Digestive Endoscopy (2002) **14**, 53–60

ORIGINAL ARTICLE

THREE-DIMENSIONAL ENDOSCOPIC ULTRASONOGRAPHY FOR THE ASSESSMENT OF EARLY GASTRIC CARCINOMA INVASION: COULD IT PROVIDE DIAGNOSTIC INNOVATIONS?

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Background: This study aimed to evaluate a three-dimensional endoscopic ultrasonographic (3-D EUS) system in the assessment of the tumor invasion depth of early gastric carcinoma.
Methods: Sixty-nine macroscopically early cancer lesions in 67 patients were recruited in an *in vivo* study. The surgically resected gastric specimens of 30 of them were re-examined in an *ex vivo* study. An Olympus 3-D EUS imaging system was employed in both studies. Diagnostic accuracy for tumor invasion depth was evaluated and compared with histopathological sections stained by H&E and Masson's trichrome stain. Reconstructed surface-rendering images were evaluated and compared with the endoscopic and macroscopic findings.
Results: Three-dimensional EUS allowed rapid tomographic assessment of the lesions in both the *in vivo* and *ex vivo* studies. The accuracy of 3-D EUS for the assessment of tumor invasion depth was 87% in the *in vivo* study. The accuracy rate was significantly lower ($P = 0.03$) for the cancer lesions associated with ulcer fibrosis (74%) than for those with no fibrosis (97%). In the 30 subjects who underwent both studies, the accuracy rates were higher in the *ex vivo* than the *in vivo* study (94% *vs* 77% for all the lesions, and 93% *vs* 74% for cancers associated with fibrosis), but were not statistically significant. The rates of good surface-rendering images were 64% and 94% in the *in vivo* and *ex vivo* studies, respectively. The differences were attributed to the clearer dual-plane reconstruction images obtained in the *ex vivo* study in absence of motion artifacts.
Conclusions: Three-dimensional EUS is a promising imaging technique for the assessment of tumor invasion depth of early gastric cancer.

Key words: endoscopic ultrasonography, gastric cancer, surface-rendering images, three-dimensional.

INTRODUCTION

Two-dimensional (2-D) endoscopic ultrasonography (EUS) provides cross-sectional images of the gastrointestinal tract wall that proved useful in the assessment of tumor infiltration depth of gastrointestinal tumors.[1,2] However, it has some limitations; the presence of peptic ulceration or ulcer fibrosis at the tumor site, which is a common association with the depressed types of gastric cancer (about 59%),[3] is one major obstacle to an accurate EUS diagnosis of the depth of tumor invasion.[4] Furthermore, sites of minimal submucosal invasion are overlooked with the conventional 2-D EUS method.[5] Tomographic EUS study of the tumor at regular minute intervals might contribute to greater diagnostic accuracy.[6,7] In addition, a spatial three-dimension (3-D) view is expected to facilitate deduction of the tumor extent in relation to the mucosal surface structure and/or the surrounding vessels and organs. With advances in computer technology, clinical imaging modalities have entered the era of 3-D, which has been shown to be valuable.[8–10] Three-dimensional EUS is not a clinical reality as yet, although the preliminary results seem to be promising.[11–13] However, the 3-D techniques have yet to be fully developed.[14]

This histopathologically verified study was conducted both *in vivo and ex vivo* to evaluate the clinical usefulness and limitations of a recently developed 3-D EUS imaging system in the assessment of tumor infiltration depth of early gastric carcinoma.

PATIENTS AND METHODS

Subjects

Both the *in vivo* and *ex vivo* parts of this study were conducted between September 1998 and January 2001. The study protocol was approved by the institutional review board of our Cancer Research Institute, and all patients gave written informed consent. A total of 71 patients with gastric carcinoma among those referred to the outpatient clinic of the Cancer Research Institute Hospital of Kanazawa University during the study period were initially recruited in an *in vivo* study. The inclusion criteria were endoscopically early or early-like advanced gastric cancers that were verified histopathologically by endoscopic biopsy. Three-dimensional EUS was employed for the preoperative assessment of tumor infiltration depth in all patients. Figure 1 is a flow diagram for the patients initially included in this study. Four out of the 71 patients were finally excluded because of the absence of histopathological confirmation of the

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Received 13 December 2001; accepted 7 January 2002.

tumor infiltration depth. Table 1 shows the clinical characteristics of the patients finally included in this study. Thirty-one tumor sites in the surgically resected gastric specimens of 30 patients who underwent surgical treatment in our hospital were re-examined within 2 h after operation using the same 3-D EUS system. The latter group constituted the combined *in vivo* and *ex vivo* study group of patients.

