Original article

Proposal for two strategies to prevent remnants of gastric cancers after endoscopic mucosal resections: fluorescein electronic endoscopy and rapid stump diagnosis based on pit patterns

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Abstract

Background. The aim of the present study was to establish strategies to prevent cancer remnants after gastric endoscopic mucosal resection (EMR).

Methods. Whether surgical stumps could be diagnosed by pit patterns was examined on 38 well-fixed EMR materials. Furthermore, a rapid stump diagnostic method, which enables pit patterns of EMR material to be observed within 10 min, was developed. This rapid stump diagnosis was tested in 6 EMR cases of early gastric cancers, one of which was absolutely obscure to routine endoscopy. Fluorescein electronic endoscopy had been performed to reveal the extent of this cancer before EMR.

Results. Adenocarcinomas showed irregular pit patterns that were significantly different from those of normal gastric mucosae. Among the 38 cases, all 20 EMR materials that were diagnosed as stump (–) and 5 that were diagnosed as stump (+), based on pit patterns, were confirmed by tissue sections to be stump (–) and stump (+), respectively. Six of the 13 EMR materials diagnosed as stump (±) by pit patterns were finally diagnosed as stump (+) by tissue sections. These data indicate that cancer remnants could be avoided if the stumps were judged positive or suspiciously positive just after EMR, and additional resections were continuously done until the stumps became negative. Rapid stump diagnosis based on pit patterns was successful in all 6 tested cases. The extent of the early gastric cancer that was absolutely obscure to routine endoscopy was clearly and exactly revealed by fluorescein electronic endoscopies done before EMR and subsequent rapid stump diagnosis.

Conclusion. It is probable that fluorescein electronic endoscopy, as a precise preoperative examination, and rapid stump diagnosis, based on pit patterns, will become effective strategies to prevent cancer remnants after EMR.

Key words EMR · Cancer remnant · Pit pattern · Rapid stump diagnosis · Fluorescein electronic endoscopy

Introduction

It is now widely accepted that early-stage intramucosal cancers of the digestive tract, which do not have accompanying lymph node metastasis, can be treated and cured by therapeutic endoscopies, such as endoscopic mucosal resection (EMR) [1–3], laser irradiation [1,2], microwave coagulation [2], and photodynamic therapy [4,5]. It would be overtreating to radically operate on these kinds of cancers, considering the patient's quality of life. Among the therapeutic endoscopies, EMR is considered to be preferable, because resected material can be obtained and examined by pathologists to see whether or not there are remnants of cancer [1].

According to the treatment guidelines for gastric cancers announced in 2001 by the Japanese Gastric Cancer Association [6], EMR has become the treatment of choice for early gastric cancers limited to the mucosa and not accompanied by metastatic lymph nodes. However, curability by EMR is still lower than that by surgical resection [7]. The recurrence of gastric cancers after EMR is reported to be between 4.2% and 22.3%, with an average of 11.9%, and is considered to be the result of cancer-tissue remnants [6]. From the authors' experiences as pathologists at Tsuchiura Kyodo General Hospital, one of the four cancer centers in Ibaraki prefecture, it has become clear that avoiding cancer-tissue remnants at the first EMR is very important. This is because there are few recurrent cases that could be curably treated by a second EMR only, due to the submucosal fibrosis following the first EMR (Figs. 1 and 2). Most of the recurrent cases were treated by EMR combined with other therapeutic endoscopies, such as microwave coagulation, in which there was no means of confirming that the cancers had been completely removed. In fact, the authors occasionally encounter biopsy materials of re-recurrent gastric cancer after EMR performed as the first treatment and microwave coagulation as the second treatment. The authors

Fig. 1A–F. A case of recurrent gastric cancer due to cancer remnants after endoscopic mucosal resection (EMR). An elevated lesion (arrowhead), IIa, is obvious by conventional endoscopy (A) and chromo-endoscopy (B). It seemed that this lesion had been completely resected by the first EMR, but to be sure, two small pieces on the anal side were additionally resected (C). However, pathological examination revealed that most of the surgical stump was positive (asterisks), especially the two small pieces, where the stump was positive in all

directions (D). Thus, the endoscopist had failed to recognize the flat lesion, IIb, located anally to the elevated lesion. Three and a half months later, recurrent cancer was detected by biopsy anal to the EMR scar (arrows; E). The recurrent cancer was found to be a slightly depressed surface (broken line) anally adjacent to the EMR scar (arrows) on the surgical material (F). The tissue section on line, observed from the bottom (large arrow), is shown in Fig. 2

