

## Format for ANSWERING REVIEWERS

July 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 19935-revised manuscript).

**Title:** Management and associated factors of delayed perforation by gastric endoscopic submucosal dissection

**Author:** Haruhisa Suzuki, Ichiro Oda, Masau Sekiguchi, Seiichiro Abe, Satoru Nonaka, Shigetaka Yoshinaga, Takeshi Nakajima, Yutaka Saito

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 19935

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Reviewer 02941580. Major point 1.** In table 3, the author performed multivariate analysis. In page 8, they state that they performed multivariate analysis using 1) outside clinical indications, 2) gastric tube cases, 3) location, and 4) a procedure time  $\geq 2$  h included that stomach status. In my opinion, it would be better to perform multivariate analysis including histologic type because it is significant in univariate analysis ( $p=0.09$ ) and they must show each odd ratio and p-value in Table3.

**Answer.** As we indicated in “Statistical analysis” of the MATERIALS AND METHODS section, Page 8, a value of  $P<0.05$  was considered statistically significant in this study. Therefore, we did not include histologic type in multivariate analysis because it was not statistically significant ( $p=0.09$ ) in univariate analysis. In addition, we added the results of multivariate analysis (NS: Not significant) in the relevant portions of this table.

(2) **Reviewer 02941580. Major point 2.** The author reported cases treated conservatively by the endoclips and endoloop. However, recent article by Kang SH et al (Clin endoscopy: 2015;48(3):251), suggest that treatment with emergency surgery should be used instead of conservative management in cases of delayed perforation after ESD because delayed perforation may be associated with excessive thermal damage and necrosis of the muscle layer. What is the reason of this discrepancy?

**Answer.** As above-mentioned case report by Kang SH described, we also consider that emergency surgery should be performed in the case of delayed perforation with panperitonitis or severe mediastinitis (gastric tube cases) with remarkable physical findings, although we fortunately experienced a small number of cases of delayed perforation managed conservatively without surgical intervention. Thus, we have already indicated the necessities of emergency surgery in the DISCUSSION section, Page 11 as follows:

“Thus, although a small number of cases of delayed perforation might be successfully managed conservatively..., we need to remember that in delayed perforation cases, emergency surgery may be required with a high probability and conservative management might not always be feasible.”

In addition, we added the above-mentioned paper in the REFERENCE section as follows:

“**Kang SH**, Lee K, Lee HW, Park GE, Hong YS, Min BH. Delayed Perforation Occurring after Endoscopic Submucosal Dissection for Early Gastric Cancer. *Clin Endosc* 2015; **48**: 251-255 [PMID: 26064827 DOI: 10.5946/ce.2015.48.3.251.]”

(3) **Reviewer 02941580. Minor points 3.** In the introduction section, it would be better to include the paper that deals with the indication for endoscopic submucosal dissection outside Japan. (e.g *J Gastroenterol Hepatol* 2011;26(5):884)

**Answer.** According to the reviewer’s comment, we added the indication for endoscopic submucosal dissection outside Japan in the INTRODUCTION section, Page 4 and REFERENCE section as follows:

“Endoscopic submucosal dissection (ESD) is widely used in East Asia (e.g., Japan and Korea) as an initial... lesions<sup>[1-4]</sup>.”

“**Chung JW**, Jung HY, Choi KD, Song HJ, Lee GH, Jang SJ, Park YS, Yook JH, Oh ST, Kim BS, Kim JH. Extended indication of endoscopic resection for mucosal early gastric cancer: analysis of a single center experience. *J Gastroenterol Hepatol* 2011; **26**: 884-887 [PMID: 21198830 DOI: 10.1111/j.1440-1746.2010.06611.x.]”

(4) **Reviewer 02941580. Minor points 4.** In the text, the term “Gastric tube” seems very hard to understand. In the context, it means “the stomach remained in thorax after esophagectomy.” I suggest authors that they should explain that in the material and method section.