Endoscopic ultrasonography

An Olympus 3-D EUS imaging system was employed in this study. The system comprises a 3-D EUS probe (UM-3-D 3R: 20 MHz) covered with a water-filled sheath (MAJ-356RN and MAJ-356RJ) and connected with an outer sheath connector (MAJ-357) to a probe-driving unit (MAJ-355). A 3-D EUS image-processing unit (EU-IP2) installed on a computer system was responsible for 3-D image reconstruction. Figure 2 shows the method of data acquisition and 3-D image reconstruction. Real-time 2-D radial images were obtained during helical scanning and a reconstructed linear image was displayed by the end of the scanning cycle (dual-plane reconstruction, DPR). A single scanning cycle is completed within 8–24 s according to the adjusted distance between two successive radial images. Three-dimensional reconstruction was performed after finishing the scanning process. To provide the necessary acoustic coupling, the water-filling method was used during the *in vivo* examination, and *ex vivo* specimens were examined in a saline bath.

The criteria for EUS determination of tumor invasion depth are shown in Fig. 3. They were generally based on the previously reported 9-layer EUS structure of the gastric wall visualized by high-frequency catheter probes.[15] A tumor is usually visualized as an irregular hypoechoic mass disrupting the normal echo layers. Whenever the muscularis mucosa (mm) could be delineated, we considered its visualization intact along all the DPR image sections as a sign favoring the diagnosis of mucosa-limited (m) lesion.[16] In cases associated with ulcer scar, cancerous infiltration within the fibrous tissue was usually visualized as hypoechoic spots. In such cases, the depths of ulcer fibrosis as well as of tumor invasion were identified.[17]

The accuracy of 3-D EUS for estimating tumor invasion depth was assessed by comparison with the histopathological sections. The 3-D reconstructed surface-rendering images were evaluated by comparison with the endoscopic features and the macroscopic findings of the resected specimens. When the reconstructed surface-rendering images closely mirrored the mucosal surface structures, they were described as good, whereas those with no similarity to the mucosal area included in the scan were described as poor images.

Pathological analysis

The lesions were macroscopically evaluated based on the classification system of the Japanese Research Society for Gastric Cancer.[18] Macroscopically early gastric cancers, which are defined as a superficial tumor with or without minimal elevation or depression, were only included in this study. Ulceration at the tumor site was diagnosed macroscopically by the presence of ulcer fur and/or fold convergence.

Histopathological study

The entire cancerous lesion resected endoscopically or surgically was examined in a stepwise manner. Paraffin sections (2 μm thick) were stained with H&E and examined carefully for the assessment of tumor infiltration depth and presence or absence of ulcer scar. The criteria for diagnosis of tumor invasion depth were based on the methods established by the Japanese Research Society for Gastric Cancer.[18] When submucosal (sm) tumor infiltration was detected microscopically, the depth of tumor invasion under the level of the mm layer was measured in micrometers using a microscopic scale and accordingly classified into 3 grades (sm_{1-3}). Additional staining of tissue sections with Masson's trichrome (MT) stain was performed to allow quantitative assessment of submucosal fibrosis at the tumor site.[19] Image analyzer software (V10 C version 1.07; Toyobo, Osaka, Japan) was employed to determine the density of fibrous tissue, which was expressed as a percentage of the total.[20] We evaluated the density of fibrous tissue in each tissue section by obtaining the ratio between its percentage at the tumor site and at the normal non-cancerous area. We considered a ratio greater than 2 as an indicator of pathological fibrosis (F +), which was subsequently graded as minimal (+/–), moderate (+) and severe (++).

Statistical analysis

Statistical analysis was performed using StatsDirect statistical software (version 1.9.8). The age of patients and duration of scanning were expressed as mean ± SD. The results in different groups were compared with the χ^2-test or Fisher's exact test, as appropriate. Approximate (Wilson) 95% mid-*P* confidence intervals (CI) were evaluated. Significance was established at values of $P < 0.05$.

RESULTS

In vivo study

As shown in Table 1 and Fig. 1, 69 lesions in 67 patients were finally included in the *in vivo* study. Three-dimensional EUS scanning could be performed successfully for all the lesions and no complications were encountered during the examinations. Helical scanning allowed rapid tomographic examination of the cancer sites; because the data were stored digitally, evaluation of the DPR images as well as computer processing of 3-D views were done after finishing the scanning process, resulting in a reduction of the time needed for each examination. The 3-D EUS examination time was 12.1 ± 4.2 min for each patient. The linear reconstructed images were sometimes disfigured during the *in vivo* scanning by undesirable motions that made it barely interpretable; hence, many scanning cycles were necessary. Clinically valuable linear reconstructed images were obtained in 52 of the 69 lesions (75%). The mm layer could be identified in the DPR images of 51 of the 69 lesions (74%). Table 2 shows the 3-D EUS accuracy in the determination of tumor invasion depth in the *in vivo* study. The accuracy rate for the diagnosis of m lesions seemed to be greater than that for sm invasion (91% *vs* 75%); however, the difference was not statistically significant ($P = 0.15$). Figure 4 shows the features of a lesion