have also sometimes experienced stomachs that were operated on not long after EMR, due to cancer remnants that were usually minute, a few millimeters in size. Moreover, it was also reported by Nakamura et al., at the 63rd meeting of the Japanese Gastroenterological Endoscopy Society, in a panel discussion on how to treat remnant and recurrent cancers of the digestive tract after EMR (Gastroenterol Endosc, 2002; 44, pp. 421, in Japanese), that some patients with early gastric cancers, which were absolute indications for EMR, died of recurrent cancers after EMR. Considering that the purpose of EMR is to cure early gastric cancers without operation, and considering that almost 100% of patients with early gastric cancers for which EMR is indicated would be completely cured if they received a gastrectomy, endoscopists should take full responsibility and

do everything they can to prevent cancer remnants at the first EMR.

Excluding the technical problems of EMR and the lack of experience of endoscopists, the main cause of cancer remnants after EMR is the misreading of cancer extent before EMR (Fig. 1A–D). It is widely accepted that it is very difficult to determine the extent of early gastric cancers in the mucosa by routine endoscopies, including chromo-endoscopy, as in some cases they are flat, slightly depressed, or slightly elevated compared to normal mucosae (Fig. 1A,B). New techniques are, therefore, needed to reveal the exact extent of early gastric cancers not shown by routine endoscopy. Fluorescent endoscopies, such as autofluorescent endoscopy [8,9] and photodynamic diagnosis [10–12], are promising new techniques. However, the

Fig. 2. Tissue section of the recurrent gastric cancer after EMR, shown in Fig. 1F. The arrowhead indicates the border-line between the normal gastric mucosa on the right and the recurrent intramucosal cancer on the left. Note that significant fibrosis (asterisk) developed in the submucosal layer (SM) under the EMR scar and the recurrent cancer, and adhered firmly to the cancerous mucosa and the muscularis proprium (MP). Therefore, it would be difficult to treat this recurrent gastric cancer by a second EMR. H&E, $\times 10$

fluorescent endoscopic systems commercially available today are performed with a combination of fiberscopes and highly sensitive cameras; thus, they are expensive and clumsy to operate. Moreover, the images obtained are rather dim and not of high quality; thus, it is not easy to tell from where the fluorescence occurs. Because of these problems, it seems that fluorescent endoscopies will not become routine examinations. However, some of the problems have been resolved recently. We discovered that fluorescent endoscopies can be performed with an electronic endoscopic system, the Olympus Evis (Olympus, Tokyo, Japan), which is already widely used, especially in Japan, just by attaching a barrier filter to the objective lens of the electronic scope and fixing a light-balancing blue glass filter to an already equipped frame in front of the light source [13]. The images obtained are clear and bright enough to perform a biopsy under fluorescent observation; thus, it is easy to recognize from where the fluorescence occurs.

Another possible strategy to prevent cancer remnants is to perform rapid diagnosis on the surgical stumps of EMR materials. Routine histopathological examination of surgical stumps of EMR material is based on paraffin tissue sections, and usually takes a few days. Moreover, the exact extent of the cancer in EMR material is not obvious using this conventional examination. If there was any way that clinicians themselves could, just after the EMR, detect how the lesions had spread on the surface of the EMR material, based on pit patterns, they could determine the necessity for and location of additional resections in cases of suspiciously positive surgical stumps. Therefore, remnants of cancer after EMR could be avoided. Histological diagnosis of the colon

based on pit patterns has been well established and is routinely used in colonic magnifying endoscopy [14–17]. Compared to those of the colon, the pit patterns of the stomach have not yet been thoroughly examined, even by stereo-microscopy. This is probably because of the mucus on the gastric mucosal surface, which interferes with clear observations of pit patterns.

Recently, we succeeded in removing the mucus on fixed gastric mucosal surfaces by treatment with peracetic acid to detach the mucus, which was then brushed away with a drawing brush. In the present study, through the examination of the pit patterns of 38 gastric EMR materials, we found that it was easy to distinguish cancerous tissue from normal gastric mucosa and to determine cancer extent based on pit patterns. Furthermore, we developed a method for rapid stump diagnosis of EMR material based on pit patterns. This method was successfully tested in six cases of early gastric cancers treated with EMR. Among these six cases, one case was completely obscure on routine endoscopy. This early gastric cancer was clearly revealed by fluorescein electronic endoscopies performed before EMR.