**Answer.** According to the reviewer’s comment, we added the explanation of the term “Gastric tube” in the MATERIALS AND METHODS section, Page 6 as follows:

“The definitions for the characteristics of EGC lesions... treatment guidelines<sup>[3,25]</sup>. The term ‘gastric tube’ refers to a stomach conduit that has been pulled up into the thorax for use as an esophageal substitute after an esophagectomy<sup>[23,24]</sup>.”

In addition, we added the reference about “Gastric tube” as follows:

“**Mukasa M**, Takedatsu H, Matsuo K, Sumie H, Yoshida H, Hinosaka A, Watanabe Y, Tsuruta O, Torimura T. Clinical characteristics and management of gastric tube cancer

with endoscopic submucosal dissection. *World J Gastroenterol* 2015; **21**: 929-925 [PMID: 25624726 DOI: 10.3748 / wjg.v21.i3.919]”

(5) **Reviewer 02955019. Comment 1.** Materials and methods; Page 6, Line 6: the marking of dots around it→ Please mention the margin-distance (cm) between the marking and the tumor edge.

**Answer.** According to the reviewer’s comment, we included additional information of the marking of dots in the MATERIALS AND METHODS section, Page 6 as follows:

“The ESD procedure began... and the marking of dots at a distance of about 5 mm outside of the lesion.”

(6) **Reviewer 02955019. Comment 2.** Materials and methods; Page 7→ Please describe the detail of endoloop-endoclip closure technique, as well as the detail of the conservative management without the endoloop-endoclip technique (PPI and antibiotics infection, etc).

**Answer.** According to the reviewer’s comment, we described the detail of endoloop-endoclip closure technique, as well as the detail of the conservative management without the endoloop-endoclip technique in “Incidence and actual management of delayed perforation” of the RESULTS section, Page 8-9 as follows:

“Among the 4 cases.... using an endoloop-endoclip technique<sup>[23,26]</sup>. In this technique, the endoloop snare was anchored with some clips to the normal mucosa around the delayed perforation defect. The endoloop snare was tightened slightly, approximating the borders of the defect. Finally, additional clips were placed to achieve complete closure.”

“As for the management... 4 were conservatively managed with nasogastric tube placement, fasting, and the use of intravenous antibiotics and proton pump inhibitors.”

In addition, we added the reference about “endoloop-endoclip closure technique” as follows:

“**Matsuda T**, Fujii T, Emura F, Kozu T, Saito Y, Ikematsu H, Saito D. Complete closure of a large defect after EMR of a lateral spreading colorectal tumor when using a two-channel colonoscope. *Gastrointest Endosc* 2004; **60**: 836-838 [PMID: 15557972] ”

(7) **Reviewer 02955019. Comment 3.** Materials and methods; Page 7, “Definition of delayed perforation induced by gastric ESD” → Please mention the blood test data and clinical symptoms (fever up, muscular defense, etc) for diagnosis of delayed perforation.

**Answer.** Delayed perforation was identified by the sudden appearance of abdominal pain or chest pain, although no remarkable clinical symptoms were observed, suggesting perforation, just after the ESD procedures. As for the pyrexia and abnormal blood test data, there were a number of varieties of these findings in each case. Especially in cases of panperitonitis, the clinical symptoms including pyrexia and abnormal blood test data were remarkable.

We added the detail information of the definition of delayed perforation in the MATERIALS AND METHODS section, Page 8 as follows:

“Delayed perforation was identified by the sudden appearance of symptoms of peritoneal or mediastinal pleura irritation (gastric tube case) after the completion of gastric ESD, with free air visible on X-ray or computed tomography (CT) images and/or with a gross defect observed endoscopically, although endoscopically visible perforations did not occur during the ESD procedure and no remarkable clinical symptoms were observed, suggesting perforation, just after the ESD procedures.”

(8) **Reviewer 02955019. Comment 4.** Results; Page8, “Incidence and actual management of delayed perforation” Line 18→Please mention the blood test data and clinical symptoms (fever up, muscular defense, etc) for diagnosis of delayed perforation which are the criteria for the emergency surgery.

**Answer.** We consider that emergency surgery should be performed in the case of delayed perforation with panperitonitis or severe mediastinitis (gastric tube cases) with remarkable clinical findings such as diffuse and severe tenderness and/or defense musculaire.