Table 1. Clinical characteristics of the patients finally included in the study

Number of patients	67
Number of cancer lesions*	69
Age (mean ± SD)	64.3 ± 12
Gender	
Male	51 (76%)
Female	16 (24%)
Macroscopic type†	
Protruded	5 (7%)
Superficial (elevated, flat or depressed)	57 (83%)
Excavated	7 (10%)
Macroscopic features of ulceration or ulcer scar	
Absent (Ul–)	35 (51%)
Present (Ul+)	34 (49%)

* Two patients had double carcinoma lesions. † According to the classification of the Japanese Society for Gastric Cancer.[19]

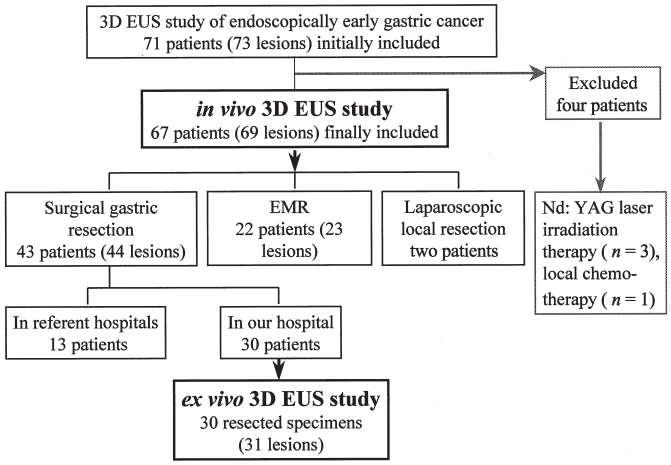

Fig. 1. Flow diagram of the 71 patients initially recruited in the study.

Table 2. 3-D endoscopic ultrasonography accuracy in the diagnosis of tumor invasion depth according to the histopathological findings in the *in vivo* study

Histopathological diagnosis	3-D endoscopic ultrasonography diagnosis m*	sm*	≥mp	Accuracy (%)	CI (%)
m ($n=55$)	50	3	2	91	80–96
sm ($n=12$)	3	9	–	75	47–91
sm_1 (≤1000 µm)	2	5	–	71	36–92
sm_2 (>1000–2000 µm)	1	3	–	75	30–95
sm_3 (>2000 µm)	–	1	–	100	–
≥mp ($n=2$)	1	–	1	50	–
Total accuracy ($n=69$)				87	77–93

CI, confidence interval; m, mucosa; sm, submucosa; mp, muscularis propria.
* ms *vs* sm, statistically not significant ($P=0.15$).

Table 3. Accuracy of 3-D endoscopic ultrasonography in the assessment of tumor infiltration depth in the *in vivo* study according to the intensity of submucosal fibrosis

Fibrosis intensity	*In vivo* 3-D endoscopic ultrasonography accuracy	CI (%)
F–	33/34 (97%)*	85–99
F+	28/35 (74%)*	64–90
Minimal (+/–)	5/5 (100%)	–
Moderate (+)	9/11 (82%)	52–95
Severe (++)	13/19 (68%)	46–85

CI, confidence interval.
* F– *vs* F+, statistically significant ($P = 0.03$).

assessed accurately as m cancer by 3-D EUS, resulting in a successful endoscopic mucosal resection.

As shown in Table 1, ulceration or converging folds in and around the cancer site was documented endoscopically in 34 out of the 69 cancer lesions examined *in vivo* (49%). However, pathological fibrosis (F+) was detected in the sm layers of 35 of them (51%). Table 3 shows the 3-D EUS accuracy for the diagnosis of tumor invasion according to the intensity of submucosal fibrosis in the *in vivo* study. The accuracy rates were significantly higher in the F– than in the F+ lesions in the *in vivo* study ($P=0.03$).

After finishing each examination, the best DPR images were reviewed and used for 3-D reconstruction. Good surface-rendering images could be obtained for 44 of the 69 lesions (64%); one image is shown in Fig. 4c.