Materials and methods

Stump diagnosis based on pit patterns on EMR materials

Firstly, to determine the effect of mucus on pit-pattern diagnosis, some EMR materials that had been fixed in formalin overnight were washed under running tap water for 10 min (a mucus-removal method used in other studies), stained with 0.25% methylene blue for 5–10 s, and observed and photographed under water, using a stereomicroscope (Olympus B071; Olympus). The same EMR material was then bleached with peracetic acid (acetic acid/hydrogen peroxide, 100:100 ml, with sulfuric acid, 0.5 ml, as a catalyst) until it became white. During this process, the mucus became degenerated, and appeared as a white membrane, which detached from the mucosal surface. The mucous membranes were then brushed away with a drawing brush under water. The EMR material was again stained with methylene blue, and observed and photographed under water, using a stereomicroscope.

EMR materials, resected by cap-assisted EMR (C-EMR) [18] between 2000 and 2003 were well fixed in formalin overnight and examined. Most of them were differentiated adenocarcinomas with diameters less than 2 cm, the absolute indication approved by the Japanese Gastric Cancer Association for EMR, and some were adenomas. They were bleached with peracetic acid, underwent mucous brushing, were stained

with methylene blue, and observed under water and photographed using a stereomicroscope, as described above. Finally, 38 EMR materials, in which the pit patterns were not collapsed by EMR procedures, were used. Among these 38 EMR materials, 22 were resected by en bloc-EMR (only one piece), 13 were resected by unplanned fractionated EMR (at the beginning, the lesion was intended to be resected as one piece, but after the main large piece was resected, more than one additional small piece was resected for fear of cancer remnants), and 3 were resected by planned fractionated EMR (from the beginning, the lesion was planned to be resected as more than two pieces). EMR materials were diagnosed, based on pit patterns, as stump (–) when the lesions were completely surrounded by normal mucosa, as stump (+) when the lesions were obviously seen reaching the surgical stumps, and as stump ($\pm$) when the lesions appeared to be reaching the surgical stumps or there were some areas with collapsed, unrecognizable pit patterns located between the lesions and the surgical stumps. The EMR materials were then sliced under a stereomicroscope, and routine pathological examinations were performed. The final results of the stump diagnosis, based on tissue sections, were compared to those based on pit patterns.

Rapid stump diagnosis based on pit patterns on EMR materials

In order to establish a method for rapid stump diagnosis, three conditions were set up. First, all processes had to be finished within 20 min. Second, pit patterns had to be observed clearly. Third, the structures of the EMR materials submitted to the processes of rapid stump diagnosis had to be well preserved, so that there would not be any problems in making a pathological diagnosis based on tissue sections.

Fresh gastric mucosa from surgical materials was stripped to roughly round pieces 2–3 cm in diameter. The stripped gastric mucosa was pitched (with stainless pins) to a soft vinyl plate, with the mucosal surfaces upward, and tested as follows:

1. The mucosal surface was brushed with a drawing brush and stained with 0.25% methylene blue, and the pit patterns were observed while they were still fresh.
2. Immersion in 20% formalin and irradiation with microwaves for 2 min was performed, then bleaching with peracetic acid was done for 2 min, and the mucosal surface was brushed with a drawing brush under water followed by staining with 0.25% methylene blue for 5–10 s. The pit patterns were then observed under water; the materials were fixed overnight, and subjected to routine examinations.

3. Immersion in hot (over 80°C) 20% formalin was done for 2 min, followed by bleaching with peracetic acid for 2 min, and brushing of the mucosal surface with a drawing brush under water; then staining with 0.25% methylene blue was done for 5–10 s. The pit patterns were then observed under water; the materials were fixed overnight, and subjected to routine examinations.

We found that method 3, by which pit patterns could be clearly observed within 10 min, gave excellent results. This method was actually tested in the endoscopy rooms, as to whether it worked clinically, in six cases of early gastric cancers treated by C-EMR.

Fluorescein electronic endoscopy for determining the extent of an early gastric cancer obscure to routine endoscopy before EMR

Fluorescein electronic endoscopy had been performed before EMR in one of the six EMR cases in which rapid stump diagnosis based on pit patterns was tested, as requested by the endoscopist, because the lesion was absolutely obscure to the previous routine endoscopy. Informed consent was obtained from the patient. An electronic endoscope (GIF-XQ 200; Olympus) with a barrier filter (Wratten Filter No. 15; Eastman Kodak, Rochester, NY, USA) attached to the objective lens was passed to the stomach. An adjuster filter (light-balancing, blue [LBB 200]; Olympus) was then placed in front of the light source, and, after a single intravenous injection of 10% fluorescein sodium (7 ml) was made over a few seconds through a plastic catheter inserted into a forearm vein, observations were then performed with the blue channel of the monitor being maximized and the red channel minimized. The findings were recorded by videotape, color film, and a video color printer. Markings were then made around the lesions, using an electric wire on the normal mucosa at a certain distance from the lesion. The lesion was subsequently submitted to C-EMR and the resected material was subjected to rapid stump diagnosis based on pit patterns, and subsequently, to standard light microscopy.

