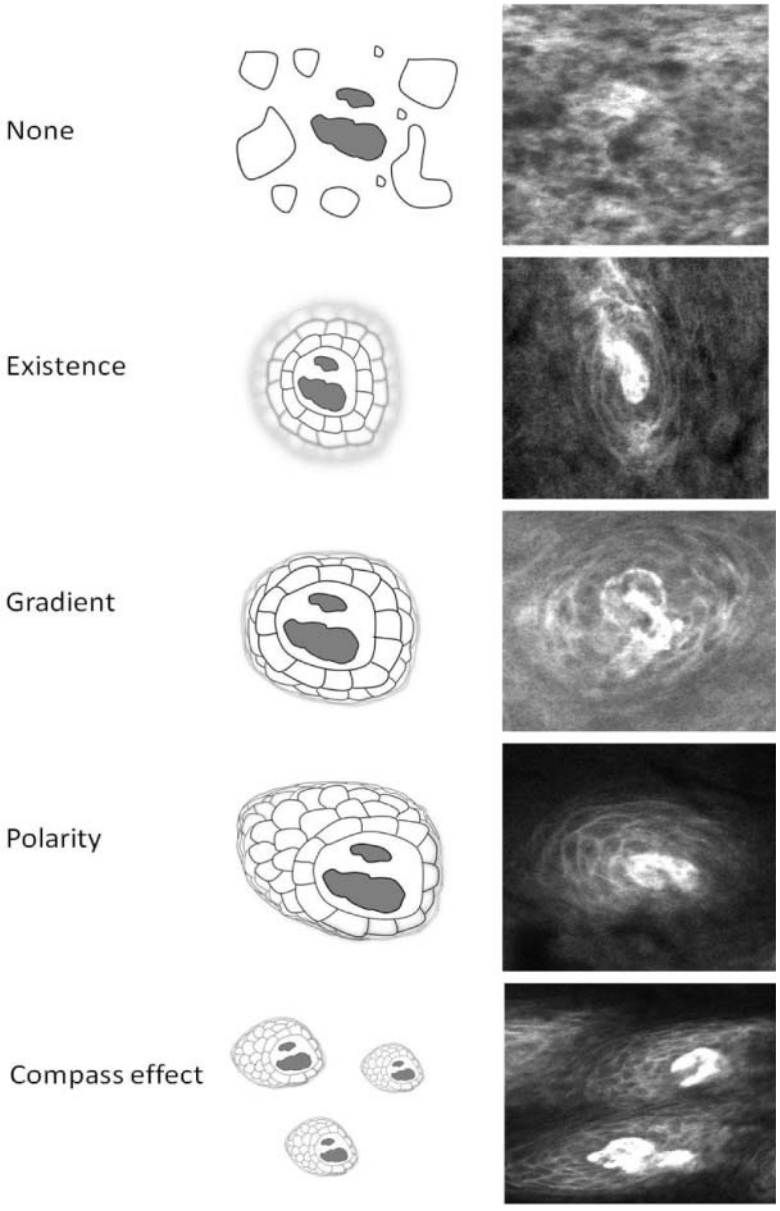

Figure 2 Features of surface maturation of oesophageal squamous epithelium on confocal endomicroscopic images.

endomicroscopic criteria for early ESCC, did not learn of the newly proposed SMS method until the final results were disclosed. During the present study, all of the confocal endomicroscopic images from each site were stored in a specific folder in JPG format after their acquisition and then delivered to an image selector (C-QL, >10 years' endoscopic experience, performing >10 000 oesophagogastroduodenoscopies and 200 CLEs). Two images with IPCLs and cells from each site were selected based on the principle that IPCLs and cells were interpretable. These twins of images were rearranged in random order and given to the CLE image evaluator for further evaluation.