Combined *in vivo* and *ex vivo* studies

Thirty-one lesions in 30 patients were reassessed postoperatively using the same 3-D EUS system. The 3-D EUS scanning time was 4 ± 1.6 min in the *ex vivo* study. High-quality DPR images were easily obtained in all cases (100%). The mm layer was delineated in 20 of the 31 lesions in the *in vivo* study (65%), whereas it was visualized in 26 of the same lesions in the *ex vivo* study (84%). The diagnostic accuracy for determination of tumor infiltration depth in the *in vivo* and *ex vivo* studies of the same lesions is compared in Table 4. Although the difference between the accuracy rates was not statistically significant ($P=0.07$), an accurate *ex vivo* diagnosis was achieved in 5 cases that were misdiagnosed during the *in vivo* study. However, misdiagnosis of one case of early-like advanced cancer *in vivo* was not corrected in the *ex vivo* study. Only 4 cases with F– cancer lesions were included in the

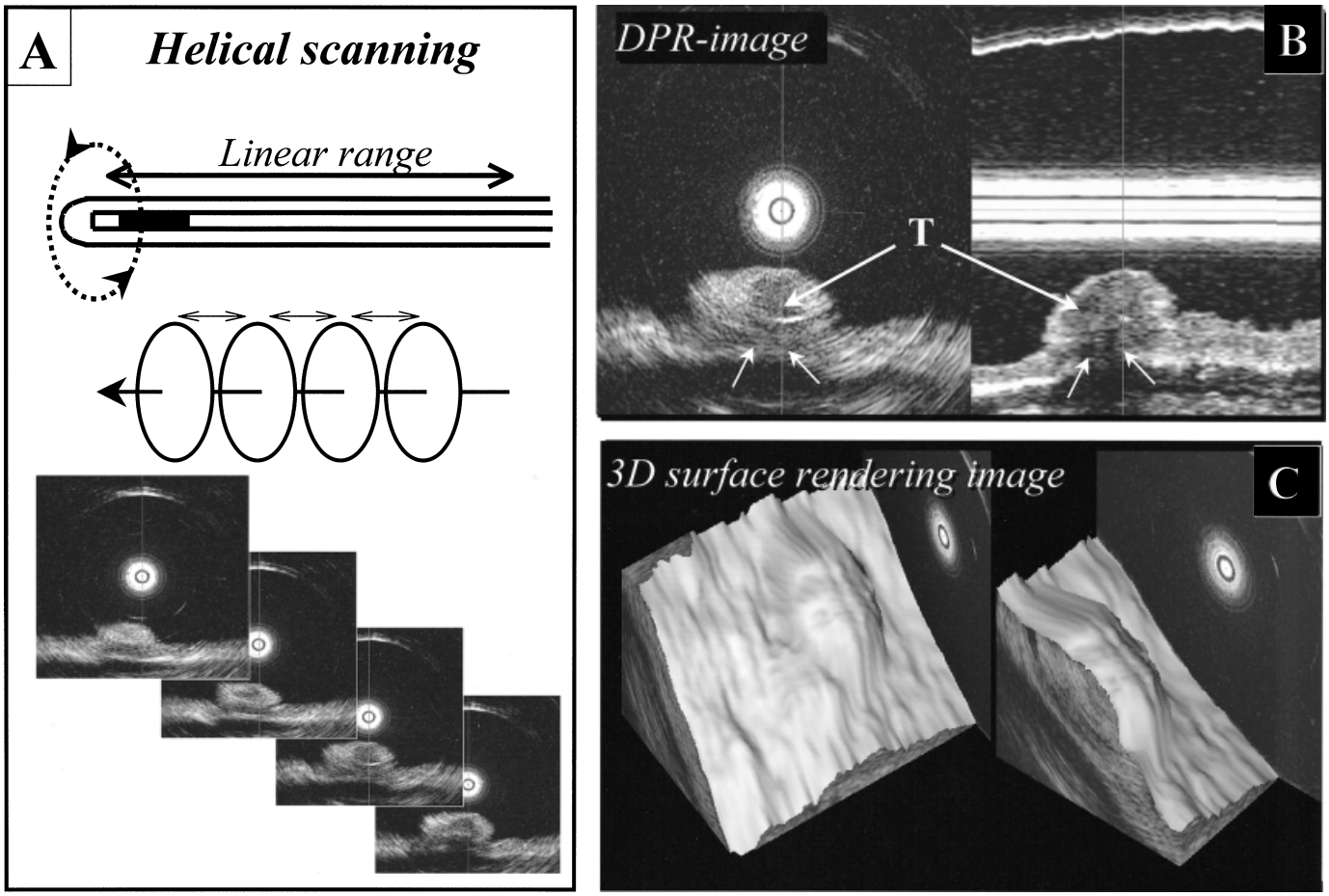

Fig. 2. Three-dimensional imaging by the Olympus 3-D endoscopic ultrasonography system. (a) Helical scanning is achieved by automatic spiral withdrawal of the 3-D-endoscopic ultrasonography probe within its sheath for 1.5–4 cm (linear range). Serial radial image sections (40–120) are recorded at fixed intervals along the linear range. (b) A linear image is reconstructed from the serial radial image sections. A selected radial image and the linear image were displayed simultaneously as a dual-plane reconstruction image (DPR), which facilitates the assessment of tumor extent. T, tumor; short arrows point to the maximum infiltration depth (submucosa). (c) The recorded images are subsequently reconstructed into a 3-D view such as a surface-rendering image.