Results

Accuracy of stump diagnosis based on pit patterns on EMR materials

When EMR materials were not treated with peracetic acid, the mucosal surfaces were still covered with a large amount of mucus, so, although samples had been washed under running tap water for 10 min, it was impossible to observe pit patterns clearly (Fig. 3A,B). Among the 38 examined cases, the final diagnosis by

Fig. 3A,B. Stereomicroscopic photographs of gastric EMR material; **A** taken before the removal of mucus from the surface. It is impossible to observe the pit pattern accurately with mucus on the surface. **B** Stereomicroscopic photograph of the same EMR material as in **A**, taken after removal of the mucus. Now, the pit pattern is clearly observed. The *arrowhead* indicates a small amount of residual mucosa. *Arrows* indicate the borderline between normal mucosa *on the right*, showing

regular pit and interpit processes with a soft image, and adenomatous mucosa *on the left*, with some areas showing densely packed small round pits and others showing stone-like interpit processes, with rigid images. *On the left side*, the sub-mucosal layer is seen through a crack in the mucosal layer in the middle of the EMR material. **A** and **B** methylene blue, $\times 15$

Fig. 4. A,B Stereomicroscopic photographs, showing an example of en-bloc EMR material judged as stump (–) by pit patterns. **A** A lesion is seen *in the middle* as an area with irregular pit patterns surrounded in all directions by gastric mucosa with regular pit patterns. Parts of the surgical stump are seen in the *upper and lower right corners*. *Arrowheads* point to the borderline between the lesion and the normal mucosa. *Asterisks* show erosive areas, caused by burning with electric wire as markings. *Arrows* denote holes made by stainless pins used for fixation. **B** Higher magnification of the *rectangle* in **A**. *Arrowheads* indicate the borderline between the

lesion and the surrounding normal mucosa. Note that a marking (*asterisk*) is placed on the lesion just next to the borderline. *Arrows* show the cutting lines for paraffin sections. **C** Photomicrograph of tissue section cut on the *black-and-white line* in **B** and observed from the bottom (*large arrow*). *Asterisk* denotes the erosive area without surface epithelium adjacent to the normal mucosa *on the right*. Histologically, the lesion is well-differentiated adenocarcinoma. Note how precisely the pit patterns reflect the histological structures of the lesion (compare **B** and **C**). **A** Methylene blue, $\times 9$; **B** methylene blue, $\times 13$; **C** H&E, $\times 25$

tissue sections was well-differentiated adenocarcinoma (tub 1), in 32 cases, moderately to well-differentiated adenocarcinoma (tub 2 = tub 1) in 1 case, cancer in adenoma, in 1 case, and adenoma in 4 cases. Under

stereomicroscopy, adenomas and differentiated adenocarcinomas showed irregular pit patterns that were significantly different from the regular pit patterns of the surrounding normal mucosa; thus, it was easy to

Fig. 5. **A,B** Stereomicroscopic photographs demonstrating an example of unplanned fractionated EMR material judged as stump (+) by pit pattern. **A** The smaller piece of material was additionally resected for fear of cancer remnants after the larger one had been resected. **B** Higher magnification of the top view of the EMR material. The smaller piece shows regular normal pit patterns with medium-sized interpit processes, while the larger piece has very irregular patterns and shapes of pits, together with small interpit processes. Side views of the larger piece are shown in Fig. 6. **C** Cut lines for paraffin tissue blocks on the EMR material. A tissue section on the *black-and-white line* in **C**, observed from below (arrow), is shown in Fig. 7. **A** Methylene blue, $\times 3$; **B** methylene blue, $\times 13$

distinguish between them at first glance. In only a few cases was more time needed to differentiate normal mucosa from atypical epithelia.

All of the 20 EMR materials diagnosed as stump (–) based on pit patterns were confirmed to be stump (–) by tissue sections (Fig. 4A–C). All of the 5 EMR materials diagnosed as stump (+) based on pit patterns were also finally stump (+) (Figs. 5A–C; 6A,B; and 7A–C). Six of the 13 EMR materials diagnosed as stump ($\pm$) based on pit patterns were found to be stump (+). These data indicated that cancer remnants could be avoided if the stumps were judged as positive or suspiciously positive just after EMR, and if additional resections were then continuously performed until the stumps became negative.