Image evaluation

After the completion of all these procedures, the 64 twins of 2D pictures were blindly shown to two senior investigators (RJ and ZL) for SMS. One of these two investigators was randomised to be evaluator B, and 2 weeks later he/she re-evaluated these twins of pictures in a different random order for the purpose of intraobserver agreement analysis. The same set was shown to another senior investigator (HL), who was the proposer of heterogeneous cells and irregular IPCL criteria. The features of heterogeneous dark cells and irregular IPCLs were blindly evaluated using the same criteria as in our previous study for early ESCC.[10] 'Irregular IPCLs' include IPCLs with an increased diameter, massive IPCLs with tortuous vessels and long branching IPCLs. 'Heterogeneous dark cells' refer to darker epithelial cells of uneven size and irregular arrangement. Presumptive confocal diagnoses were independently made based on these three different approaches (SMS, heterogeneous dark cells or irregular IPCL). The sensitivities, specificities and accuracies derived from these three approaches were evaluated and compared.

Histopathological evaluation

The biopsy specimens obtained from each examined lesion underwent routine H&E staining for histopathological analysis. The gastrointestinal pathologist (C-JZ, average 200 oesophageal specimens reviewed/year, >10 years of practice) who was unaware of the patients' clinical or endoscopic information examined all of the specimens. The diagnosis and graduation of

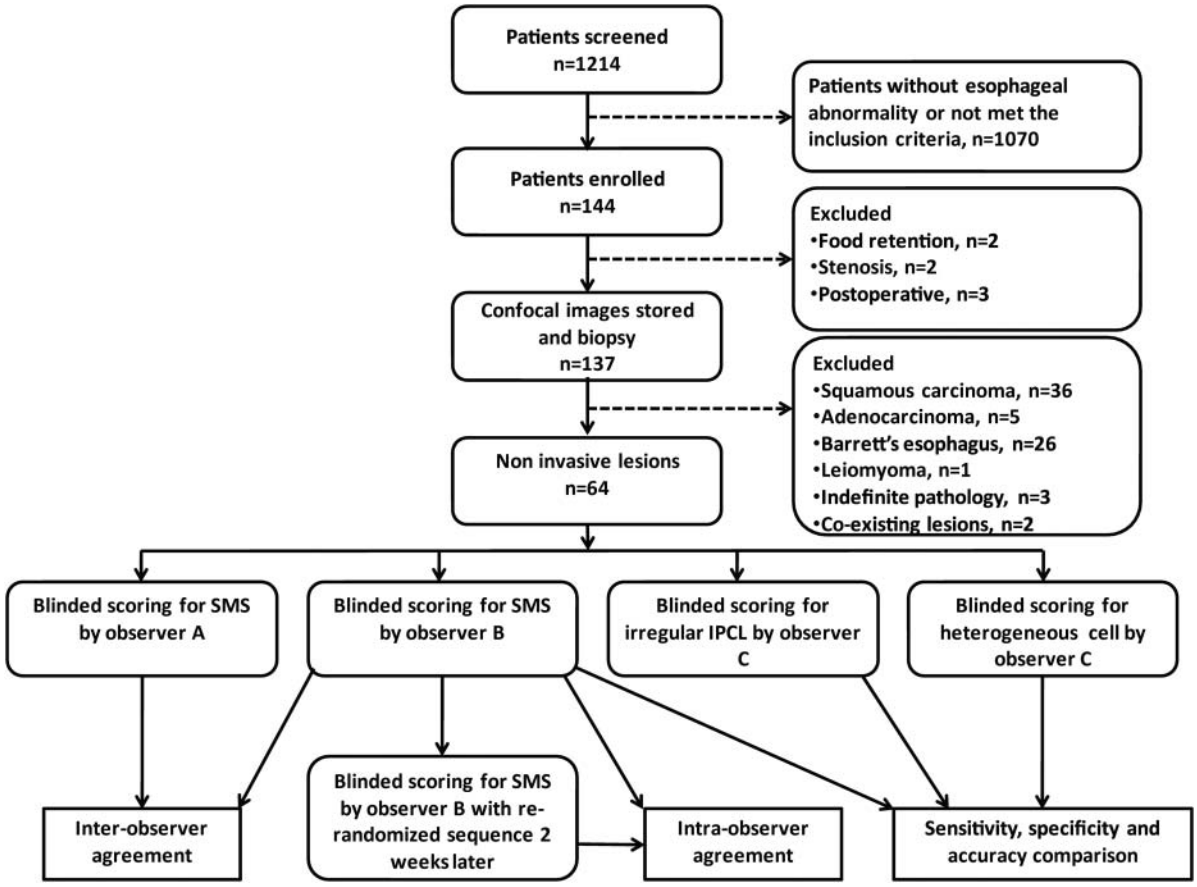

Figure 3 Flow chart of patient disposition and data analysis. IPCL, intrapapillary capillary loop; SMS, surface maturation score.