Table 4. Comparison between the *in vivo* and *ex vivo* 3-D endoscopic ultrasonography (EUS) accuracy in the determination of tumor invasion depth of the cancer lesions examined pre- and postoperatively

Histopathological diagnosis	*in vivo* 3-D EUS diagnosis					*ex vivo* 3-D EUS diagnosis					*P* value
	m	sm	≥mp	Accuracy (%)	CI%	m	sm	≥mp	Accuracy (%)	CI (%)	
m ($n=19$)	16	1	2	84	62–94	18	1	–	95	75–99	0.3
sm ($n=10$)	3	7	–	70	40–89	–	10	–	100	–	0.1
sm_1 (≤1000 μm)	2	3	–	60	23–88	–	5	–	100	–	0.22
sm_2 (>1000–2000 μm)	1	3	–	75	30–95	–	4	–	100	–	0.44
sm_3 (>2000 μm)	–	1	–	100	–	–	1	–	100	–	–
≥mp ($n=2$)	1	–	1	50	–	1	–	1	50	–	–
Total ($n=31$)				77	60–89				94	79–98	0.07

CI, confidence interval; m, mucosa; sm, submucosa; mp, muscularis propria.
* *In vivo vs ex vivo.*

combined *in vivo* and *ex vivo* study group, and all of them were limited to the mucosa. This was because a large number of patients with m-limited lesions that were not associated with ulceration or ulcer scar on the basis of endoscopic and/or endosonographic findings were considered candidates for endoscopic mucosal resection[17] (23 of 69 lesions; 33%) and hence they were not available for an *ex vivo* study. The proportion of F+/total number of lesions in the combined study group was 87%. Table 5 compares the accuracy of estimates of tumor invasion depth in the two studies according

to the intensity of sm fibrosis. Figure 5 shows the difference in the quality of DPR images obtained *in vivo* and *ex vivo* for the same lesion. Good surface-rendering images were obtained for all except two cases with polypoid features (94%) in the *ex vivo* study.

DISCUSSION

Studies on 3-D EUS have been reported since 1995.[21–25] In most of them, the third plane was obtained by manual linear withdrawal of the transducer within the straight parts of the GI tract such as the esophagus and rectum. Nishimura *et al.* were the first to develop software capable of displaying a surface-rendering image as well as 3-D EUS sections.[11] Unfortunately, however, the *in vivo* images were of poor quality because of manual traction of the probe. Hence, a method of automatic withdrawal was needed to obtain an accurate 3-D EUS image. Yoshimoto[12] and Yoshino *et al.*[13] introduced preliminary clinical data on automatic 3-D EUS. In the present study, we evaluated the clinical utility and limitations of this newly developed 3-D EUS system in the assessment of gastric carcinoma. The 3-D EUS scanning unit is basically composed of a high-frequency miniature probe. As miniprobe ultrasonography was reported to be less reliable for staging of advanced carcinoma owing to its limited depth of sonographic penetration,[26,27] we therefore limited the patients included in this study to those with endoscopically early diseases, although two of them proved to have advanced lesions. Indeed, the EUS diagnosis of tumor infiltration depth was accurate in only one of the two cases with advanced disease in both the *in vivo* and *ex vivo* studies.

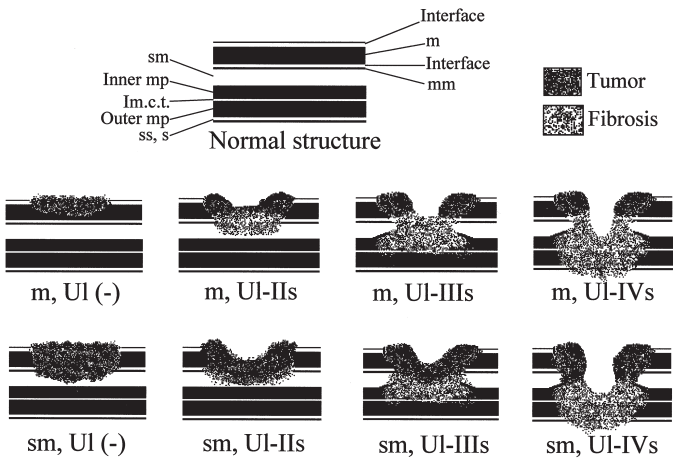

Fig. 3. Schematic presentation of the endoscopic ultrasonography diagnostic criteria for the judgment of tumor infiltration depth of early gastric carcinoma in accordance with the presence or absence of ulceration in and around the tumor site. m, mucosa; mm, muscularis mucosa; sm, submucosa; mp, muscularis propria; Im.c.t., intermuscular connective tissue; ss.s, subserosa and serosa; Ul (–), absence of ulceration or ulcer scar; Ul-IIs, associated with an ulcer scar limited to the sm layer; Ul-IIIs, ulcer scar reaching the mp layer; Ul-IVs, ulcer scar involving the whole gastric wall up to the serosa.