The stump positivity rates, according to the method of EMR material resection, were as follows: 4 of the 22 EMR materials resected by en bloc-EMR, 7 of the 13 EMR materials resected by unplanned fractionated EMR, and none of the 3 EMR materials resected by planned fractionated EMR were finally

Fig. 6A,B. Stereomicroscopic photographs, showing side views of the larger piece of EMR material seen in Fig. 5. **A** The side without additional resection. The lesion, with remarkably irregular pit patterns, reaches the surgical stump to a widespread extent. **B** Additionally resected side. There is an area with normal pit patterns between the lesion and the surgical stump. Arrowheads indicate the borderline between the lesion and the normal mucosa **A** and **B** methylene blue, $\times 16$

diagnosed stump (+). The overall positive rate was 29% (11/38).

Rapid stump diagnosis based on pit patterns on gastric EMR materials

The fastest way to observe the pit patterns of EMR material is while they are still fresh (method 1). It was found that the fresh gastric mucosal epithelium did not stain with methylene blue or other stains; thus, the pit pattern could not be clearly observed. Therefore, the EMR materials had to be fixed. In order to shorten the fixation time, we first immersed the stripped fresh gastric mucosa in 100 ml of 20% formalin, then irradiated it under microwaves for 2 min (method 2). By this method, the stripped gastric mucosa was well fixed and pit patterns could be clearly observed. However, when tissue sections were made, the structure of the glands in the lower one-third of the gastric mucosa was found to be damaged. Irradiation of 100 ml of formalin for 2 min

Fig. 7A–C. Photomicrographs of the tissue section cut on the line in Fig. 5C. **A** Well-differentiated adenocarcinoma is seen reaching the surgical stump of the side without additional resection in the larger piece. **B** Higher magnification of the rectangle in **A**. Note the high nuclear/cytoplasmic (N/C) ratio and villo-tubular growth of the cancer cells. **C** This shows the additionally resected side of the larger piece and the additionally resected smaller piece. Arrowhead indicates the borderline between cancer on the left and normal mucosa adjacent to the resected end of the larger piece. Note that the additionally resected small piece is also normal mucosa. It is worth pointing out that this EMR material is also an example of surgical stump (+) due to preoperative misreading of cancer extent. **A** H&E, $\times 17$; **B** H&E, $\times 80$; **C** H&E, $\times 15$

resulted in boiling. Therefore, we tried fixing the stripped gastric mucosa in hot (over 80°C) formalin for 2 min (method 3). This time, the fresh gastric mucosa was well fixed and no structural damage was observed. When we performed rapid stump diagnosis on stripped gastric mucosa from a stomach with signet-ring cell carcinoma, the pit pattern could be clearly observed within 10 min (Fig. 8). The mucosal structures were so well preserved that, on frozen sections subsequently made, isolated signet-ring cells were clearly observed invading the stroma, which was covered with normal epithelium (Fig. 9A,B).

When we tested the rapid stump diagnostic method in the endoscopy room, the fresh EMR materials were first dipped in MgSO_4 1%, in saline, for 1 min, to relax the

mucularis mucosae, which always constricts after being stripped by electric wire in C-EMR, thus making it easier to spread and pitch the EMR material on the soft vinyl plate. The materials were then immersed in hot (over 80°C) formalin, prepared in a thermos bottle, for 2 min for fixation. After a brief wash with water, the EMR material was put into peracetic acid for 2 min, the mucus on the surface was brushed off with a drawing brush under water, and the material was then stained with 0.25% methylene blue for 5–10 s and observed under water with a stereomicroscope. For each piece of EMR material, pit patterns could be clearly observed within 10 min from the time the material was taken from the patient's body.