oesophageal epithelial neoplasia were made according to the Vienna classification.[15]

Statistics

Computer generated randomisation sequence was used to decide the sequences of image sets and the evaluator sequences. SAS V9.13 statistical software was used for data analysis. Fisher's exact test was used to determine the correlation between SMS diagnosis and ESIN. To compare the sensitivity, specificity and accuracy of each diagnosis approach, a U test was used. To examine inter-observer and intraobserver agreement, the weighted κ values along with 95% CI were calculated.[16] The strength of agreement was considered as follows: slight (κ 0.01–0.2), fair (κ 0.201–0.4), moderate (κ 0.401–0.6), substantial (κ 0.601–0.8) and almost perfect (κ 0.801–1.0).

RESULT

Diagnostic value of SMS

To further investigate whether this newly proposed method based on surface maturation could improve the diagnostic yield of ESIN, we conducted an offline prospective trial from May 2008 to April 2010. The flow chart of patient disposition and data analysis of this trial are demonstrated (figure 3). A total of 1214 consecutive outpatients were screened in this trial and 64 oesophageal non-cancerous lesions were analysed. Histopathology showed 21 of the 64 suspected lesions were ESIN, including nine low-grade and 12 high-grade intraepithelial neoplasia. The remaining lesions included 35 inflammatory, seven hyperplastic and one normal epithelium (table 1).

The confocal images of these lesions were blindly evaluated according to the SMS method. The close correlation between SMS diagnosis and ESIN was statistically significant ($p<0.001$) for both observers (table 2). The examples of non-neoplastic epithelium and ESIN by CLE and corresponding histopathology are demonstrated (figure 4). An SMS of 0 is suggestive of ESIN.

Table 1 Patient demographics and clinical features of the analysed lesions in this study

Total number of patients	64
Gender (male/female), n/n	42/22
Mean age, years (range)	55 (23–81)
Mean size, mm (range)	17 (2–60)
Macroscopic type, no.	
0–I	3
0–IIa	27
0–IIb	23
0–IIc	11
Location	
Upper segment oesophagus	1
Middle segment oesophagus	22
Lower segment oesophagus	41
Histological type	
Oesophageal squamous intraepithelial neoplasia	21
Low-grade	9
High-grade	12
Inflammatory lesions	35
Hyperplasic lesions	7
Normal epithelium	1

Table 2 Comparison of SMS and histology for diagnosis of ESIN

Histology	0	1	2	3	4
	Surface maturation score (SMS) from Observer A				
ESIN (+)	16	0	0	1	4
ESIN (–)	4	0	0	6	33
	Surface maturation score (SMS) from Observer B				
ESIN (+)	17	1	0	0	3
ESIN (–)	4	1	4	2	32

The correlation between SMS diagnosis and ESIN was significant ($p<0.001$) from both observers. The cut-off value between ESIN lesions and non-neoplastic lesions was primarily set between 0 and 1.
ESIN, oesophageal squamous intraepithelial neoplasia.

Observer A had a diagnostic sensitivity for ESIN of 76.2% (95% CI 52.8% to 91.7%), specificity of 90.7% (95% CI 77.9% to 97.4%), accuracy of 85.9% (95% CI 75.0% to 93.4%) and positive likelihood ratio of 8.19. Observer B had a diagnostic sensitivity for ESIN of 81.0% (95% CI 58.1% to 94.6%), specificity of 90.7% (95% CI 77.9% to 97.4%), accuracy of 87.5% (95% CI 76.8% to 94.4%) and positive likelihood ratio of 8.70. The diagnostic values of other cut-off points were plotted in receiver operating characteristic curves (figure 5). The inter-observer and intraobserver agreements for SMS were both substantial, of weighted κ value 0.687 (95% CI 0.530 to 0.844) and 0.601 (95% CI 0.434 to 0.768), respectively.