In the current study, although a direct comparison between the 2D EUS and the 3-D EUS methods was not performed, we could appreciate that automatic withdrawal of the probe allowed tomographic assessment of the depth of tumor invasion in a relatively short examination time, with marked reduction in patient's burden and without complications. Linear reconstructed images were frequently disfigured by cardiac, respiratory or peristaltic motions during the *in vivo* study. Conversely, all the images were of good quality in the *ex vivo* study owing to the absence of motion artifacts.

In the *in vivo* study, the total 3-D EUS accuracy in the assessment of tumor infiltration depth was 87%. The accuracy rate was higher when the lesions were limited to m lesions (91%). The imaging quality including the ability to delineate the mm layer in the DPR images was thought to contribute to the high diagnostic value.[28] In our study, when the mm layer was visualized intact in all the DPR image sections, sm invasion could be accurately excluded. This might be of value in deciding the candidates for local endoscopic therapy as radical treatment could be achieved only in m-limited lesions.[29] Considering the depth of sm invasion as a factor, a minimal sm invasion of less than 1000 μm was detected in 3 out of 5 patients in the *in vivo* study, however, it could be visualized in all 5 patients in the *ex vivo* study. Although the figures are too small to provide statistical significance, it might be promising. The difference was related to the high-resolution linear reconstructed images obtained in all the *ex vivo* examinations; as there was no interference by undesirable motions, automatic spiral scanning could be performed at shorter intervals resulting in clear linear images that were more accurately correlated with the pathological features.[14] Consequently, sites of submucosal invasion, even when they were minimal, could be accurately depicted.

The presence of ulcer or ulcer fibrosis is one of the limiting factors for the accurate determination of tumor infiltration

Table 5. Comparison between the *in vivo* and *ex vivo* 3-D endoscopic ultrasonography (EUS) accuracy in the determination of tumor invasion depth according to the intensity of submucosal fibrosis in the lesions examined pre- and postoperatively

Fibrosis intensity	*In vivo* 3-D EUS study		*Ex vivo* 3-D EUS study		*P* value*
	Accuracy	CI (%)	Accuracy	CI (%)	
F–	4/4 (100%)	–	4/4 (100%)	–	–
F+	20/27 (74%)	55–87	25/27 (93%)	77–98	0.07
Minimal (+/–)	4/4 (100%)	–	4/4 (100%)	–	
Moderate (+)	6/8 (75%)	41–93	8/8 (100%)	–	0.23
Severe (++)	10/15 (67%)	42–85	13/15 (87%)	62–96	0.19

CI, confidence interval.
* *In vivo vs ex vivo*.