Rapid stump diagnosis was successful in all six cases tested. These cases were: one signet-ring cell carcinoma (IIc; 5 mm) and five differentiated adenocarcinomas (two IIa + IIc, one IIa, one IIb, and one IIc lesions; diameters ranging from 5 to 16 mm). The signet-ring cell carcinoma was completely resected by two-piece EMR. The entire depressed lesion was seen to be inside the first piece, surrounded by normal mucosa. Because the signet ring cells may have invaded the surrounding mucosa without causing any changes to the pit patterns of the covering foveolar epithelium, the second piece was additionally resected on the side where the lesion was near the surgical stump. Histopathological examination made a few days later showed that signet ring cells existed only in the first piece. The two cases of differentiated adenocarcinomas that were endoscopically considered as IIa + IIc (elevated lesion with a depressed area in the middle) were completely resected by en-bloc EMR. It was revealed by rapid pit pattern diagnosis, and later confirmed by tissue section, that the cancers were limited only to the depressed parts (IIc), while the elevated parts (IIa), together with the surrounding marginal zone, consisted of normal mucosa. One case of differentiated adenocarcinoma, an elevated IIa lesion, was completely resected by four-piece EMR. In the fifth case, rapid stump diagnosis was done on the second EMR 5 days after the initial one, because the stump had been diagnosed as positive by tissue section. The second time, the endoscopist had no idea exactly where the remnant cancer was exactly located. Four pieces of gastric mucosa were stripped from the area close to the ulcer formed from the first EMR. By the rapid stump diagnostic method, the remnant cancer was found in one piece of the stripped gastric mucosa with one side adjacent to the ulcer and the other sides surrounded by normal mucosa. Therefore, this time, the stump was judged negative by pit patterns, and later tissue sections confirmed this finding. The sixth case is described in the next section.

Fig. 8. The processes of rapid stump diagnosis based on pit pattern. A piece of stripped gastric mucosa from an operated stomach with signet-ring cell carcinoma was used. Within 10 min, pit patterns could be observed clearly under stereomicroscopy. At the marginal zone, normal gastric mucosa with a regular pit pattern was seen between the stump and the area with a collapsed pit pattern. After observation of the pit pattern, the stripped mucosa was cut into 2-mm-wide ribbons. Three cutting lines are seen in this photograph. A frozen tissue section taken along the line indicated by the vertical arrow and observed from the bottom (large arrow) is shown in Fig. 9. Methylene blue, $\times 20$

Fig. 9. **A** Frozen tissue section made on the indicated line drawn in Fig. 8. Arrowhead indicates the borderline between the area with a collapsed pit pattern and normal mucosa. **B** Photomicrograph of the rectangle in **A**, showing signet ring cells (arrowheads) invading the stroma, which is covered by normal foveolar epithelium. Compare signet ring cells (arrowheads) with plasma cells (arrows). Inset, Photomicrograph at higher magnification (H&E, $\times 500$) of the signet ring cells shown by the arrowheads in **B**. **A** H&E, $\times 25$; **B** H&E, $\times 200$

Fluorescein electronic endoscopy for determining the extent of early gastric cancers obscure to routine endoscopy before EMR

During a follow-up endoscopy of an early gastric cancer in the antrum previously resected by EMR, another cancer had been found incidentally in the biopsy material from the gastric angle. However, cancer was not found in biopsy material taken from nearby mucosa with the same appearance as the gastric angle under routine endoscopy (Fig. 10A,B). In order to determine the exact location and extent of the cancer, fluorescein electronic endoscopy was performed before further EMR and subsequent rapid stump diagnosis.

Upon observation under the light passing through the adjuster filter, and with the barrier filter in front of the objective lens, a few minutes after the injection of fluorescein sodium, the contour of the surface microstructures, i.e., pits and interpit processes, became evident, brought about by the contrasting effect of fluorescein sodium. The cancer then appeared as a depressed surface with irregular surface microstructures, showing somewhat weaker fluorescence intensity compared to the surrounding normal mucosa (Fig. 10C). After markings were made around the lesion (Fig. 10D), the electronic endoscope with a barrier filter was changed to one with no filter but with a clear cap attached to its head to perform EMR under conventional observation. It was found that the markings on the anal side still remained after the first resection; therefore, an additional resection was done (Fig. 10E). By rapid stump diagnosis based on pit patterns, the lesion was found to be suspiciously positive; it reached the anal side stump of the first piece of resected mucosa, i.e., surgical stump ($\pm$) (Figs. 10F and 11A), but no lesion was found