We added the detail information of the criteria for the emergency surgery in “Incidence and actual management of delayed perforation” of the RESULTS section, Page 9 as follows:

“The reason for the emergency surgery in these three cases was panperitonitis with remarkable clinical symptoms, such as diffuse and severe tenderness and/or defense

musculaire.”

(9) **Reviewer 02955019. Comment 5.** Results; Page8, “Incidence and actual management of delayed perforation” , Line 20→Please describe the statistical difference of hospital stay or other clinical short outcomes between the cases treated by endloop-endoclip closure technique and those by conservative management without the endloop-endoclip technique.

**Answer.** Because of too few cases (delayed perforation cases treated by endloop-endoclip technique and delayed perforation cases managed conservatively without endloop-endoclip technique were only two cases, respectively), we decided the statistical calculation was not suitable. Actually, as for the median hospital stay, there was no significant difference of two groups (the median hospital stay in the delayed perforation cases treated by endloop-endoclip technique was 21.5 days, while that in the delayed perforation cases managed conservatively without endloop-endoclip technique was 31.5 days (no significant difference)).

(10) **Reviewer 02955019. Comment 6.** Figure legends; Page18, Figure 1A→ Please describe additionally the tumor size, histopathological diagnosis.

**Answer.** We indicated the Figure legends briefly so we described the detailed information in the RESULTS section of the main text, Page 9.

(11) **Reviewer 02955019. Comment 7.** Figure legends; Page18, Figure 1B→ Please describe the length and circumference of the ESD ulcer.

**Answer.** According to the reviewer’s comment, we revised the Figure IB legend as follows:

“Mucosal defect just after the completion of ESD (60 mm in size and half circumference).  
ESD: Endoscopic submucosal dissection.”

In addition, we added the more detailed information of the Mucosal defect in “Factors associated with delayed perforation” of the RESULTS section, Page 10 as follows:

“ESD was performed.... complications. As for the mucosal defect just after the completion

of ESD, the size of the defect was 60 mm, and the circumferential extent of the defect was one half of the lumen of the gastric tube.”

(12) **Reviewer 02955019. Comment 8.** Figure legends; Page18, Figure 1D→ Please explain the actual process of endoloop-endoclip closure (the number and places of applied the clips, and the method to bundle the clips by endoloops, etc).

**Answer.** According to the reviewer’s comment, we added the actual process of endoloop-endoclip closure in the RESULTS section of the main text, Page 10 as follows:

“However, this patient did not develop... the endoloop-endoclip technique was attempted and was successfully performed (Figure 1D). In detail, the endoloop snare was anchored with some clips to the normal mucosa around the delayed perforation defect. The endoloop snare was tightened slightly, which approximated the borders of the defect. To achieve complete closure, two endoloop snares with additional clips were needed.”

(13) **Reviewer 02941342. Comment 1.** Due to one of the factors involved in acute and late perforation is the current type (cut / coagulation) used during the stage of dissection of the submucosa, and also because of the low rate of perforation reported by the authors, I think it would be useful to readers to explain briefly what kind of power was usually used during the dissection phase. I think this could be stated in the part of ESD procedure.

**Answer.** According to the reviewer’s comment, we added the Table of the set-up for the high-frequency generators for ESD at our hospital and added this information in the “ESD procedure” of the MATERIALS AND METHODS section, Page 6 as follows:

“Cases with the bleeding ... and/or hemostatic forceps (Coagrasper (FD-410LR; Olympus Optical, Tokyo, Japan) and Radial Jaw hot biopsy forceps (Boston Scientific Japan, Tokyo, Japan)), or by grasping them with endoclips. The set-up for the high-frequency generators for ESD along with the IT knife for early gastric cancer (ICC200 Erbe Elektromedizin, Tübingen, Germany, ESG100 Olympus Medical and VIO300D Erbe Elektromedizin, Tübingen, Germany) is shown in Table 2.”

(14) **Reviewer 02941342. Comment 2.** Also, the authors could say (although this is shown

in the results), no differences in the rate of delayed perforation were found in the group of patients with absolute indications and expanded indications.