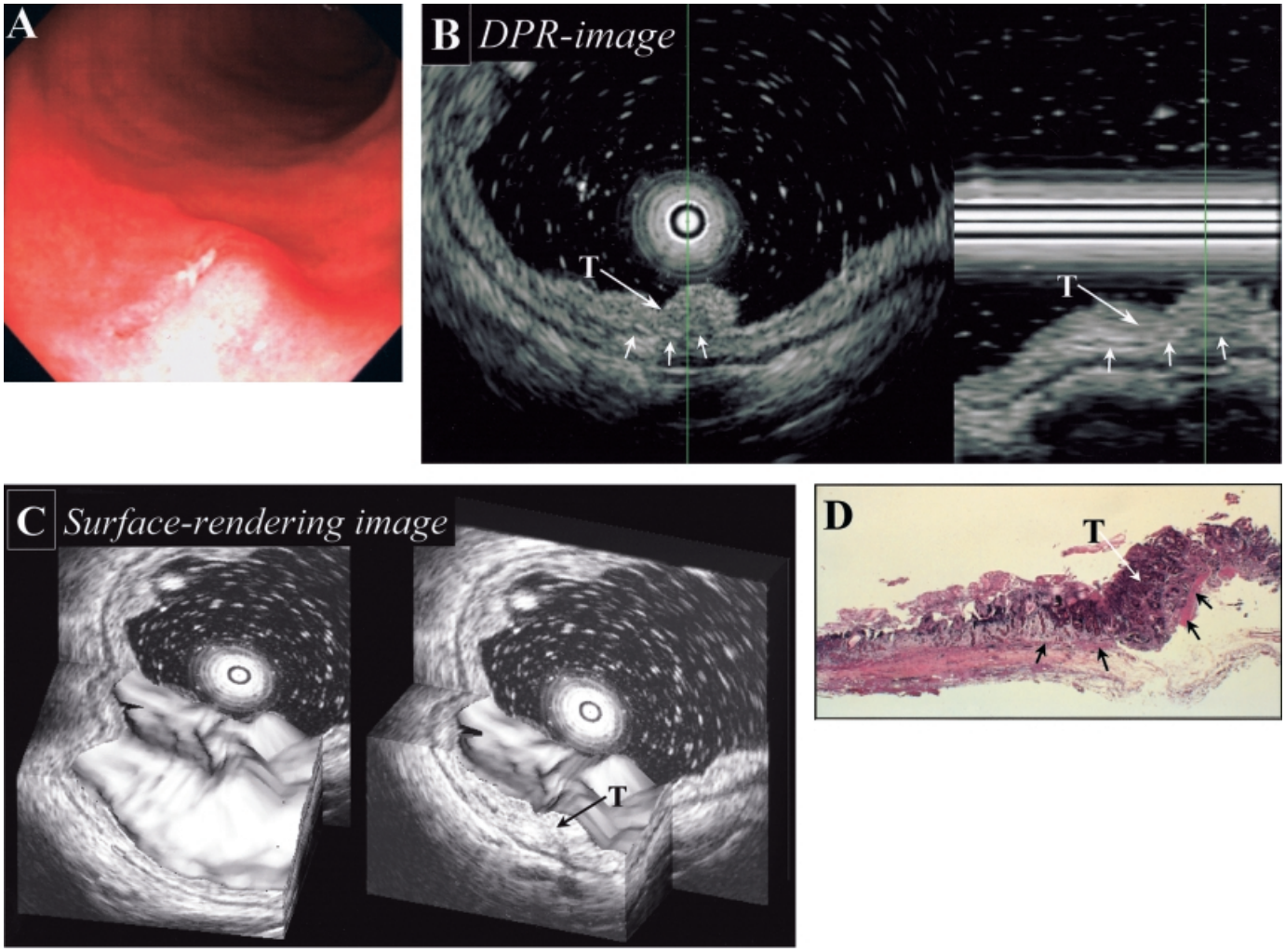

Fig. 4. *In vivo* 3-D endoscopic ultrasonography (EUS) of a mucosa-limited cancer (by a 20-MHz 3-D EUS probe). (a) Endoscopic view showing a superficially elevated gastric cancer. (b) The DPR image showing a hypoechoic tumor (T) occupying the first and second echo-layers. The mm layer (short arrows) is preserved intact in both radial and linear images, thus a mucosa-limited carcinoma was diagnosed. (c) Three-dimensional EUS surface-rendering image (left) closely similar to the endoscopic view. A radial cut section (right) shows the EUS features at the tumor center. (d) Histopathological section at the center of the tumor (T) resected by endoscopic mucosal resection shows the preserved mm layer (short arrows). The tumor was proved to be mucosa-limited. (H&E, original magnification ×15).

depth in gastric carcinoma.[30] In the present study, we tried to elucidate the effect of ulcer fibrosis at the site of cancer, as identified in the MT-stained histopathological sections, on the 3-D EUS diagnostic accuracy. To date, there is no standard EUS criterion for the diagnosis of ulcer-associated lesions; the available reports were dependent on individual observations.[31] In fact, we observed that despite the variations in the fibrous tissue-induced echo pattern, malignant infiltration of fibrous tissue is usually visualized as low echoic spots or patches with ill-defined margins. Such patterns were not always clearly detectable by radial EUS, but it was clearly observed in the linear reconstructed images if they were of good quality. In the present study, the presence of ulcer fibrosis significantly reduced the accuracy of tumor infiltration depth in the *in vivo* study because high-resolution DPR images could frequently not be obtained. However, higher accuracy for F+ lesions was obtained in the *ex vivo* part of the study. We therefore think that motion artifacts are the main obstacles to the reconstruction of clear linear images clinically and their elimination might provide higher accuracy rates. Improvement of the instrument is necessary and is expected to overcome this problem. For example, if the probe withdrawal rate is speeded up, the examination could be performed without interference by respiration or peristalsis. Synchronization of the scan with cardiac beats might be possible if the software is developed. The use of a water-filled balloon to maintain the distance between the probe and mucosal surface might also decrease the effects of motion artifacts.