Fig. 10A–F. A case of early gastric cancer not evident by conventional endoscopy, in which fluorescein electronic endoscopy was performed before EMR and rapid stump diagnosis, based on pit patterns. **A** Conventional endoscopy, showing the gastric angle and lesser curvature of the antrum. No significant changes could be seen. **B** Two biopsies were randomly performed on the angle (*arrow*) and an area nearby on the anal side (*double arrows*). It was found that the specimen from the angle was cancer, but the other area was not. *Arrowheads* in **A** and **B** point to the same line. Note that it was not possible to recognize or determine the extent of the cancer by conventional endoscopy. **C** Under fluorescein electronic endoscopy, the cancer appeared as a slightly depressed surface with irregular surface microstructures, i.e., pits and interpit processes, and showed somewhat weaker fluorescence intensity

compared to the surrounding normal gastric mucosa with regular surface microstructures. **D** Before EMR, markings were made under fluorescein electronic endoscopy. The *markings*, shown by *asterisks*, served as landmarks compared to the stereomicroscopic photograph of the resected material (shown in **F**). **E** The lesion was resected in two pieces. The second piece (2) was additionally resected because it was found that some markings on the anal side remained on the gastric mucosa after the first resection (1), and rapid stump diagnosis, based on the pit pattern, also showed that the anal surgical stump of the first piece was suspiciously positive. **F** Stereomicroscopic photograph, showing low magnification of the first piece of resected gastric mucosa (1). *Asterisks* denote the same landmarks as in **D**. Higher magnification of the *rectangle* is shown in Fig. 11A. **F** Methylene blue, $\times 5$

in the second piece of resected mucosa. The stump was, therefore finally, judged to be negative. Tissue sections showed that the lesion was well-differentiated adenocarcinoma located only in the first piece of resected mucosa, with no part reaching the surgical stump (Fig. 11B).

Discussion

The data in the present study support the idea that the preoperative misreading of cancer extent is a major

cause of gastric cancer remnants after EMR. The stump positive rate (7/13) was highest in the unplanned fractionated EMR materials, in which cancer tissue was usually not found in the additional pieces resected for fear of cancer remnants (Figs. 5, 6, and 7). This means that the extent of the cancers had not been correctly recognized. Although there were only three cases of planned fractionated EMR, no stump-positive cases were found. This implies that there would be few remnants after EMR if the cancer extent were clearly recognized. Based on the 38 EMR materials thoroughly examined both by pit patterns and tissue sections, the

Fig. 11. **A** Higher magnification of the *rectangle* shown in Fig. 10F, with the anal stump on the *right hand side*. Arrows show the borderline between the normal mucosa with regular pit pattern, *at the bottom*, and the lesion with a somewhat irregular pit pattern, *on the top*. Note that this is an example of a rare case, as the pit pattern of the cancer is not easily distinguished from that of the normal mucosa. From the top view, the lesion is seen to reach some parts of the surgical stump, and was thus judged as stump ($\pm$) by the pit pattern. **B** Tissue section cut *on the line in A* and observed from below (*large arrow in A*). It became clear that a small part of the normal mucosa not seen under stereomicroscopy intervened between a well-differentiated adenocarcinoma and the cut end. The *arrow-head* denotes the borderline between the lesion *on the left* and normal mucosa *on the right*. **A** Methylene blue, $\times 9$; **B** H&E, $\times 12$

overall stump (+) rate reached 29%. Therefore, there would have been two to three cases out of ten in which the extent of the cancer had been incorrectly recognized.

As reported in the present study and in our previous study [13], fluorescein electronic endoscopy was effective in detecting and determining the extent of early gastric cancers not evident with routine endoscopy. After reaching the stomach, fluorescein sodium leaks from blood vessels and accumulates more in mucosa with low glandular density and large stromal volume. Usually, most gastric adenomas and differentiated adenocarcinomas show higher glandular density and less stromal volume than normal mucosa. Under fluorescein electronic endoscopy, these entities therefore show weaker fluorescence than the surrounding normal mucosa. On the other hand, differentiated adenocarcinomas with lower glandular density and higher stromal

volume show stronger fluorescence than the normal mucosa. Areas with undifferentiated adenocarcinomas sparsely invading stroma that is covered with normal epithelium also demonstrate stronger fluorescence, because normal glands collapse following cancer invasion; thus, the stromal volume is increased. In any case, borderlines between the cancer and the surrounding normal gastric mucosae can be clearly observed. It should be emphasized here that the findings of fluorescein electronic endoscopy are not diagnostic. However, there is no doubt that it is a powerful tool to determine the extent of known lesions previously diagnosed by biopsy but with unclear borderlines, as was demonstrated in the present study.

Another possible method for the preoperative detection of the extent of early gastric cancer not evident by routine endoscopy may be the use of magnifying endoscopy. This method, however, has some problems. Unlike stereomicroscopy, the field of vision under magnifying endoscope observation is very narrow, and the movement of the stomach walls due to respiration has to be suppressed while observing *in vivo* in order to obtain clear images. Therefore, it may take quite a long time to determine the entire borderline of the lesions by magnifying endoscopy.