**Answer.** According to the reviewer's comment, we added the rate of delayed perforation in the group of patients with absolute indications and expanded indications in "Factors associated with delayed perforation" of the RESULTS section, Page 9 as follows:

"Based on univariate analyses,... with a delayed perforation (Table 4). No significant difference between the rates of delayed perforation was observed when the absolute indications (0.1%) and the expanded indications (0.1%) were applied."

(15) **Reviewer 02941342. Comment 3.** Some minor spelling errors were underlined and corrected in the text.

**Answer.** We revised the relevant portions accordingly.

(16) **Reviewer 02840060. Comment 1.** First, because complications may be associated with operator experience, the authors should state information of operators. For example, are there significant between >50 of gastric ESD and <50 in regard to operator experience?

**Answer.** In this study, we did not assess the association between the delayed perforation and operator experience. But as our previously report indicated (Oda I, Odagaki T, Suzuki H, et al. Learning curve for endoscopic submucosal dissection of early gastric cancer based on trainee experience. Dig Endosc 2012; 24: 129-132), less experienced endoscopists could perform gastric ESD with higher resectability in addition to lower complications rates (because the step-by-step training system in our center has been highly effective with an en bloc resection rate of 100% and a low complication rate).

(17) **Reviewer 02840060. Comment 2.** Second, resection margin differs whether differentiated or undifferentiated. Even if tumor size of two lesions are equal, there is a potential for a difference between each resection size of two lesions.

**Answer.** We make marking of dots at a distance of about 5 mm outside of the lesion, followed by a circumferential mucosal incision about 5 mm outside the marking dots irrespective of the histological type. Actually, we compared the resection size of

differentiated-type early gastric cancers achieving curative resection for absolute indications ( $\leq 2$ cm) (n=781) with that of undifferentiated-type early gastric cancers achieving curative resection for expanded indications of undifferentiated-type ( $\leq 2$ cm) (n=43) using our data of previously report (Haruhisa Suzuki, et al. High rate of 5-year survival among patients with early gastric cancer undergoing curative endoscopic submucosal dissection. Gastric cancer 2015). As a result, the median lesion size of differentiated-type cancers and undifferentiated-type cancers were 10mm and 10mm, respectively (not significant difference) and then the median resection size of differentiated-type cancers and undifferentiated-type cancers were 35mm and 35mm, respectively (not significant difference).

(18) **Reviewer 01047630. Comment 1.** The authors classified the cases of perforation into perforation during ESD and delayed perforation. However, the definition of delayed perforation is somewhat vague in this study. If an undetected perforation is found after ESD (within 12 hours), is this case regarded as delayed perforation? Therefore, more strict definition of delayed perforation should be stated in the method section. Furthermore, the definition of delayed perforation should be differentiated from undetected perforation during ESD. After that, the data should be re-analyzed.

**Answer.** In this study, we did not check undetected perforation because in most of the cases undergoing gastric ESD in our hospital, we did not perform chest radiography or CT examination. However, according to the reviewer's comment, from the aspect of developing delayed perforation, we added the possibility of the existence of severe damage to the surface of the muscularis propria with transmural air leak in the Discussion section, Page 12 and REFERENCES section as follows:

"In addition, Hanaoka et al<sup>[14]</sup>..., resulting in necrosis. Furthermore, Onogi et al. reported the existence of a "Transmural air leak" after gastric ESD, as detected by a CT examination<sup>[28]</sup>. In the present study, we cannot rule out the possible existence of severe damage to the surface of the muscularis propria with a transmural air leak, since we did not perform a CT examination in most of the cases undergoing gastric ESD. Thus, there might be a possibility of developing delayed perforation from severe damage to the

surface of the muscularis propria with transmural air leaks after the ESD procedure.”