As 3-D images were reconstructed from the recorded DPR images, their quality is also dependent on motion artifacts. Good surface-rendering images were reconstructed for 64% of the cancer lesions in the *in vivo* study, but in all except two (94%) of the studied lesions in the *ex vivo* study. The 3-D surface-rendering images might provide a useful tool for candidates of local endoscopic management as they

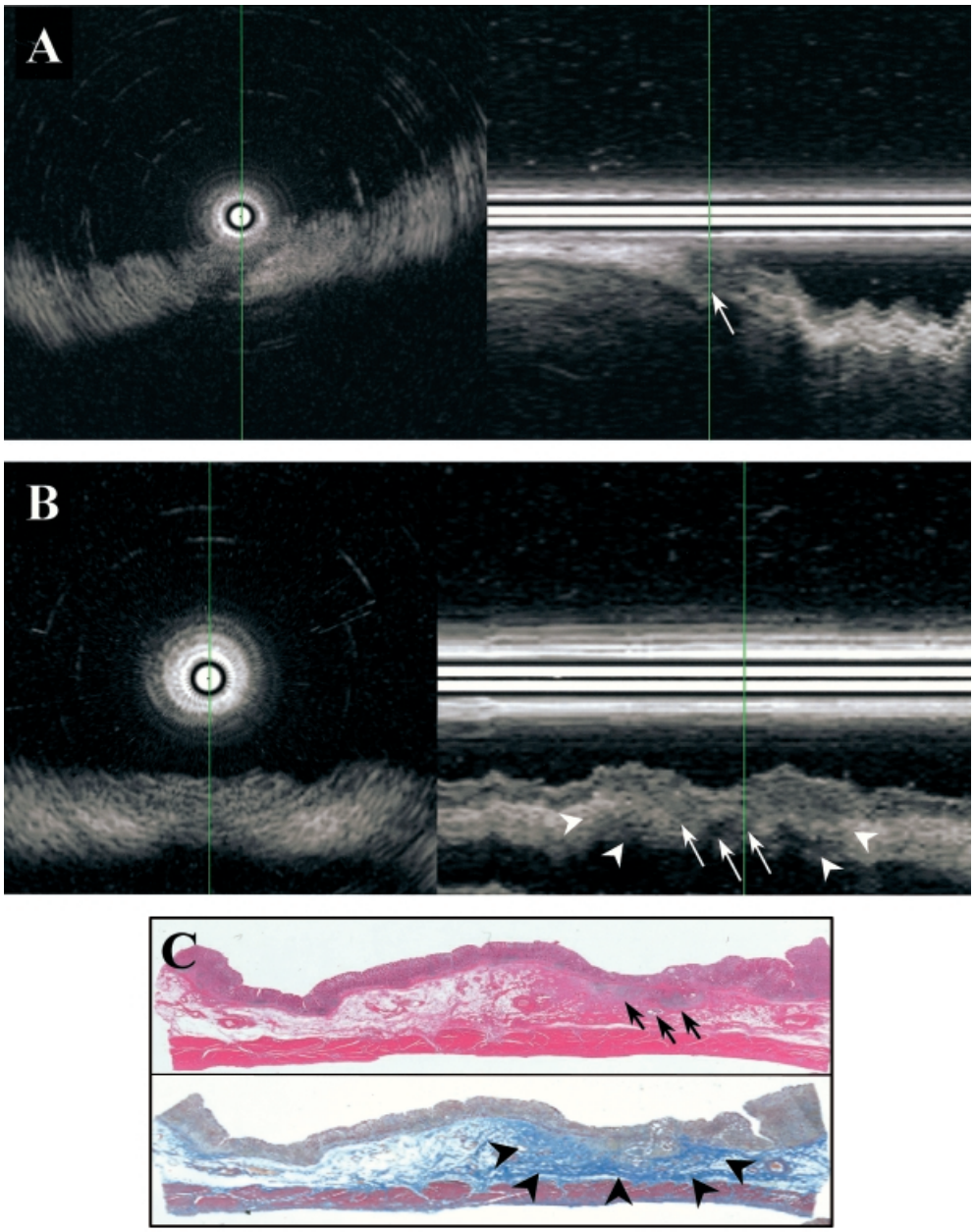

Fig. 5. Comparison between the *in vivo and ex vivo* 3-D endoscopic ultrasonography (EUS) of an early gastric carcinoma lesion invading the submucosa. (a) The DPR image obtained during the *in vivo* examination; motion artifacts interfered with the quality of the linear reconstructed image; however, the lesion was visualized as invading the submucosa (arrow). (b) The *ex vivo* DPR image shows a high-quality linear reconstructed image with clear demonstration of the layer structure; the site of submucosal invasion (arrows) as well as ulcer fibrosis (arrow heads) could be seen. (c) Histopathological study confirmed the 3-D EUS diagnosis; upper, H&E, original magnification ×5; arrows point to the site of sm invasion; lower; MT, original magnification ×5; arrow heads point to sm fibrosis. The *ex vivo* 3-D EUS features were more accurately correlated to the pathological findings.

combine the mucosal topographic features with the underlying EUS findings. Further such investigations are needed to define its role in the diagnostic or therapeutic fields.

In conclusion, 3-D EUS may have a beneficial role for the evaluation of gastric carcinoma, especially early lesions, and for planning their management. However, it is still a matter of debate as to whether the DPR images really could add valuable clinical information regarding the diagnosis of minimal submucosal tumor invasion or cases associated with ulcer fibrosis.

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