Diagnostic value comparison

We compared the diagnostic value for ESIN between the SMS and the previous microvasculature or the individual cell evaluation method. The irregular IPCLs and heterogeneous dark cells were evaluated according to the criteria in our previous study[10] by the observer who had proposed the previous criteria but was not informed of the newly developed SMS. Compared with irregular IPCL criteria, SMS was superior in sensitivity ($p<0.05$) and accuracy ($p<0.01$) (table 3). Compared with heterogeneous dark cells, SMS was superior in sensitivity ($p<0.05$).

DISCUSSION

To our knowledge, this is the first application of 3D confocal imaging within the human body using an endomicroscope.

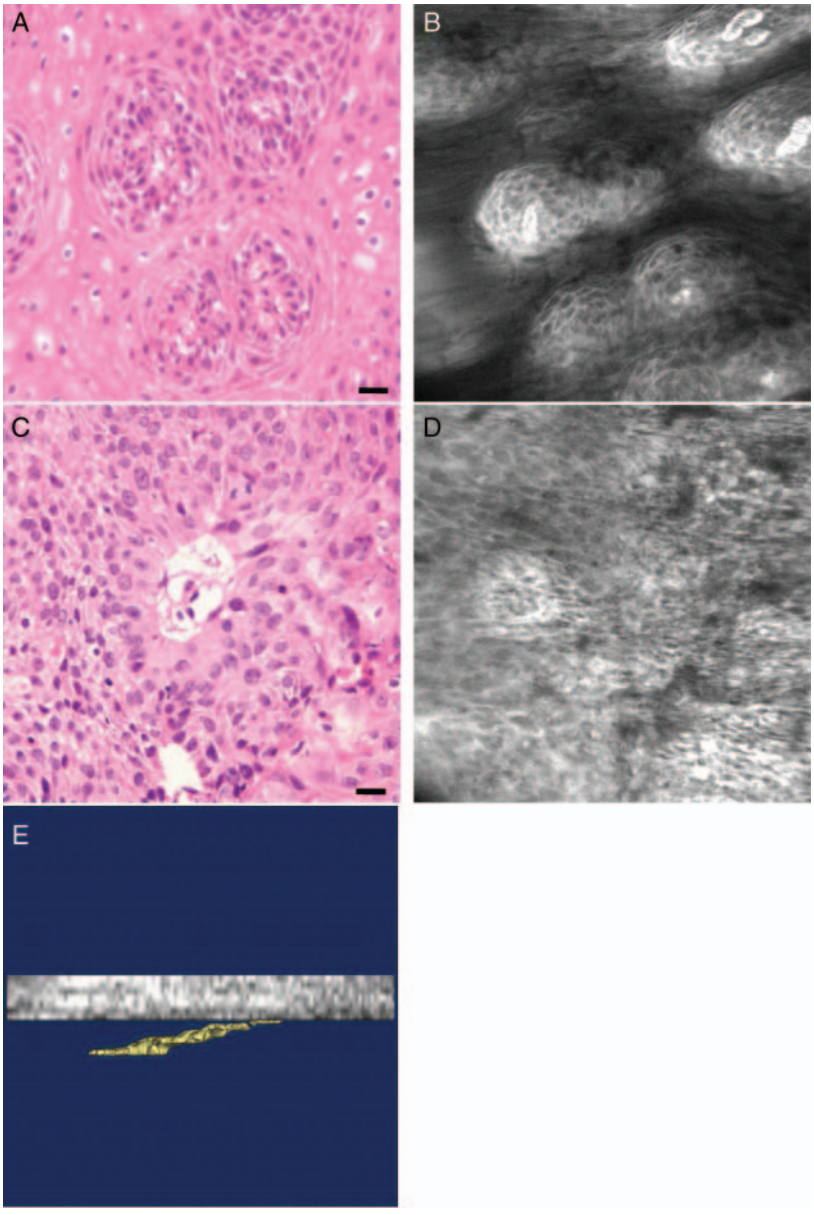

Figure 4 Comparison of histological section and confocal endomicroscopic images between non-neoplastic and oesophageal squamous intraepithelial neoplasia (ESIN) epithelium. (A) Transverse section of non-neoplastic inflammatory oesophageal epithelium (H&E stain). Scale bar: 10 μm. (B) Corresponding confocal laser endomicroscopy (CLE) image of non-neoplastic inflammatory epithelium. Four features of halos were obvious and surface maturation score (SMS)=4. All halos around the intrapapillary capillary loops (IPCLs) extended