“Onogi F, Araki H, Ibuka T, Manabe Y, Yamazaki K, Nishiwaki S, Moriwaki H. "Transmural air leak": a computed tomographic finding following endoscopic submucosal dissection of gastric tumors. *Endoscopy* 2010;**42**: 441-447 [PMID: 20432207 DOI: 10.1055/s-0029-1244013]”

In addition, we added the detail information of the definition of delayed perforation in the Materials and methods section, Page 8 as follows:

“Delayed perforation was identified by the sudden appearance of symptoms of peritoneal or mediastinal pleura irritation (gastric tube case) after the completion of gastric ESD, with free air visible on X-ray or computed tomography (CT) images and/or with a gross defect observed endoscopically, although endoscopically visible perforations did not occur during the ESD procedure and no remarkable clinical symptoms were observed, suggesting perforation, just after the ESD procedures.”

(19) **Reviewer 01047630. Comment 2.** In the representative case of this study, it is hard to diagnose the delayed perforation. In the Figure 1B, some concave area suggesting undetected perforation is seen at the proximal edge of the ulcer base. It would be better to change the figures of more typical case.

**Answer.** As we commented below (Answer 18), this case of delayed perforation had sudden appearance of chest pain. Endoscopically visible perforation did not occur during ESD procedure and there were not any findings of remarkable clinical symptoms, suggesting perforation, just after the ESD procedures. According to the reviewer’s comment, we described that severe damage of surface of the muscularis propria due to electrical cautery was seen at the proximal edge of the ulcer base in “Factors associated with delayed perforation” of the RESULTS section, Page 10 as follows:

“At the proximal edge of the ulceration, severe damage to the surface of the muscularis propria as a result of electrical cautery was seen, but no remarkable clinical symptoms, suggesting perforation, were observed (Figure 1B).”

(20) **Reviewer 01047630. Comment 3.** The authors stated that gastric tube cases are

associated with delayed perforation. The possible reasons why delayed perforation increased in gastric tube cases should include the gastric wall thickness (including muscle layer) decreases because of stretching the gastric tube after esophagectomy.

**Answer.** We considered that the reason for the high frequency of delayed perforations in the gastric tube was reduced vascular circulation of the reconstructed gastric tube as we have already describe in the DISCUSSION section, Page 12.

(21) **Reviewer 01047630. Comment 4.** In addition, more important issues are the risk factors for delayed perforation in the normal stomach. More analysis is strongly recommended for this.

**Answer.** We performed additional analysis to clarify the risk factors for delayed perforation in the normal stomach. But all of the clinicopathological factors were not significant on univariate analysis so we cannot calcify the risk factors for delayed perforation in the normal stomach (because of too small number (delayed perforation cases in the normal stomach was only 5 cases)).

(22) **Reviewer 01047630. Comment 5.** On Table 3. the ORs for clinicopahtologic findings which were significant on univariate analysis should be stated in the multivariate analysis.

**Answer.** We added the results of multivariate analysis (NS: Not significant) in the relevant portions of this Table.

(23) **Reviewer 01047630. Comment 6.** On the statistical section, more detail description for multivariate analysis is needed. Especially, which factors are included in the multivariate analysis?

**Answer.** We added the more detail description for multivariate analysis in “Statistical analysis” of MATERIALS AND METHODS section, Page 8 as follows:

“The Fisher exact test...perforations. We performed a multivariate analysis for clinicopathological factors that were significant in univariate analyses.”

(24) **Reviewer 01047630. Comment 7.** Although this study is a retrospective observational study, the permission of IRB should be included in the paper.

**Answer.** Based on the Ethical Guideline for Epidemiological Research, this study was conducted at 2014. In this Guideline, it was not necessary to request the permission of IRB for retrospective observational study in single center. Thus, we did not request the permission of IRB. If your journal require the permission of IRB, we would like to request the permission of IRB although it will take about 1-2 month.

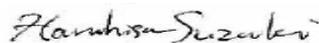
(25) **Reviewer 01047630. Comment 8.** Some errors are seen the Reference section. Please refer to attached file (yellow mark).

**Answer.** We revised the relevant portions accordingly.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



Haruhisa Suzuki, MD  
Endoscopy Division  
National Cancer Center Hospital  
5-1-1 Tsukiji, Chuo-ku, Tokyo  
104-0045, Japan  
Fax: +81-3-3542-3815  
E-mail: harusuzu@ncc.go.jp