Whether early gastric cancers with obscure extent under routine endoscopy should be examined by new methods such as fluorescein electronic endoscopy before EMR is not the question. The question is whether fluorescein electronic endoscopy should be performed before EMR even in cases of early gastric cancers appearing on routine observation. Tada et al. reported, in their presentation at the 78th Kanto Area Meeting of the Japanese Gastroenterological Endoscopy Society, in a symposium on the verification of gastric and esophageal EMR, that the recurrence rate due to lateral stump positivity was nearly 10% in the gastric EMR cases in which preoperative endoscopic examinations, including chromo-endoscopy, were thoroughly done. Considering this fact, fluorescein electronic endoscopy should be performed to determine the exact extent of early gastric cancers before EMR in every case, if possible. In cases where fluorescein electronic endoscopy is not performed before EMR, the endoscopist should at least perform rapid stump diagnosis based on pit patterns to prevent cancer remnants after EMR. The processes of this examination are so simple and fast that any medical staff, or nurses, could prepare EMR materials for the endoscopist to observe their pit patterns while the endoscopist is observing whether there are any problems, such as bleeding or perforation, after EMR. Therefore, soon after finishing this, he or she should be able to see whether or not the stump is negative. In the authors' routine histopathological work, they had experience in examining, by stereomicroscopy

and tissue sections, total gastrectomy materials which had been treated by EMR 2 weeks previously for an early gastric cancer in the cardiac area. The whole stomach had been resected because cancer remnants had been found in the biopsy material taken after EMR. It was shown that a cancer only a few millimeters in size remained adjacent to the fresh EMR scar. This could have been prevented if the endoscopist had done rapid stump diagnosis based on pit patterns just after EMR.

EMR techniques can be divided into two types: cap-assisted EMR (C-EMR) [18–20] and endoscopic submucosal dissection (ESD) [21–23]. In the first type, C-EMR, the lesions are aspirated into a cap attached to the head of an endoscope, are ligated by a wire, and electrically resected. In the second type, ESD, the lesions are stripped, little-by-little, from the gastric wall at the submucosal layer by a diathermic knife. C-EMR has the advantage of having relatively easy-to-master techniques, and the operative time is shorter. However, the size of the gastric mucosa resected in one resection is smaller. Therefore, it usually becomes a fractionated resection when applying these techniques to large lesions. The recurrence rate is higher when the lesions are subjected to fractionated resection, probably due to residual cancerous tissue [6,22]. ESD is considered to be preferable for large lesions because the en-bloc resection rate is higher than that of C-EMR (22). With the lesions resected in one piece, it is easier for pathologists to examine whether or not the stump is negative, or whether there are cancer cells invading the venous and lymphatic vessels of the submucosal layer. However, it is more difficult to master ESD techniques, and the operative time is longer; also, side effects, such as bleeding and perforation, seem to occur more frequently. For these reasons, ESD might not come into widespread use [18,22]. Needless to say, it does not matter if an early gastric cancer is resected by C-EMR or ESD; accurate preoperative diagnosis, by fluorescein electronic endoscopy, of the cancer extent, and rapid stump diagnosis, based on pit patterns, will help to prevent a cancer remnant. Theoretically, practicing rapid stump diagnosis will dramatically diminish the residual rate on the lateral cut ends of fractionated resections of large lesions by C-EMR. If the marginal zone of a large lesion, together with the surrounding normal mucosa, is resected and the borderline between the lesion and normal mucosa on the EMR material is confirmed individually immediately under a stereoscope, and the marginal zone is completely resected, the cancer tissue left in the middle can then be resected without fear of a cancer remnant. It was reported at the 67th Congress of the Japan Gastroenterological Endoscopy Society, by the Endoscopic Therapeutic Efficacy Evaluating Committee that, based on long-term data from multiple hospitals, although early gastric cancers with diameters

between 2 and 4 cm were subjected to fractionated resection by C-EMR, there were no recurrences in EMR materials that were diagnosed as stump (–) by the pathologists.

Nowadays, the importance of placing patients in medical care centers has been demonstrated many times. Concerning the treatment of very early gastric cancers, in order to allow patients to receive the full benefits of EMR techniques, the authors would like to propose that the Japanese Gastric Cancer Association should test the usefulness of fluorescein electronic endoscopy and rapid stump diagnosis based on pit patterns in preventing cancer remnants. If these techniques are accepted, the Association should ask the Ministry of Health and Welfare to cover the cost of these techniques in the health insurance plan. If this is done, endoscopists will have to take more responsibility to prevent cancer remnants after EMR.

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