further in one common direction, at the 11 o'clock position in this image. (C) Transverse section of high-grade ESIN (H&E stain). Scale bar: 10 μm. (D) Corresponding CLE image of high-grade ESIN. None of the four features of halos were present, and SMS=0, while the IPCL irregularities were inconspicuous. Surface maturation gradient could be recognised in (A) and (B), while absent in (C) and (D). (E) Lateral view of 3D reconstructed and coexhibited image of IPCL and epithelial tissue of the high-grade ESIN lesion. The optical heterogeneous atypical cells further aggravated light scattering and tissue opacity, impairing the imaging depth and quality of reconstructed 3D image.

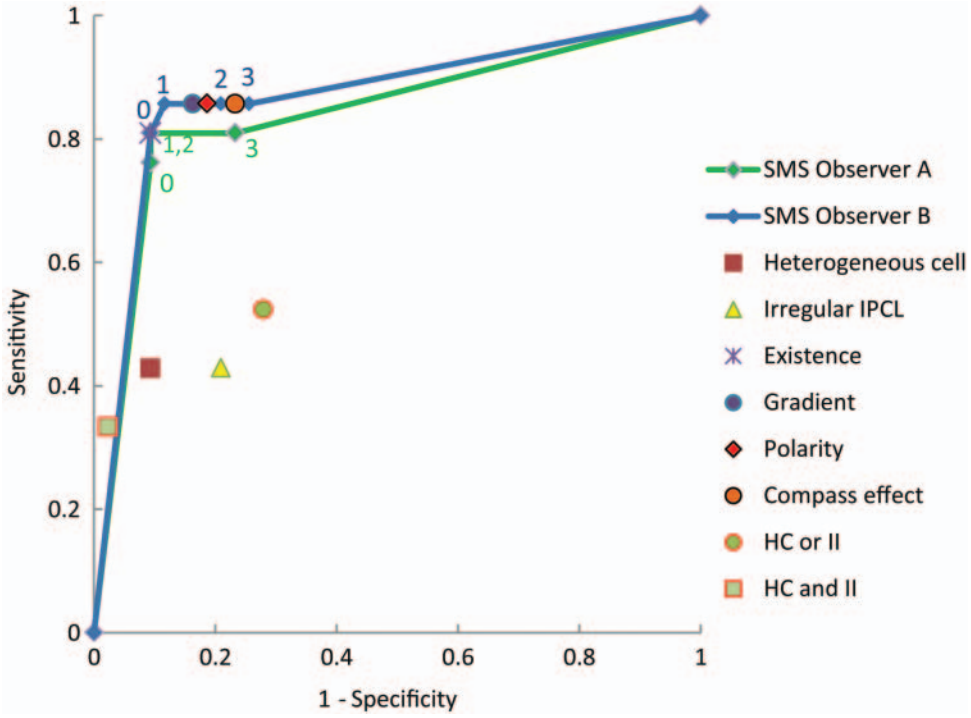

Figure 5 Diagnostic values of different approaches. The diagnostic yields of surface maturation score (SMS), irregular intrapapillary capillary loop (IPCL) and heterogeneous cell, four features of surface maturation, as well as the combination of irregular IPCL and/or heterogeneous cell were plotted on a receiver operating characteristic curve for comparison. SMS=0 was the criteria of oesophageal squamous intraepithelial neoplasia, and other cut-off values are labelled next to the corresponding patches. HC, heterogeneous dark cell; II, irregular IPCL.

This technical leap enables us to investigate the surface maturation gradient for a better diagnosis of ESIN. Surface maturation represents the morphological transition from the cuboidal basal layer to the flatter superficial layer in the squamous epithelium.[12] It is a key feature of non-neoplastic lesions in the pathological diagnosis. However, surface maturation and its potential diagnostic value had not been explored before the advent of 3D confocal endomicroscopic imaging. Previously, Microfil (Canton Bio-Medical Products Inc., Boulder, CO, USA) was used to visualise fine microvessels in the oesophageal mucosa. It is an opaque silicone compound, which coagulates to form a 3D cast of the vascular network after injection into the microcirculation,[17] but it falls short of exhibiting the microvessels and the squamous epithelium simultaneously. In this study, we took advantage of 3D confocal imaging to demonstrate the spatial relationship between the IPCLs and the squamous epithelium, and recognised four features of surface maturation on plane images. The subsequently developed SMS method is totally compatible with plain confocal images and clinical application to support on-table decisions is easy.

We conducted this prospective study and validated a good performance of the newly developed SMS method in the diagnosis of ESIN. Previous endomicroscopic diagnosis of ESIN was largely based on the microvasculature shift[5 9–11 18] because it is well established that unrestricted growth of tumours is dependent on angiogenesis.[4 19] However, its performance in low-grade ESIN diagnosis had not been investigated.[10] It was primarily restricted by two factors: microvascular changes are often too subtle to be effectively detected in intraepithelial neoplasia and non-neoplastic processes are also associated with angiogenesis.[20] In contrast, loss of surface maturation is more directly associated with intraepithelial neoplasia. In non-neoplastic epithelial lesions, including normal, hyperplastic or inflammatory ones, surface maturation is not affected by either the proliferation status or vasculogenesis.

Table 3 Diagnosis utility of SMS, irregular IPCL and heterogeneous cell

		Histology		Sensitivity (%)	p Value	Specificity (%)	p Value	Accuracy (%)	p Value
		ESIN (+)	ESIN (–)	95% CI	versus SMS	95% CI	versus SMS	95% CI	versus SMS
SMS	0	17	4	81.0	–	90.7	–	87.5	–
	1–4	4	39	58.1 to 94.6		77.9 to 97.4		76.8 to 94.4	
Irregular IPCL	+	9	9	42.9	0.011	79.1	0.132	67.2	0.006
	–	12	34	21.8 to 66.0		64.0 to 90.0		54.3 to 78.4	
Heterogeneous dark cell	+	9	4	42.9	0.011	90.7	1.000	75.0	0.070
	–	12	39	21.8 to 65.9		77.9 to 97.4		62.6 to 85.0	
II and HC	+	7	1	33.3	0.002	97.7	0.167	76.6	0.107
	–	14	42	14.6 to 57.0		87.7 to 99.9		64.3 to 86.2	
II or HC	+	11	12	52.4	0.050	72.1	0.027	65.6	0.003
	–	10	31	29.8 to 74.3		56.3 to 84.7		52.7 to 77.0	

ESIN, oesophageal squamous intraepithelial neoplasia; HC, heterogeneous dark cell; II, irregular IPCL; IPCL, intrapapillary capillary loop; SMS, surface maturation score.

This study suggests that SMS could serve as a new 2D endomicroscopic diagnostic method of better performance.

This study added to the diagnostic armamentarium of ESCC/ESIN diagnosis. Pech *et al* first proposed the cellular and vascular criteria for early ESCC.[9] The studies by Pech *et al*[9] and Liu *et al*[10 18] included ESCC and controls, and the study of Iguchi *et al*[11] focused on ESCC depth prediction. Low-grade intraepithelial neoplasia has the potential to progress into high-grade intraepithelial neoplasia and calls for surveillance, but had not been involved or regarded as a negative lesion in previous research.[9–11 18] In the present study, the issue of intraepithelial neoplasia, rather than invasive oesophageal cancer, was especially addressed.

Some lesions were misdiagnosed by the newly proposed SMS criteria. There were two false-positive cases from both observers. In one case, the scanning plane was so deep that the maturation gradient was inconspicuous (basal layer squamous cells are evenly immature despite X/Y location). In the other case, excessive fluorescent sodium extrusion affected the image reading. There were four false-negative images from both observers, and they were too dark for evaluation. In these cases, the application of SMS was affected by low image quality (either too dark or too bright) and image depth (too deep).

The axes of the IPCLs were demonstrated to be not strictly vertical to the imaging plane of confocal endomicroscope in the 3D reconstructed images of the oesophageal epithelium (figure 1A,E). We speculate that such an oblique spatial relationship might possibly be due to two factors: the IPCL axis was hardly vertical to the basement membrane or the top surface of the epithelium in the demonstrated pathological sections[21] and Microfil-fixed vasculature specimen.[17] Then, the rigid tip of the confocal endomicroscope cannot be placed precisely vertical to the parietal wall of the oesophagus due to its physical dimension. This asymmetric structure around the IPCLs enables surface maturation to be interpreted in plane confocal images.

Several limitations of this study merit discussion. First, FocusClear (CelExplorer, Hsinchu, Taiwan),[14] an optical clearing solution, cannot be used within the human body, and this limited the depth of light transmitted in the present study. It greatly increases image resolution with an ex vivo thick tissue due to more efficient excitation, less signal absorption and deflection, and better signal detection.[22] While in our study, especially in ESIN cases, the optical heterogeneous atypical cells further aggravated the light scattering and tissue opacity, preventing indepth and sharp 2D confocal images, which are essential for high-quality 3D reconstruction. Fortunately, these did not limit our analysis of the spatial relationship between the microvasculature and the nearby surface maturation gradient. Another limitation of this study is the exclusion of invasive cancerous lesions in an offline fashion. The ability of SMS to distinguish ESIN and non-neoplastic lesions should be prospectively assessed online in all kinds of oesophageal lesions. A third limitation of this study is the lack of comparison between endomicroscopy using SMS and other imaging modalities, such as high-definition white light, chromoendoscopy or electronic chromoendoscopy. Finally, we would like to point out that because of the in vivo resolution limitation, halos around the IPCLs cannot be precisely reconstructed in 3D with the present technology. Undoubtedly, in the future, the endoscopist will be able to interpret the real-time reconstructed vertical sections of the gastrointestinal epithelium as the pathologist does.

3D endomicroscopic imaging was helpful to develop the plain-image-compatible SMS criteria but was not directly employed as a diagnostic approach. Computer processing time to generate 3D images, potential decreased accuracy and inter-observer agreements when a real-time diagnosis is made, potential learning curve, and additional procedure time needed to obtain confocal images are all barriers that need to be tackled.

In conclusion, 3D confocal endomicroscopic imaging provides a unique insight into the stereo configuration of the human oesophageal epithelium. We based the development of the SMS system on 3D images, and applied the system to 2D CLE images. The SMS CLE criteria performed better than the irregular IPCL/dark cell criteria for the detection of ESIN and should be further validated in future prospective studies.

Significance of this study

What is already known on this subject?

- Oesophageal squamous intraepithelial neoplasia is a precancerous lesion that can be treated endoscopically.
- Confocal endomicroscopy gives real-time optical sectioning images; thus, it is speculated to have the potential to visualise the 3D microstructure of the human gastrointestinal tract.
- The current confocal endomicroscopic diagnostic yield of oesophageal squamous intraepithelial neoplasia needs to be improved.

What are the new findings?

- 3D endomicroscopic imaging of the human gastrointestinal tract has been used for the first time to investigate the stereo configuration of the epithelium.
- The surface maturation scoring system performs better than the old irregular intrapapillary capillary loop/dark cell system.

How might it impact on clinical practice in the foreseeable future?

- 3D endomicroscopy may provide a novel approach to investigate the human gastrointestinal tract in various pathological conditions in the near future.

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